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NATURE

A WEEKLY JOURNAL OF SCIENCE

"To the solid ground
Of nature trusts the Mind that builds for aye"—WORDSWORTH

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Volume 192

OCTOBER 7, 1961, to DECEMBER 30, 1961

LONDON
MACMILLAN AND CO., LTD.
NEW YORK. ST. MARTIN'S PRESS, INC.

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$$w(z) = e^{-z^2}\left(1 + \frac{2i}{\sqrt{\pi}}\int_0^z e^{t^2}\,dt\right)$$

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Printed in Great Britain by FISHER KNIGHT & CO LTD , The Gainsborough Press, St Albans,
and published by MACMILLAN & CO LIMITED, at St Martin's Street, London, W C 2,
and ST MARTIN'S PRESS, INC , 103 Park Avenue, New York 17, N Y , U S A.
Saturday, April 28, 1962

CREATIVE ABILITY

IN his presidential address to Section F (Economics) at the British Association meeting at York in 1959, Prof John Jewkes asked some searching questions about accepted correlations between industrial output and the number of scientists and technologists, and suggested that we needed better criteria for determining how much of its resources the community should devote to science and technology Prof Jewkes's views on the shortage of scientists were promptly challenged by Sir Solly Zuckerman, particularly in the light of figures afterwards issued by the Scientific Man-power Committee of the Advisory Council on Scientific Policy, although Sir Solly agreed entirely as to the need for more satisfactory criteria In the past two years there has been no serious suggestion that the supply of scientists or technologists is increasing unduly Shortages of particular categories of scientists, as, for example, teachers of mathematics, have, however, emphasized the soundness of some of Prof Jewkes's remarks about the decisions required in striking the right balance between the numbers of scientists and technologists

This is equally true of Prof Jewkes's comment on the extreme importance of employing the scientists and technologists we already have to the best effect, a matter on which Prof Jewkes already had some doubts This year, at Norwich, his successor as president of Section F devoted the whole of his presidential address to an examination of the conditions which affect the economic use of intellect Prof C F Carter also has reservations as to the extent of the alleged shortage of scientists and technologists He finds no evidence that the present educational system of Britain is biased towards arts subjects On the contrary, he believes that the specialized study of science and technology is in danger of attracting an undue proportion of able minds, and that, at the higher levels of ability, it is more significant to examine the general shortage rather than the specialist deficiencies

Prof Carter begins, indeed, by stressing the importance of the supply of first-rate ability All too often in discussions on trained man-power, or university or technical education, or in such matters as training for management, there is a disposition to ignore the importance of really first-rate ability and its implications to-day and to concentrate on the needs and the supply of the middle and lower ranges of ability Prof Carter rightly points out that, leaving aside for the moment the question of how limited is the supply of such ability, its importance relative to other natural resources increases as technology becomes more complex, and because ideas and methods of organization and working are capable of being copied, those of the highest ability, ingenuity and inventiveness, who are an example to others, are of special importance It is the supply of first-rate ability which determines the speed of change, all other shortages being of secondary importance

Nevertheless, Prof Carter contends that we have given surprisingly little thought to the nature and proper use of this factor, and that there is considerable need for further study and research It is, of course, difficult to define what is meant when in common speech we talk of an 'able man' We do not mean one whose only skill is the perfect performance of a very narrow task The shortage of the higher forms of ability, Prof Carter points out, is a shortage of men capable of dealing with new and unexpected tasks within a certain range, and who are not confined within too narrow a specialization We are looking for an intelligence which is capable of analysing a new situation and reacting to it in a sensible way, and this quite clearly is something which will be found independently of the particular disciplines in which that intelligence has been trained

We are thus at once reminded of the importance of general education itself—but Prof Carter is not deflected from his main discussion Something more is required than developed intelligence, and he notes that since people can be intelligent but ineffective, the causes of ineffectiveness themselves may well be worthy of more attention In fact, serious errors are frequently made in both directions in our judgment as to how intelligent men will react to the stress of responsibility, while Prof Carter does not doubt that scientific selection procedure can reduce these errors, he also considers that personnel selection at the highest levels has far to go before it can claim to be very scientific

Properly speaking, the waste of ability due to failure to find appropriate occupation—because of adverse social or economic conditions during the educational period—is scarcely a matter of the economic use of brains, though it is the aspect that has most commonly been considered Prof Carter, moreover, is hesitant in accepting the conclusion, drawn from reports such as *Early Leaving*, which demonstrate a continuing connexion between a long and successful educational career and high social class of the parents, that the supply of able people will necessarily be increased by a wider and better educational system He questions, in fact, whether improved educational provision will necessarily produce this result, though he agrees that we might be able to alter the supply of ability if we could alter the social structure or environment in some relevant way As yet, however, we do not know which of the inter-related characteristics of a poor social environment are most important in restricting the development of a child, and it is highly probable that while change of this kind may, in fact, be proceeding, it is a long-term process. Further, it is equally possible that the ultimate result of all such changes will be increased numbers in the middle ranges of ability rather than in the high levels of ability with which Prof Carter is primarily concerned in this address

For such reasons, Prof Carter is indisposed to accept, on the evidence available, that there is plenty of high ability available, at present running to waste, and easily conserved, and he turns to discuss the question of what is the best use of an able man's time Prof Carter at once recognizes the extreme difficulty of finding any satisfactory criteria, quite apart from the extent to which the personal idiosyncrasies of ability must be recognized and given due allowance The complex nature of intellectual effort does not permit any ready separation of easy or routine tasks and those of greater complexity or strenuous activity While it is important that the best qualities of mind and experience should be available to deal with difficult, controversial and complicated matters, it is seldom possible to filter out all lesser problems and deal with them at a lower level in the organization It might be possible to design information systems which could help here in taking care of the lesser problems, but there would remain the problem of how experience is to be gained by the high ability which is coming on; and there is also liable to be disagreement as to function and ability Prof B R Williams and Prof Carter found in their industrial studies, in fact, that very little attention had been given to the science of using scientists productively, and he suggests in the present address that in all forms of work which use high ability, there is need for much more study of the conditions which favour effective work. He considers that there are valuable principles to be discovered and reforms to be made

It is curious, in view of the dearth of material to which Prof Carter refers, that he makes no reference to the report of the Barlow Committee on Scientific Staff, issued in 1945, which provided one of the best analyses of the conditions for effective scientific work, at least in the Government service, which has yet appeared He notes, indeed, that men of high ability are often in positions in which they are able to create their own conditions of work, though at the same time he points out that the economic use of brains may involve protecting a man from the self-imposed wastage of his talents, as well as giving him freedom to use them to the full What needs to be said here, above all, is that it is not so much fresh knowledge about the conditions conducive to effective work—whether it be creative or administrative—at the highest level that is required, but the perceptive application of existing knowledge So far as the Government is concerned, disregard of what is already known about such conditions is largely responsible for much of the present frustration and wastage, particularly among scientific staffs, and it would seem a sufficiently important matter to warrant attention by the Minister for Science or the Advisory Council for Scientific Policy

From this aspect of the efficient use of distributed man-power, Prof. Carter turns to discuss the distribution of ability between different occupations Here he suggests that the determinant factor is the initial choice of occupation, and he stresses the desirability of greater attention to factors which promote mobility between different occupations Desirable in itself on the grounds of experience, such mobility is a corrective to mistaken or excessive choice of particular occupations, and all the more in that there is still much to learn about factors influencing the initial choice of occupation Moreover, the choice at present seems often to be needlessly haphazard, it should be possible even with existing knowledge for this choice to be much better informed both in regard to the potentialities of the boy or girl, and the advantages or responsibilities to be found in the occupations to which specialization leads

The present shortage of mathematics teachers underlines the importance of Prof Carter's remarks on the distribution of high ability between the universities and elsewhere Surveys of the employment of graduates made by Political and Economic Planning and other similar surveys have given no full answer to such questions as the distribution of high ability between expanding and declining, or modern and traditional industries, between large and small firms, between different parts of Britain, between the nationalized industries and other parts of the public service Yet under certain conditions this could be the decisive factor in the success or failure of an industry or a firm, and despite the imperfections of definitions of ability, more research on these matters and on the trends in the demands of various industries and services is needed

By and large, Prof Carter outlines very fairly the problems in this field that require attention, although he is inclined to make too little of what could be done with existing knowledge and resources. Curiously enough, while he takes a much less optimistic view as to the existence or extent of the pool of ability on which we can draw than did Prof. G Seth in addressing Section J (Psychology), he ignores completely, as such, the reservoir of ability to be found in the employment of women which, Prof Seth notes, could go far to meet any existing shortage of skilled talent Further, he does not discuss the contribution which improved methods of teaching could make, either in the development of the student or in the more efficient use of the time of the trained teacher Two points, however, should be noted in conclusion Prof Carter fully recognizes that the effective use of trained man-power in the universities is not simply a matter of using the university teacher's time to the best advantage, but is also a part of the national effort in research and the right use of man-power in relation to scarce and expensive scientific equipment —a matter to which also more critical and constructive thought must be given

Finally, there is the question of what economies of scale may be possible in the use of high ability

Prof Carter does not discuss that difficult question in any detail Indeed, he seems to raise it only to illustrate the complexity of all these issues and how readily they may incline to decisions which are self-frustrating or defeating. It is only too easy to take decisions for one purpose only to find that they have created conditions which destroy the incentives it is sought to provide We must indeed recognize the existence of these economic problems and give them the study and attention they need, but it cannot be too carefully kept in mind that nothing is easier than to plan out of existence the conditions in which high, and especially creative, ability functions most effectively and efficiently

PHASES IN EVOLUTION

Grossablaufe der Stammesgeschichte
Erscheinungen und Probleme Von Prof Arno Hermann Muller. Pp vii+116 (Jena Gustav Fischer Verlag, 1961) 18 20 D M

THE work under review is a revised edition of one originally published in 1955 , some of its subject-matter and diagrams have also been incorporated in Vol 1 of its author's *Lehrbuch der Palaozoologie* (1957) Its purpose is to study the fluctuating fortunes, during their long geological histories, of those major animal groups of which abundant fossil remains are preserved, and to draw such general conclusions as seem possible The author uses as data the number of genera recorded in successive subdivisions of the geological column, each representing some 15–18 million years He is not the first palæontologist to have taken up this line of investigation, for an account of very similar work by a group of United States authors has appeared in the *Journal of Paleontology,* 26 (1952) For presentation of the facts he relies mainly on symmetrical diagrams in which the number of genera, grouped according to their time-ranges in differently shaded areas, is plotted horizontally across a median axis representing geological time

The obvious objection to the choice of genera as the basis of the study is that of differing taxonomic standards among workers who have specialized (as most modern palæontologists tend to do) in fossils belonging to particular families or geological formations, for irresponsible genus splitting might well become interpreted as evidence of unusually intense evolutionary diversification By taking his data from treatises in which the material has already been digested according to more or less uniform standards the author has probably avoided this pitfall to some extent In discussing the trilobites he gives two diagrams, based respectively on modern works in which 996 and 1,395 genera are recognized, and he shows that both present the same general picture In the case of Mollusca other than Cephalopoda his information does not appear to be derived from up-to-date sources Thus on p 94 the number of known gastropod genera is given as about 700, whereas in the Wenz–Zilch treatise on this class (1938–60) in the *Handbuch der Palaozoologie* the number of shell-bearing genera recognized is 3,400, the figure 296 given for known genera of Bivalvia (Lamellibranchia) is similarly disproportionately low

It is probable (although some authors deny it) that the concept of a species is less subjective than that of a genus, so that a census of species would have been a more satisfactory basis for the present study According to a recent estimate, however, among invertebrates alone the total number of extinct species with hard parts capable of becoming fossilized has been 7–8 millions, and, even though (according to estimates from other sources) those so far described number only from one-hundredth to one-twentieth of the whole, it would clearly have been beyond the capacity of a single worker to assemble the necessary data about them A count of genera was the only practicable approach to the investigation. One thing is clear , the number of genera and species living at any period does not necessarily bear any relation to the total of adult individuals belonging to a group or give any indication of its relative competitive success The former is incalculable, the latter unassessable

In the work under review the diagrams already mentioned show a succession of bulges and contractions , not one is a steadily broadening triangle, although there is some approach to this with the Gastropoda and Bivalvia A marked bulge corresponds to what the author terms a "Virenzphase", a phase of profuse mutational changes. The most abrupt and intense of such phases are the 'explosive' or 'eruptive bursts' of United States and British writers According to the author, three types of evolutionary history are distinguishable. In the "regressive" type (Trilobita, Graptolithina, Nautiloidea) there is a series of "Virenzphasen" decreasing in intensity and duration and leading eventually to extinction In the "progressive" type there is a succession of such phases increasing in intensity and duration, although ultimately the group may fail to survive a subsequent phase of shrinkage and crisis (For example, during the two intervals, at the close of the Permian and of the Triassic periods respectively, between their three "Virenzphasen" the Ammonoidea almost died out, but two or three genera just managed to survive in each case ; a third crisis at the end of the Cretaceous, however, sealed the fate of the whole order) In the third type of evolutionary history, the "intermediate" type (Brachiopoda, Amphibia), there is a series of complex "Virenzphasen" each consisting of a relatively weak initial phase, an intense main phase, and a weak late phase.

Applying these results to groups now living, the author considers the Nautiloidea, reduced to a single genus following what he believes to be the final phase of a history of the "regressive" type, to be probably well on the way to extinction. The Echinoidea and Reptilia, however, although now on the decline, would appear to have further "Virenzphasen" ahead The Brachiopoda may pass through a weak phase of increase before their final extinction The Mammalia are now passing through the main phase of a "progressive" series , at least, they were doing so before the advent of man

Chapters are devoted to a survey of knowledge of pre-Cambrian life (illustrations of recent finds in South Australia are reproduced in some of the plates) and to a discussion of the various factors which may have combined to determine the history of life through the ages A study of the diagrams in this book suggests to me that the author's three-fold classification is an over-simplification The future, in fact, seems to be entirely unpredictable. L R. Cox

CELLS IN EVOLUTION

Cytology and Evolution

By Dr. E N Willmer Pp x+430 (New York Academic Press, Inc , London · Academic Press, Inc (London), Ltd , 1960) 70*s*

THIS is a scholarly work, for Dr. Willmer has given thought to his subject for many years Inevitably, it is not an easy book to read and assimilate *Cytology and Evolution* is a challenging title , at one extreme, it is concerned with the techniques required to reveal the nature of cellular physiology, and at the other, the infinitely complicated problem of cell evolution in higher organisms Characteristically, Dr Willmer introduces the subject by visualizing "the conversion of the Victorian carriage into the racing car of to-day" as a lesson in evolution He is intrigued by "the mystery of a common organization", and his argument opens by examining the similarity in the fine structure of cilia throughout the lower and higher forms of life

The author knows as much as anyone could about the behaviour of cells isolated from their normal environment and grown in tissue culture From his experience, he has developed the interesting simplification that tissue culture encourages the growth of three stem-lines of cells These are the epitheliocytes, the mechanocytes, and the amœbocytes, and the photographs selected are most effective in illustrating his point of view

The book begins its own organic growth with the definition of these different cell forms, and here the reader meets the first complications in the argument. What, the author has asked himself, are the morphological and physiological features which define an epitheliocyte, a mechanocyte, and an amœbocyte? Many examples can be cited which apparently define or separate one cell from another, such as the strange phenomenon that an epitheliocyte will be unaffected by the death of its neighbour (punctured through his nucleus), while a fibroblastic cell will choose to die along with its neighbouring cell There is also the problem of the permanent cell lines which have been maintained for many years in tissue culture and which appear to have become a 'classless' society

The basic ideas of the author take up one-third of the book , their effect on the analysis of embryogenesis is developed in the remaining two-thirds of the book Dr Willmer takes the reader as gently as possible through the reversible transformation of flagellate cell types to amœboid cell types, as found in certain lower organisms, particularly *Naegleria*, on which he has himself done fascinating work One reader, at least, is concerned that he has chosen only to discuss the transformations in terms of homoiostasis, and does not envisage, for example, the possibility that the ionic environment might alter the templates for enzyme formation within the cell

In developing his theme, Dr Willmer seems at first to be 'Lamarckian' rather than 'Darwinian' in his outlook He instances the fact that "many animals independently under Arctic conditions cease to produce melanin in their skins, and so all become white", and evidently considers this to be an adaptation to the iciness of their environment instead of natural selection How else can one construe the observation on the same page (227) that "when phenyl-alanine is added to tissue cultures of even the ventral ectoderm, the cells of which would never normally produce melanin, some of these cells can actually be induced to do so"? Perhaps it is only a lapse in analogy, for clearly the author favours natural selection when he later describes the disadvantages of "poor form-vision under critical conditions of hunting or being hunted"

In discussing the development of the kidneys, gonads, adrenal cortex, and other cell populations closely related to them, he envisages their origin from the cœlomoducts and the nephridia of the more primitive invertebrates This last third of the book is a mine of valuable information hidden in catacombs of unresolved definition and delineation When, for example, at page 274, the reader finds that the nephron of kidney tissue would best be described as "some sort of nephromixium", he later has to try to understand why the spermatic tubules and their mesonephric elements should be described as a "mixonephridium".

Appropriately the book ends on the subject of light and evolution of vision, and the final chapter is an illuminating summary of the author's concepts of evolutionary cytology Here, Dr Willmer states concisely the principles on which he bases his view about the evolution of cell types and embryogenesis As a cytologist he treats cell structure with reverence and appears to be horrified by the butchery involved in the biochemical analysis of sub-cellular fractions Nevertheless, the book concludes that there is an exciting 'jungle pathway' ahead for microbiochemistry and cellular physiology. Dr Willmer has done a great service by creating a new and well-indexed route through the jungle of cytological embryology.

I Leslie

A SURVEY OF EXTRA-SENSORY PERCEPTION

The Hidden Springs

An Enquiry into Extra-Sensory Perception By Renée Haynes. Pp. 264+9 plates (London Hollis and Carter, Ltd , 1961) 30*s* net

ALTHOUGH this book is said on the title-page to be an inquiry into extra-sensory perception, it is much more a descriptive historical summary than an inquiry Miss Haynes, writing from the point of view of a Roman Catholic, traces the belief in extra-sensory perception from early times, showing how the faculty was believed to exist and how it was described and became a part of various theological and philosophical systems Passing from the supposed appearance of extra-sensory perception in animals, insects, etc., Miss Haynes proceeds to describe its occurrence and treatment among people in ancient, medieval and modern times, and traces the story from the days of the Roman Augurs to the late Prof Jung's theory of synchronicity.

This book, like others on the same subject recently published, illustrates a trend in psychical research which is becoming increasingly apparent Although there have been some trenchant criticisms of some of the more famous and supposedly 'water-tight' experiments, the tendency is to ignore and belittle such work and to proclaim that extra-sensory perception is now an established fact, and that, as Miss Haynes puts it, resistance to the idea springs from dread of the acute intellectual discomfort involved in recognizing its implications, a feeling which would be new to many scientific men What the author fails to realize is that the resistance in the mind of the well-informed reader is engendered by precisely the kind of book that she has written Thus when she speaks of certain early experiments where the methods of the 'exact sciences' were used to organize the tests and evaluate the results, it can scarcely be thought that she can be ignorant of the nature of these tests and of the damaging criticisms levelled against them Nevertheless, if the book is to be read as a popular and well-written historical introduction to the subject, it will serve at least one of its main purposes, since it provides the reader with a clear sketch of the development of the study of extra-sensory perception accompanied by a series of interesting religious and philosophical speculations

The book is well produced and indexed and would have been improved had the author taken a little more care with her proper names and a good deal more caution in the treatment of some of her material

E J Dingwall

EFFECTS OF CLIMATE ON VEGETATION

Klimadiagramm-Weltatlas

Von Prof Heinrich Walter und Dr Helmut Lieth 1 Lieferung Mit einer Deutschen Einleitung und Auszugen in Englischer, Franzosischer, Russischer und Spanischer Sprache 11 Karten und 65 Beiblattern (Jena Gustav Fischer Verlag, 1960) 120 D M

GERMAN work on the relationship between climate and vegetation is of long standing, stemming from the celebrated work of Humboldt ; and our ideas with regard to the classification of climates owe much to Koppen One turns accordingly with keen expectation to the first section of this newly produced and bulky atlas

Prof H Walter, as director of the Botanical Institute of the Landwirtschaftlichen Hochschule at Stuttgart-Hohenheim, with his assistant Dr H Lieth, has embarked on the mapping of all the meteorological stations for which information is accessible, and on the presentation for each station of 'climate graphs' to illustrate the extent of characteristic types of climate This is to fulfil the need to compare the climates of different regions in which the vegetation is very similar They quote as an example a recent inquiry as to where a climate corresponding to that of the central Anatolian steppe might be found in the United States, or in Central Asia, moreover, regions with similar types of vegetation offer the same possibilities for land utilization, for example, in agriculture or forestry An explanatory introduction is given in four languages , it is hoped that the diagrams will also yield a basis for a more accurate classification of climates

This characteristic aim is supported by the admirably clear printing of a very large number of station diagrams showing the annual march of the monthly means of temperature and precipitation , indeed, an elaboration of the familiar diagrams of present-day school atlases Vertical scales are so chosen that 10° C corresponds to a precipitation of 20 mm This is said to derive from a paper by Gaussen, which demonstrates that in the Mediterranean region aridity can be said to prevail when on such a scale the precipitation curve falls below the temperature curve, and vice versa Additional information with regard to excessive rainfall, the length of the frost-free season and severity of winter frost is provided ; and Prof Walter also introduces his concept of the limit between forest-steppes and woody regions

This first selection of maps and diagrams gives a pleasant impression of useful and orderly compilation; one can run one's eye along scores of 'climatic graphs' with interest But many will dispute whether, in the light of our knowledge of evaporation-losses, humid and arid months can be distinguished over a wide range of latitudes on such a very simplified basis Such detailed mapping of climatic regions also begs the question of purpose; is it in order to tell American troops at what point they must put their boots on ? Some of the subdivisions are quite fascinating; that an outlier of the maritime climate of North France appears to be found at Cologne raises the interesting question of where the observations are kept It is curious to find no use made of the long-standing record at Stanley in the Falkland Islands This atlas seems in these days to be a little old-fashioned , it makes a very fine compilation of information and the pictorial presentation has its advantages , but it is decidedly expensive and bulky for the more general reader, and lacks the detail for which some specialists will probably ask

G Manley

HISTOLOGY AND ELECTRON MICROSCOPY

Histological Techniques for Electron Microscopy

By Daniel C Pease Pp xii+274 (New York Academic Press, Inc , London Academic Press, Inc (London), Ltd , 1960) 7 50 dollars

IN spite of the extensive use of the electron microscope in biological and medical research in the past decade, there has been a complete lack of a comprehensive guide to techniques since Drummond edited *The Practice of Electron Microscopy* in 1950 Presumably all the leading exponents of the subject have been too busy cutting ever thinner and thinner sections in the exciting exploration of the ultrastructure of tissues and micro-organisms Certainly there has been no lack of publication in the way of original papers , but for one reason or another no general survey of methods has been compiled One of the pioneers of ultra-microtomy has now remedied the situation The text under review starts from the proposition that a novice should be able "to gain reasonable competence through his own ingenuity if he has a guidebook to help with the maze of detail" This function the author proceeds to fulfil in the course of nine chapters and two appendixes After general advice on the organization and management of an electron microscopical laboratory, the fixation, embedding and sectioning of tissues are discussed in great detail The mounting of sections and their examination in the microscope are then considered, and a further chapter is devoted to photographic techniques The final chapter describes preparation procedures other than sectioning, including shadow-casting and replica making The appendixes give useful guidance to the beginner about the literature of the subject and available sources of equipment and materials

The treatment is clear and thorough, being illustrated with photographs of various operations, as well as with typical micrographs The author is at pains to describe many "seemingly small, but actually critical details" of preparation This is a praiseworthy aim, but it opens the way to dogmatism and the glossing over of differences of opinion between rival schools of thought However, the author is usually frank in stating where his own experience has been limited, and he also provides a list of references at the end of each chapter, so that the novice can seek alternative advice In the almost complete absence of regular courses of instruction in the techniques of electron microscopy, the present text provides as comprehensive a handbook as can be expected from a single pen It is designed to be used at the bench rather than in the library, and should be beside every electron microscope in use in a biological or medical laboratory

V E Cosslett

RADIATION PROTECTION

Radiation Protection and Recovery

Edited by Alexander Hollaender (International Series of Monographs on Pure and Applied Biology Division Modern Trends in Physiological Sciences, Vol 7) Pp v+392 (London and New York. Pergamon Press, 1960) 70*s* net

Problems of Infection, Immunity and Allergy in Acute Radiation Diseases

By N N. Klemparskaya, O G Alekseyeva, R V Petrov and V F Sosova Translated from the Russian by Lydia Venters Translation edited by R Clarke. Pp viii+165 (London and New York Pergamon Press, 1961) 50*s*

The Delayed Effects of Whole-Body Radiation

A Symposium Edited by Bernard B Watson (Jointly sponsored by the Operations Research Office and the Walter Reed Army Institute of Research) Pp ix+80. (Baltimore, Md The Johns Hopkins Press, London Oxford University Press, 1960) 36*s* net

The Entry of Fission Products into Food Chains

Edited by J F Loutit and R Scott Russell (Progress in Nuclear Energy Series 6 Biological Sciences, Vol 3) Pp vii+154+xii (London and New York Pergamon Press, 1961) 45*s* net

Radioactivity for Pharmaceutical and Allied Research Laboratories

Edited by Abraham Edelmann (A Symposium sponsored by Nuclear Sciences and Engineering Corporation, held in Uniontown, Pennsylvania) Pp xii+171. (New York. Academic Press, Inc, London Academic Press, Inc (London), Ltd. 1960) 6 dollars

CURRENT and forthcoming legislation, in the form of Factories Acts such as the "Ionising Radiations (Sealed Sources) Regulations 1961", and its companion dealing with the use of unsealed radioactive substances in industry, require the appointment of a medical officer, who will carry out examinations on those workers exposed to radioactive materials Much of this work will fall on the industrial medical officer already connected with the firm, or the local medical officer of health, but it will be extremely unusual to find one with any knowledge on the effects of radiation It is true that a set of notes (*Medical Supervision in Radiation Work*) has been drawn up by the World Health Organization, but the keen medical officer will generally wish to supplement this knowledge with information on the effects of relatively high doses or chronic exposure to relatively low doses of radiation

The subject is, however, still in its infancy, and much of the information is contained only in professional journals or proceedings of learned societies. The production of any text-book on the subject is therefore of considerable interest at the present time, though from this point of view the books under review are rather too specialized for the average medical officer On the other hand, there is much to be learned from them, even by the non-specialized reader, and while the first three are primarily medical in character, the health physicist can also find much to interest him here

All five books are the products of a team of authors, each a specialist in some particular part of the field

Probably the most important is *Radiation Protection and Recovery* This is not a comprehensive text-book, rightly concentrating on biological, physiological and biochemical methods of protection and leaving discussion of shielding, etc, to physicists Its stated aim is to present some of the background of a rapidly developing subject, and in this it succeeds. A considerable amount of space is devoted to experimental treatment of acute whole-body radiation injury in mammals and to the allied problem of antibody formation, which is of great importance in connexion with bone-marrow implantation

A remarkably extensive bibliography of some 1,500 references is included; this alone should make the book a valuable source of information in this field

The second book is a translation, sponsored by the U S Atomic Energy Commission, of a monograph published in Moscow in 1958 and is probably not, therefore, fully representative of current thought in the Soviet Union It deals exclusively with the results of animal experiments on auto-infection following irradiation, natural and induced immunization, allergic reactivity, and infectious diseases in radiation sickness, again there is an extensive bibliography, about half the 326 references being non-Russian in origin.

From the research worker's point of view, *The Delayed Effects of Whole-body Radiation* will be disappointing, though for those generally concerned with radiation protection there is some interesting material Effects on life-span, the induction of malignant diseases and the production of cataracts are each covered in two chapters, one on animals and the other on man, genetic aspects are treated jointly

The Entry of Fission Products into Food Chains is not directly comparable with the previous three books, but it is justifiable to include it in this review since the subject is so closely connected with radiation protection As one might expect of the editors, who are authorities in this field, we have here a most informative group of papers, which were originally produced as reports by the Atomic Weapons Research Establishment There are two sections on the retention of fall-out on herbage and ripe cereals, but the majority of the papers deal with animal experiments connected with actual nuclear weapon tests. This is definitely a specialist's book, but should also be of great interest to anyone concerned with the wider aspects of radiation protection and civil defence

The last book is unfortunately titled It is certainly not a text-book for pharmaceutical laboratories, being simply the proceedings of a symposium, it is very mixed in character and is too varied in standard to be of great interest

Hugh D Evans

Structural Concrete

By Prof Kurt Billig Pp xx+1020 (London. Macmillan and Co , Ltd , New York St. Martin's Press, Inc , 1960) 84*s*. net

PROF. BILLIG is a well-known author of books and papers on reinforced concrete and prestressed concrete His present volume aims to provide a "minor encyclopædia on structural concrete", and this is indeed a fair description of the work. It is divided into three sections, the first dealing with materials, the second with structural design and the third with design and construction. In attempting to cover such a wide field in a single volume of manageable size the author is inevitably faced with very difficult problems of selection and condensation On the whole, Prof Billig has tackled these problems successfully although the depth of treatment varies somewhat from one chapter to another

Part 1 is devoted mainly to concrete technology, that is to a discussion of the properties, selection and testing of cements and aggregates and the techniques of mixing, placing and consolidating concrete A chapter is devoted to the important question of durability Part 2 considers first the basis of structural design in concrete and goes on to discuss in considerable detail the methods of proportioning practically every kind of structural element in reinforced concrete, including beams, columns, slabs, arches and shells A great amount of ground is covered, and numerous diagrams, tables and examples are included The third section of the book discusses the use of structural concrete for a wide variety of building and engineering structures, with reference to actual examples

A very good balance has been achieved between structural analysis, practical design considerations and concrete practice The book is a veritable mine of information for structural engineers and architects, to whom it can be highly recommended

A W HENDRY

An Electronic Outline of Organic Chemistry

By Dr. S Horwood Tucker Pp xvi+478 (London · University of London Press, Ltd , 1959.) 63*s* net

THIS is a book, written for undergraduates, in which the reactions of the commoner types of organic compounds are interpreted in terms of the electronic theory Dr. Tucker is to be congratulated for having rescued so effectively the "gems of electronic exposition" from submergence in the "heavy matrix of comprehensive fact". Not that the importance of experimental fact is in any way diminished indeed, just sufficient significant facts have been carefully selected and lucidly expounded to present to the undergraduate a clear and reasonable account of the essentials of organic chemical theory

There are, however, certain omissions which, though they do not detract from the overall value of the book, nevertheless seem surprising, especially since these topics are now included in undergraduate lecture courses Thus, the reactions of benzyne, and of carbenes (even in connexion with the Reimer–Tiemann reaction, p 407) are neglected ; likewise Bender's work on ester hydrolysis receives no mention

The text is well supplied with references to both individual papers and review articles

It is a pity that several misprints remained undetected in the proofs Fortunately, most of them are relatively trivial and they do not seriously affect the sense of the text

The book is well produced and possesses a good index.

While this book can be strongly recommended to students, it is likely that its price will deter all but the most affluent, or the most self-denying of the impecunious, from purchasing it. NEVILLE H. P SMITH

Proceedings of the 1960 Annual International Conference on High Energy Physics at Rochester

N.Y., August 25–September 1, 1960. Edited by E C G Sudarshan, J H Tinlot, and A. C. Melissinos Pp. xxv+890 (Rochester, N Y University of Rochester, 1960 Distributed by Interscience Publishers, Inc , New York and London) 13 50 dollars

THE International Conference on High Energy Physics which was held in Rochester, N.Y , was the tenth in the series This annual conference is acknowledged to be the most important one in this field Its purpose is to assemble a representative group of workers from high-energy laboratories throughout the world to discuss the experimental results obtained and the theoretical developments which have occurred during the previous year

The *Proceedings* are divided into three sections The first section covers the initial "working" or "S" sessions, several of which were usually held concurrently The second, "R" section, contains the reports of the *rapporteurs* Finally, in the last three chapters are contained, in full, the papers presented at three "plenary" sessions The subject-matter of the conference was limited to the following topics the strong interactions of pions and nucleons ; the strong interactions of strange particles , weak interactions ; the structure of elementary particles , new results at super-high energies, and finally, theories of elementary particles

The editors, secretaries and all concerned with the publication of the *Proceedings* deserve the highest praise for producing such a magnificent record within two months of the end of the Conference No physicist interested in elementary particle physics can afford to be without the volume close at hand

G H STAFFORD

Probability Theory

By Prof. Michel Loève Second edition (The University Series in Higher Mathematics) Pp. xvi+685. (Princeton, N J D Van Nostrand Company, Inc , London · D Van Nostrand Company, Ltd , 1960) 111*s*

THE first edition of this book appeared in 1955 and went a long way to fill a serious gap in the mathematical literature The new edition, revised in detail and longer by two chapters, comes a little nearer to closing the gap and is still the only (and an excellent) approximation to a comprehensive textbook on mathematical probability. The new chapter 11 (64 pages) deals with the generalities of the theory of stochastic processes (including separability and measurability), continuous-parameter martingales, and decomposability theorems The new chapter 12 (95 pages) deals with Markov processes It starts with an account of the 'strong' Markov property, and passes on to a discussion of the differentiability of transition functions before taking up the theory of one-parameter semi-groups of operators, which it pursues as far as a proof of the basic Hille–Yosida theorem Finally, there is a valuable synthesis of the recent work by Feller, Dynkin and others on diffusion theory. D G. KENDALL

PROF. A. J. EAMES AND THE MORPHOLOGY OF THE ANGIOSPERMS

By Prof F. C. STEWARD, F.R S
Cornell University

By a happy combination of circumstances, the McGraw Hill Publications in the Botanical Sciences have issued *Morphology of the Angiosperms*, by emeritus professor of botany, Arthur J Eames, in the author's eightieth year* It is therefore fitting that botanists should congratulate Prof Eames on October 10, his eightieth birthday, on an active and fruitful life devoted to the study of plants and to the teaching of botany His latest book, which is a crowning achievement in an already distinguished career, is also a proper subject for congratulation

For almost fifty years (since 1912) Prof Eames has been identified with botany at Cornell University He joined the staff of that University, fresh from his first travels to Australia and New Zealand, and, when botany was transferred wholly from the College of Arts and Sciences to the College of Agriculture in 1913, Prof Eames formed one of that group which was to make the newly constituted Cornell Botany Department known and respected the world over by the mid-1920's and '30's Now, in his eightieth year, Prof Eames is the only one of that first group who is still actively engaged in botanical research on the Cornell campus Throughout that near half-century, his presence has lent distinction to Cornell botany, and his work has enriched the subject everywhere This distinction was recognized by the University of Glasgow in 1956 by the award of an honorary LL D degree

Although Prof. Eames retired from active teaching in 1949, he has led a full life since, and the title 'emeritus professor' has been to him a challenge and an opportunity to pursue further scholarly work, of which the *Morphology of the Angiosperms* is a product Never was the title 'emeritus professor' more richly deserved, nor more gracefully borne, for throughout this period of retirement he has set an example of continued dedication to his lifelong study of plants He has also been ever ready to share his knowledge, whether in the casual conference or the country walks in which his love of Nature excels These brief congratulations would, however, be incomplete—and even inappropriate—without reference to Mrs Eames, who was herself technically trained in botany and has been a great help to Prof Eames throughout, and especially so in the laborious task of producing this latest book

It is appropriate at this point to look back on Prof Eames's career and on his influence on teaching and research in botany Together with the late Prof. Wiegand, he produced the *Flora of the Cayuga Lake Basin*—in the obvious desire to know first the plants which were closest at hand Indeed, many of the early collections which formed the basis of the present Wiegand herbarium were a part of this relationship Although morphology has always been his consuming interest, Prof Eames first became most widely known for the *Introduction to Plant Anatomy*, first published in 1925 and revised in 1947 This book, compiled with his first graduate student, Prof. McDaniels (also now an emeritus professor) was, and in many places still is, the essential modern text on which successive generations of botanical students have become aware of plant anatomy Those who, in the 1920's and 1930's, were privileged to take Eames's course in plant anatomy know that much of the wealth of illustrative material in the form of prepared slides and sections had actually been produced by Eames and McDaniels It was a rich experience for the student to see not merely a stem, or one example of this or that anatomical feature or tissue, but a rich array of representative examples In this, as indeed in the study of morphology, Prof Eames's outlook was always comprehensive Even then, however, the book on anatomy was essentially the background, or working manual, for it had distilled the essence of Eames's experience of anatomy, and his lectures clothed this framework with much more that had been gained from a rich observational experience Yet, with this far-ranging interest to comprehend the plant kingdom as a whole, Prof Eames has in all his books shown that rare ability to state the essentials, to distil it down to its minimum, so that often a paragraph presupposes the equivalent of a research investigation before it could be written

The new *Morphology of the Angiosperms* is again in this style, though necessarily it is a larger work than its predecessor on the *Morphology of Lower Vascular Plants*, which was published in 1936 The new book comprises 518 pages, 147 well-chosen figures, with often several pages of selected references at the end of each chapter The *Morphology of the Angiosperms* is again remarkable for its concise style and for the way its author has been able to compress the knowledge gained from the literature and his own experience. Again one senses that, over the years in which this volume has been prepared, Prof. Eames had conducted the equivalent of many research projects, each worthy of separate review, on subject-matter which in the book is concisely treated

Prof Eames's first book on morphology dealt with the lower vascular plants In respose to an evident need, however, the new work deals with the angiosperms—the largest in number, most complex and most important vascular plants. One wonders whether, and even hopes that, Prof Eames will now fill the gap and enrich botanical science by yet another work on the gymnosperms.

Characteristically, Prof Eames does not in his introduction enlarge on what morphology is, and in these days of ever-narrowing specialization—of plant physiology and biochemistry, of cytology and genetics, of cell physiology, cytochemistry and ultrastructure, of enzymology and molecular biology—some may even ask, what is it all about?

The book is simply and completely about angiosperms—the largest modern group of seed plants and morphology here implies strictly that the work deals with the structure and the form of these plants The work distils from the literature and from Prof.

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* *Morphology of the Angiosperms* By Prof Arthur J Eames. (McGraw-Hill Publications in the Botanical Sciences) Pp xiii + 518 (New York McGraw-Hill Book Co , Inc , London McGraw-Hill Publishing Co , Ltd., 1961) 104*s* 6*d*

Eames's own rich experience what one can interpret about angiosperms from what one observes of them, and observation here means visual observation, aided by the lens and the optical microscope Among the allied disciplines that are invoked to interpret angiosperms from this point of view, physiology, biochemistry, genetics, ultra- and sub-microscopic structure as yet find no place Prof Eames looks at plants as they are, as he sees them, and by an essentially comparative approach seeks information which is to be interpreted against the background of evolution and phylogeny In this sense the book follows a classical tradition

But it is precisely because so much modern biology and botany has become so dissected into specialties that this comprehensive work is now so valuable Indeed, it should be especially valued even by those students of plants whose interests lead them to experimentation, to culturing organs and cells, to the study of biochemistry and nutrition, and to the approach to truth at the molecular level A generation which is steeped in this sort of thing has rarely had the time, even if it had the interest, to acquire the breadth of knowledge and scholarship about plants that Prof Eames's book now contains Even a glance at the table of contents, for the headings and sub-headings of the chapters, may be a salutary reminder to some that although biological events may often be determined at the cellular and subcellular level, they finally culminate in organization that needs to be comprehended at much higher levels Those of us who attempt to exploit the new tools of physiology, biochemistry and ultra-structure need particularly to appreciate the nature and the relationships of the cells and organs with which we work

Here then is a modern work which synthesizes knowledge in a comparative way about such parts of the plant body as shoots and roots, leaves and stipules, nodes and branches, the inflorescence and the flower, the androecium, the stamens and pollen, the gynoecium, carpel and ovule, sporogenesis, fertilization, embryology, seeds and fruits, etc , and, in the course of this treatment, it introduces the reader succinctly to classical morphological concepts and to the author's contemporary views But it is in the chapters on the stamen and the carpel and their derivation from foliar organs that Prof Eames's personal interest and interpretations are particularly evident

Inevitably this work, which could not possibly be summarized here, will find a sure place among the botanical classics, and it will necessarily be read by those who make morphology their speciality But this book should do more than this, for it should be a ready means to guide those who work experimentally with angiosperms, and especially those concerned with problems of growth and reproduction, into the proper interpretation of the nature of the material with which they deal There is a dangerous and mistaken idea abroad that biology has outgrown the older descriptive disciplines of morphology, anatomy and taxonomy Prof Eames's book should dispel this idea by bringing a fresh presentation of this older subject In fact one notes, parenthetically, that Prof Eames finds that the older literature is often nearer the truth than much which has been written more recently

A less-modest man than Prof Arthur J Eames might, in his eightieth year, have titled his book "The Angiosperms as I see them", for he is one of the very few who have had the experience and have the knowledge, the vision and the understanding for this great task

Botanists everywhere will feel indebted to him for this additional achievement in a long and fruitful career

RE-EVALUATION OF SOME FACTORS IN ARTERIOSCLEROSIS

By Dr HANS KAUNITZ

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MORPHOLOGICALLY, arteriosclerosis is defined as a disease of the intimal layers of the arterial wall occurring focally and associated with intimal thickening and lipid deposition The earliest changes can be seen in infants The internal elastic membrane becomes deformed, calcified, and fibrotic These foci are eventually called atheromata Disturbances of the flow of blood may ensue Frequent sites of these changes are, in addition to the aorta, the coronary, cerebral, and lower leg arteries Any theory of the disease will have to account for these facts at least

Thickened arteries (παχειη αρτεριη) were noted nearly two thousand years ago by Aretaeus of Cappadocia The term artheromatous (or tuberculous) was used by Morgagni[1] and perhaps even by others before him, but modern ideas about the disease were developed by the great French physicians of the Napoleonic era Lobstein[2] classified the disease as a nosological entity between 1820 and 1833 and was the first to use the name arteriosclerosis He had the first inkling that the disease may be related to cerebral accidents The relation of myocardial infarction to arteriosclerosis was established much later After the great English physicians of the eighteenth century had recognized the association of coronary disease and angina pectoris, it took another century to establish that this coronary disease is arteriosclerosis

Soon after Lobstein's description, two theories as to the pathogenesis of the disease were developed which still dominate our thinking Rokitansky thought that abnormal clotting processes, resulting secondarily in the organization of thrombi, were responsible for the arterial roughness[3] Virchow assumed that, in what he called endarteritis chronica deformans[4], focal inflammatory changes are followed by deposition of lipids such as cholesterol, which had just been discovered Virchow may have been somewhat handicapped by his inability to distinguish arteriosclerosis from other arterial diseases, such as syphilitic aortitis, which became possible only at the end of the nineteeth century

In addition to syphilitic aortitis, other arterial diseases involving calcification were recognized, particularly media necrosis[5] Whether or not the latter is at all related to arteriosclerosis is by no means sure For purposes of definition it should, at the present time, be excluded This would make unnecessary the use of such terms as atherosclerosis, angiosclerosis, and the like

At about the turn of the century, it was discovered that early arteriosclerotic lesions occur in children[6], and this observation has since been extended to infants[7] However, at the same time, the rapid progress of biochemistry and nutrition began to influence the work on arteriosclerosis[8], so that, since then, most investigations have centred around the disturbances of lipid metabolism and their association with dietary fat It is curious how little attention has been paid to the early occurrence of the lesions

By the end of the nineteenth century, it had been well established that arteriosclerosis, in common with other systemic diseases, is subject to modifications by a number of general factors

The disease runs in families, and probably has a genetic factor Sex plays a part in that women below the age of fifty have fewer heart attacks than men Physical activity is also a factor because people with sedentary work show extensive lesions more frequently than people doing manual work Arterial hypertension is associated with more advanced lesions Arteriosclerosis occurs more extensively in old persons The abuse of coffee and tobacco leads to more frequent myocardial infarctions, whereas the incidence in heavy drinkers is relatively low The stress that those in managerial positions are subjected to has been cited, although not completely convincingly, as contributing to a high incidence among this group

The accummulation of fatty substances in the atheroma suggested examination of lipid metabolism At about the turn of the century, it was found that patients afflicted with advanced arteriosclerosis have lipæmic sera[9], and it was soon established that the serum cholesterol is frequently elevated[10] Further work elucidated other disturbances of lipid metabolism in arteriosclerosis Important is the elevation of the β-lipoprotein fraction of the serum[11] , it contains most of the cholesterol, two-thirds of which is esterified with oleic and linoleic acids[12] Finally, it was discovered that, in advanced arteriosclerosis, the saturated and oleic acid-levels of the serum are often elevated, whereas the polyunsaturated levels are lower than normal

When these lipid disturbances were first examined investigators considered them as the consequence rather than the cause of the lesion , and it is certainly true that many chronic diseases are accompanied by similar lipid changes However, since the fateful experiments with cholesterol feeding, which were interpreted as proof that cholesterol produces arteriosclerosis in animals[13], many leading investigators have been inclined, during the past fifty years, to accept disturbances of lipid metabolism as etiological factors in arteriosclerosis

If one were to summarize the evidence implicating cholesterol (or perhaps the β-lipoprotein fraction), one might choose these animal experiments involving the feeding of large amounts of cholesterol or the induction of an abnormal rise in serum cholesterol , its presence in large amounts in the atheroma ; higher serum cholesterol-levels in diseases predisposing people to arteriosclerosis (hypothyroidism, nephrosis, familial hypercholesterolæmia) , and the fact that its increase in arteriosclerosis patients permits some degree of predictability as to the state of the disease

With regard to animal experiments, it has frequently been pointed out that the high cholesterol diets which induce lesions having a high cholesterol content are entirely unphysiological One finds that cholesterol is deposited in most organs[14] , but it is usually the blood vessels which are singled out for discussion It has been argued by some pathologists that the morphology of the lesions bears little resemblance to arteriosclerosis[15] and that the disease scarcely ever gives rise to myocardial infarctions. A number of experiments has been described in which arterial disease associated with cholesterol deposits has been produced on cholesterol-free diets[16], whereas, in others, the serum cholesterol-level was raised to ten times its normal value without producing arterial lesions Therefore, at the present time it does not seem that the lesions induced in animals by cholesterol feeding are analogous to human arteriosclerosis

In a discussion of the presence of cholesterol in the lesions, it is well to recall some general facts about cholesterol metabolism All organs, and especially the liver, produce much more cholesterol than a human being would eat in any reasonable diet, and feeding of as much as 10 gm of crystalline cholesterol (compared with about 500 mgm in a normal diet) does not influence the serum cholesterol-level in humans (it does in animals) Of the total cholesterol produced by the body, about 2 per cent is known to be used in the synthesis of steroid hormones The function of the rest is largely unknown One may speculate that, under normal conditions, it forms a building material for all cells[17] , and this guess is supported by its role in disease As I have stated before, disturbances of lipid metabolism like those in arteriosclerosis occur in many chronic diseases In tuberculosis, for example, the onset of the disease is followed by hypercholesterolæmia and the deposition of large amounts of cholesterol in the tubercle, which, in the lungs, may afford some protection against cavitation These changes in cholesterol metabolism can be considered as a defence mechanism of the body Similar mechanisms are seen in many other chronic diseases, (nephrotic kidneys, thyroid tumours, uterine fibroids, old scars) Sometimes, the inflammatory process leads to such an accummulation of cholesterol that the repair process becomes a danger, as with gall stones and kidney stones or when cholesterol crystals break off an arteriosclerotic lesion and produce an embolism[18] If one assumes that the pathology of cholesterol metabolism in arteriosclerosis is similar to that in other chronic diseases, one could interpret the presence of cholesterol in the lesions as a process of repair

Such a hypothesis could also explain the premature arteriosclerosis associated with chronic diseases such as nephrosis and hypothyroidism These conditions are associated with generalized tissue damage which, in turn, evokes a generalized repair process, as in severe rheumatic conditions or in syphilis , this repair process may involve the arterial lesions which are present in all of us to some extent from infancy Thus, one may also understand the predictive value of elevated serum cholesterol-levels in arteriosclerosis and even of the apparently anomalous low levels seen in some advanced cases[19] Furthermore, one may speculate that therapeutic reduction of serum cholesterol may have its disadvantages unless one is sure that the lower level in serum does not reflect some damage to the animal This is not such a remote possibility in view of the fact that feeding of oxidized fats which are toxic leads to a reduction in serum cholesterol[20].

Because arteriosclerosis, in Western countries, is now the main cause of death, investigation of life expectancy can in a general way be interpreted as work on arteriosclerosis. The fact that obese people have a shorter life expectancy needs no elaboration. Careful autopsy studies immediately after the lean years of the two World Wars in Vienna and Norway respectively showed a remarkable decrease in the incidence and severity of arteriosclerosis, which manifested itself in fewer strokes and heart attacks[21]. There was a rapid increase as soon as the food intake returned to normal. This is at the bottom of all speculations about the relation of diet to arteriosclerosis.

Dietary fats have been given tremendous importance in the consideration of arteriosclerosis. They exert a two-fold influence. Unspecifically, they are the densest source of calories and their feeding reduces energy requirements which increases body-weight and arteriosclerosis. But more specifically, they wield a strong influence on serum cholesterol unrelated to their own cholesterol content. It has been claimed that the number of heart attacks is a function of the fat content of the diet, Japan, with a dietary fat-level of about 8 per cent, has the fewest, and the United States, with 40 per cent of fat in the diet, heads the list. The incidence among Japanese living in California and eating the same diet as the rest of the population is the same as for the rest of the United States[22].

This hypothesis has not remained uncontested. It has been pointed out that the incidence of strokes in Japan is higher than in the United States and that arteriosclerosis is less of a problem in Thailand than in Japan despite a much higher dietary fat-level[23]. Similarly, it has been claimed that there is no difference in the incidence of arteriosclerosis in London and Calcutta despite widely differing diets[24]. Furthermore, it has been shown that, among those countries having a daily fat intake of about 120 gm., the rate of myocardial infarction is four times as high in the United States as in the Netherlands[25]. In general, however, there is fair agreement that a very low fat intake, especially in association with a low total caloric intake, leads to fewer myocardial infarctions. With regard to strokes, it is difficult to evaluate the data.

Human life-time studies do not exist, and animal experiments are sparse and contradictory with regard to the level and kind of fat eaten. Therefore, the main argument as to the advisability of eating specific fats rests on their influence on serum cholesterol, and possibly also on blood coagulation. From the point of view of the tenuous hypothesis that serum cholesterol-levels may reflect the extent of tissue damage and in view of the over-all predictive value of these levels, the question of whether manipulation of serum-levels by fat is meaningful is of interest, but ardent public discussion is, at best, premature. Much has been made of animal versus vegetable fats[26,27], saturation versus unsaturation[28], and linoleic acid deficiency[29]. The fact that the saturated long-chain fraction of coconut oil increases and the saturated medium chain fraction decreases serum cholesterol-levels[30,31] is one of the many examples that no generalization can be made and that fats have to be considered individually. Only the theory that liquid fats have some advantages over hard ones has received some experimental support.

The discovery of heparin and the 'spoiled sweet clover disease' opened the way for an important advance in the treatment of arteriosclerosis. Based on Rokitansky's hypothesis as to the importance of thrombus formation, the newly developed anticoagulants have been used successfully in preventing many heart attacks and strokes. This approach was almost immediately influenced by prevailing ideas about the role of fat in arteriosclerosis, and a large number of papers has been written dealing with the question of whether the lipæmia occurring after the intake of fat increases the tendency of the blood to clot. Although, with respect to this, opinion is divided, most investigators agree that certain fats (especially butter) do have some effect on coagulation in that they shorten the Russell viper venom time. *In vivo* studies correlating changes in the clotting mechanism with those of the arterial wall may be of considerable interest[32] because the normal arterial wall produces enzymes which affect clotting, as in the clearing factor[33]. In the arteriosclerotic artery, the surface is more irregular, the lipid content is higher[34], the lipid breakdown products are different[35], and the atheromata themselves have a higher thromboplastic activity[36]. One should also keep in mind that the lipid composition of all organs is influenced by dietary fat, and this may in turn influence coagulation. In any event, the relation of fats to coagulation is still a wide-open field.

More recent work relating infectious processes to intra-arterial clotting may shed some light on arteriosclerosis. It is an old idea that infectious processes, by damaging the arterial wall, may be responsible for some of the changes seen in arteriosclerosis[37]. McKay has been able to produce intra-arterial platelet and fibrin deposits in pregnant rabbits by injecting endotoxins[38]. It is important that this process was observed when the organism was under the stress of pregnancy or when an endotoxin was injected together with substances of higher molecular weight[39]. It remains to be seen in which way these changes may be linked to arteriosclerosis.

The factors so far enumerated can modify the arteriosclerotic lesion, but are unspecific because they cannot account for the early occurrence of what is now believed to be the primary lesion in infants, neither can they account for the focal distribution of the lesions in the arterial system. These aspects of the disease invite the speculation that it may have a specific chronic infectious origin, as do leprosy, tuberculosis, and syphilis. Such a hypothesis could account for the early onset of the disease, for the focal distribution of the lesions, for the specificity of the organ attacked, for the eventual emergence of a histologically rather characteristic lesion from its early form, and even for the occasional disappearance of the lesion[40]. Furthermore, in view of the fact that the total population is affected by the disease at an early age, differences in the development of the lesions among members of the population would be accounted for by general factors such as heredity, nutrition, and the like. Something akin to this has actually happened in parts of the world where, at one time syphilis infected the whole population and where, before the infectious origin of the disease was known, the clinical manifestations were ascribed to factors similar to those implicated in arteriosclerosis. Even the rapid increase in the consequences of the disease in younger age-groups may be explained by the known increase of an unchecked infection if others are suppressed, as has happened in our lifetime Finally, such chronic infectious diseases as syphilis and tuberculosis have been with the human race for a long time, and their natural history was characterized by sudden epidemics Is it possible that we are witnessing such an outbreak of an old infectious disease ?

Whether or not arteriosclerosis, as it is defined to-day, will remain a nosological entity or will be seen as several diseases, or whether it will be recognized as an unspecific response (like fever) to a number of etiological factors, is uncertain Historically, the instrument of the physician of the nineteenth century was the microscope and of the twentieth century the paraphernalia of the biochemical laboratory Notions about arteriosclerosis were formed accordingly At the present time, virology and electron microscopy may enter the picture , it would be interesting to apply to these problems the methods used for the study of infectious nucleic acids[41] Again, our sense of the etiology may be influenced by the methods we use

Arteriosclerosis, being a part of the life process, can scarcely be pinned down to one etiological factor Any investigator will, according to his temperament and talent, pick out just those of the infinity of factors which he believes are important , and one would be satisfied to elucidate one facet which would permit us to influence decisively the course of the disease

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MOVEMENT IN THE ATMOSPHERE

THE Royal Meteorological Society held its summer meeting this year in the University of Exeter during July 12–14, this included two symposia, on "Winds and Circulation in the Upper Atmosphere" and "Medium Range Diffusion in the Atmosphere"

Winds and Circulation in the Upper Atmosphere

At this symposium the upper atmosphere was regarded as extending from the tropopause (10–15 km) to the ionosphere (100 km) This region is one of great interest to meteorologists since it is situated between the outer layers of the atmosphere, which are under direct solar control, and the troposphere, where the weather occurs Due to indirect measurements and the more recent rocket investigations its general composition, temperature and wind structure are now fairly well known Broadly, the composition is not very different from that in the troposphere the temperature rises from 200–230° A near the tropopause to 260–300° A around 50 km , falls to 160–230° A near 80 km and then rises again at higher levels , the winds are mainly strong westerly in the winter hemisphere and easterly in the summer hemisphere Many measurements have been made of the distribution of ozone, water vapour and atomic debris in the lower stratosphere and these have been used to infer the meridional circulations Since electromagnetic forces, dissociation, excitation and ionization are still comparatively unimportant and mean free paths small, it is possible to apply the well-known meteorological equations used for the troposphere in the examination of its dynamics and interpretation of experimental observations

The first paper, "Possible Meridional Circulations in the Stratosphere and Mesosphere", by R J Murgatroyd and F Singleton, described a calculation of the meridional circulation sufficient to transport heat between the sources and sinks of radiation in a simple model of this region The sources and sinks have been estimated from differences of solar energy absorbed by ozone and molecular oxygen and that emitted by long-wave radiation from carbon dioxide, water vapour and ozone The principal sources are near the equatorial tropopause and in the mesosphere over the summer pole The corresponding sinks are near the polar tropopause and the winter pole in the mesosphere The calculation showed the air to be rising near, and possibly through, the equatorial tropopause, spreading polewards and sinking towards the polar tropopause In the mesosphere the air rises near the summer pole, flows across the equator and subsides near the winter pole This scheme is in good general agreement with many of the observed phenomena in this region, such as noctilucent clouds at high latitudes in the summer hemisphere near 80 km, comparatively high temperatures in the winter polar mesosphere, the 'sudden warming' phenomena, and the observed distributions of ozone, water vapour and atomic debris in the lower stratosphere

The second paper, "Winds at 80–100 km as revealed by Radar Reflexions from Meteor Trails", by J S Greenhow and E L Neufeld, contained the results of systematic work carried out at Jodrell Bank during 1953–58 The observation of drifts of ionized meteor trails using high-powered radio-echo equipment has produced upper wind data of a volume and accuracy unobtainable at other levels Regular prevailing winds with westerly components of about 20 m sec $^{-1}$ have been observed each year in summer and winter with easterly components in spring and autumn Large northerly components occur in summer and smaller southerly components in winter There is a semi-diurnal periodic wind of about 20 m sec $^{-1}$ which can usually be represented by a vector which rotates in a clockwise direction with constant amplitude every 24 hr Although large departures occur from this simple model, it provides good general confirmation of the resonance theory Measurements of the 24-hr and 8-hr periodic wind systems and also non-periodic (turbulent) components were also described and comparisons made with Australian data It is clear that with world-wide measurements of this type it will soon be possible to obtain a comprehensive picture of the atmospheric circulation at these levels The final paper, "Fluctuations in Tropical Stratospheric Winds", by R A Ebdon, described detailed analyses of winds and temperatures found by radio-sonde and radar wind methods between about 15 and 25 km near the equator The winds were shown to have had a 26-months periodicity in east–west direction during the past ten years The north–south components are usually small This effect appears to be world-wide, greater at the higher levels and near the equator but observable up to 1,000–1,500 miles from it When a change-over occurs, it starts at high levels and spreads downwards At present there is no explanation for this phenomenon and there are dynamical difficulties in explaining how west winds can occur at all extensively in equatorial regions

These three papers, which are being published in the *Quarterly Journal of the Royal Meteorological Society*, provoked considerable discussion Recently, there has been increasing evidence that the humidity mixing ratio in the stratosphere may increase from about 2×10^{-6} to 4×10^{-5} between 15 and 30 km, and this is difficult to explain unless there is a large extra-terrestrial source of water vapour, but this seems unlikely Recent measurements by the Meteorological Research Flight have shown values at 15 km around 2×10^{-6} at all latitudes (although no measurements have yet been made at high latitudes in winter), so that vertical upwards moisture transfer, except perhaps by some penetrative convection, is ruled out for most latitudes Other points needing clarification include the nature of the meridional transfer mechanisms, that is, the respective roles of the mean and eddy components on all scales The next steps in the advance of our knowledge of the dynamics of this region will be to take account of the angular momentum as well as the energy balance, and to formulate criteria for the onset of dynamic instability (which depends on lapse-rates and wind shears and will therefore be different at different levels) Turbulent transfer will be more important in the lower stratosphere and the region between 50 and 80 km than in the stable region between about 25 and 50 km, but the circulation here will of course also have to fit in with that for the region above and below it

Medium-scale Diffusion in the Atmosphere

The second symposium was concerned with the propagation and spread of particles in the atmosphere up to distances of 100 miles or more from the source This subject is of very wide application (since it involves all forms of atmospheric pollution including dust, radioactive materials, condensation and freezing nuclei and spores), and the study of concentrations at different points down-wind in different meteorological situations and their possible prediction is of great importance In the first paper, "Some Observed Properties of Medium-scale Diffusion in the Atmosphere", by F Pasquill, a series of experiments were described in which zinc–cadmium sulphide, a fluorescent pigment, was used as a tracer to investigate the horizontal spread of particles both in the cross-wind and along-wind directions The particles were discharged either from a maintained point source on the ground and sampled at different heights down-wind by an aircraft, or alternatively dispensed from an aircraft flying across the wind (which could be regarded as a continuous line source) and sampled down-wind by instruments carried on the cable of a captive balloon In each case it was possible to count under a microscope by using ultra-violet light the number of particles caught in unit volume at the different locations, and to relate them in terms of bearings and spread of the plume of particles to measurements of the strength of the source and the winds and other meteorological parameters

When diffusion occurs over considerable distances and height-ranges the systematic wind shear with height affects the cross-wind and along-wind dispersion of material spread out in depth, and this effect depends on the amount of vertical mixing (both mechanical and convective) The question arises as to whether at medium ranges the horizontal spread of the particles bears a consistent relation to the apparent horizontal field of turbulence, as it does in short-range diffusion, or whether there is a contribution which cannot be provided by this turbulence and is recognizably due to shear The analysis of the observations indicated that, when vertical mixing is present, the tendency for a cloud of particles to be sheared is strikingly removed, but when vertical mixing is inhibited, distortion of the cloud takes place, yielding horizontal displacements which would become effective on the revival of the mixing Due to this effect there is usually a diurnal cycle of vertical but not horizontal spread

The details of intermediate cases require further examination for a full quantitative understanding, although it is clear that turbulent spread, especially that due to the low-frequency components, always makes a large contribution apart from the shear There was also usually only a small variation with height of the direction and speed of travel of the particles compared with that of the wind In some cases, however, the mean direction was several degrees backed from the direction of the mean wind in the layer, and even from that of the surface wind, and was difficult to account for in terms of vertical momentum transfer

Dr J M Hirst then presented a paper on "The Long-range Travel of Fungus Spores" Plant diseases such as potato blight and black rust of wheat can be transmitted hundreds of miles due to the diffusion of spores in the atmosphere, and an examination of this mechanism is of considerable economic importance At the place of origin, the spore concentration may be as much as 1/c c and the concentration in the vertical is controlled by turbulence and atmospheric stability The decrease at inversions is usually rapid Furthermore, the presence of an immigrant as opposed to a locally produced spore can often be recognized by its different vertical distribution The concentration of the former tends to increase with height over the first few thousand feet and then decrease above, an effect which appears to be due to dry deposition during its relatively long period of travel The efficiency of raindrops as collectors of spores is high, so that spores are quickly removed from the atmosphere during rain Examples were shown of the measurement of the spread of spores of black rust of wheat from Europe to Britain The disease appears in early summer in southern Europe and slowly spreads northwards and is often observed in Britain by about August Samples obtained over the English Channel using the aircraft of the Meteorological Research Flight during periods of southerly winds had produced vertical profiles of spore concentrations which illustrated their travel in the atmosphere from Europe, whereas on other occasions it had been demonstrated by back-tracking air trajectories that they were not present in air of Atlantic origin reaching samplers on the ground Work of this type is continuing, and further experiments are now in hand to examine the diurnal variation of spore content at various heights and other characteristics of their diffusion in the atmosphere.

The final paper, "Mechanisms of Atmospheric Diffusion", by R S Scorer, was concerned with diffusion by turbulent eddies and by thermals In certain meteorological situations the latter are very important and may even be the dominant mechanism When diffusion of momentum occurs following any disturbance in the flow, there will be a loss of energy as the energy of shear is disorganized into smaller fluctuating motions, a process which may best be studied in terms of the vortex lines in the flow (Scorer, R S, *Natural Aerodynamics*, Pergamon Press, London, 1958) Momentum, however, is not always transferred at the same rate as heat or material, and in the case of thermals, $\frac{K_{\text{momentum}}}{K_{\text{material}}} = \frac{\sigma}{2}$, where σ is the fraction of the horizontal area occupied by the thermals and is usually 0 1–0 01 Hence thermals are about 20 times more efficient at transferring material than momentum, whereas in the case of mechanical stirring their rates of transfer are more equal As a consequence, the wind shear can be retained, and there can still be considerable transfer of material when thermals are operating

The ensuing discussion was principally concerned with the generation of thermals, which are intermittent and frequently confined to preferred areas, the relative importance of form drag and viscous drag and the necessity of considering eddies in three rather than in two dimensions As regards the practical aspects, it now appears that, observationally and empirically, at least the problem of determining the concentration of particles down-wind from a given source in given meteorological conditions is well understood and considerable progress has now been made.

R J Murgatroyd

UNIVERSITY EDUCATION IN GHANA

By Dr C T MORTIMER

University College of North Staffordshire, Keele, Staffordshire

THE report of the Commission on University Education in Ghana, and a statement by the Government on the report*, were published on July 5, 1961 Mr Kojo Botsio, a Minister of the Ghanaian Government, was the chairman of the Commission, which included American, British, Russian and Sierra-Leonean members The original terms of reference were "to advise on the future development of university education in Ghana, in connection with the proposal to transform the University College of Ghana, at Legon, and the Kumasi College of Technology into an independent University of Ghana" Afterwards, these terms were altered slightly, because the Government later proposed that the two institutions should be independent universities They are to be called the University of Ghana and the University of Science and Technology, Kumasi

In formulating their constitutional proposals for these two Universities, the Commission was guided by two main principles These are that the Universities should be able to respond to the immediate and future needs of the community, and that they should have the greatest possible autonomy in their organization, teaching and research The report deals first with the relationship between the Universities and the Government of Ghana The prevailing opinion was that new developments in higher education should be planned and financed at a national level by a single body, which would also co-ordinate and finance research programmes, both within and outside the Universities It was recommended that a National Council for Higher Education and Research be established Prof L A Bornholdt, of the University of Pennsylvania, did not support this recommendation "If such a body were to be created and were given full responsibility for planning research and for financing university education throughout Ghana, the Commission's recommendations for the autonomy and freedom of individual institutions would become no more than pious hopes " This minority opinion suggested a division of responsibilities and the creation of three bodies in place of the National Council a Joint Research Board, a

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* Report of the Commission on University Education Pp 43 4*s* Statement by the Government on the Report of the Commission on University Education W P No 5/61 Pp 7 6*d* (Accra, Ghana Ministry of Information, London Crown Agents for Overseas Governments and Administration, 1961)

University Grants Committee, and a Research Grants Committee The Government has accepted the majority opinion and has created a National Council

Although the Commission made no specific recommendation, the Government has appointed *interim* University Councils, which have taken over the responsibilities of the College Councils until the governing bodies of the new Universities have been established The Government Statement notes that these *interim* Councils have been unable to offer appointments to five members of the existing academic staff The *interim* vice-chancellors and the Secretary of the National Council will be asked to report on recommendations of the Commission and those of committees which have been appointed by the President of Ghana Some of the functions of these committees were (1) to prepare proposals for the constitution of the National Council , (2) to draft constitutions for the new Universities , (3) to consider the supply of students , (4) to consult with College authorities on syllabuses

The Commission considered in some detail the type of university government which would best meet the needs of higher education in Ghana For the composition of the University Councils, the Commission suggested the following the Chancellor, the Vice-Chancellor, and one member appointed by the Council of the sister University Of the remaining members, one-third should be nominated by the Government, one-third by various educational bodies, and one-third should be selected by the University Senate from its membership Membership of the Senate would be broadly representative the head and two other teaching members of each Department, at least one of whom should be Ghanaian

In its statement, the Government endorses the views of the Commission on the steps which should be taken to accelerate the Africanization of University staff, in order that there will be a substantial majority of Ghanaians by 1967–70 The report notes that, at present, of a total academic staff of 201 at Kumasi and Legon, only 35 are Ghanaians Ghanaians who are of professorial calibre, working in departments which already have professors, should be appointed either as additional full professors or as associate professors When professorships fall vacant in departments in which there is no Ghanaian immediately qualified for appointment, distinguished scholars from other parts of the world might be brought in as visiting professors for periods of two or three years, while the departmental responsibilities would be entrusted to a Ghanaian lecturer The report stresses that efforts to increase the proportion of Ghanaians on the staffs of the two Universities should not involve the displacement of existing expatriate members of the staff In fact, the Government recognizes that the expansion of higher education will make it necessary to continue, for some time to come, the recruitment of overseas personnel on short-term contracts

The total 'high-level man-power' requirement during the period 1960–65 in administrative, managerial and professional employment and in secondary school teaching has been estimated at 7,000, or an average annual requirement of 1,400 To meet this there must be an increase, as rapidly as possible, in the flow of students into the two Universities Under the present entrance regulations, possible student numbers are controlled by the number of those obtaining the General Certificate of Education with two subjects at advanced level This consequently depends on the extent to which sixth-forms have been developed in the secondary schools On the basis of existing sixth forms and plans for expansion of sixth-form work, it has been estimated that the student population of the Universities in 1964 would be about 1,400, representing an annual output of some 450 graduates This estimate does not take into account courses at Kumasi in such subjects as engineering, architecture and pharmacy, which can be entered at the ordinary level of the General Certificate of Education To ensure that the rate of development of university education is not so dependent on the rate at which sixth forms can be developed, it is proposed that there should be a special entry into the University of Ghana for students who have passed General Certificate of Education (ordinary level), but who lack the normal advanced-level qualifications, similar to the arrangement which already exists at Kumasi. By 1964 the estimated annual output of General Certificate of Education ordinary-level certificates will be about 1,800

It has been agreed that the two Universities should remain predominantly residential, though making provision for a proportion of non-residential students To accommodate the increased student numbers at Legon, the Government has authorized the building of a fifth hall of residence, to accommodate 320 students At the same time plans are to be drawn up for the building of hostel-type annexes to the four existing Halls, which at present can accommodate about 700 students It has often been suggested that the system of hall membership has tended to inhibit the growth of student life and restrict students' social activities To overcome these objections, and to cater for non-residential and part-time students, the Commission has proposed that centrally situated cafeterias, common rooms, gymnasia and other common facilities for staff and students should be built at both Universities

With the ending of the 'special relationship' with the University of London, the time was considered opportune to review the structure of degree courses Many of the suggestions presented to the Commission on this matter would be met by an arrangement whereby all students in their first year would take one of a limited number of prescribed general courses Each of these courses would normally include three subjects Students could then proceed either to a two-year general degree course in two or three subjects or to a special degree course in one or two subjects, terminated by a final examination In addition, all students at both Universities would take some courses in African studies Since much of the re-organization of the structure of degree courses has already been under consideration, it is hoped that students entering the University of Ghana in October 1961 would begin at once to work for the B A and B Sc degrees of the new University

In considering the division of responsibilities for teaching and research between the existing Colleges at Legon and Kumasi there were three areas in which actual or potential overlapping existed—in engineering, agriculture, and in work for general degrees The Commission recommended that engineering be concentrated at Kumasi, together with architecture, building technology, rural and urban planning, and pharmacy The four-year diploma course in agriculture at Kumasi should be strengthened, although the degree course in agriculture should be confined to Legon It is hoped that with the development of science laboratories at Kumasi, it might be possible to start general degree courses in some combination of science and technology , metallurgy, wood technology, agricultural engineering and industrial chemistry are cited as examples The Government accepts the Commission's proposal for the establishment of an Institute of African Studies, based on the University of Ghana, but having some measure of autonomy The future of the College of Business Management at Achimota remains uncertain, and the National Council will examine ways of associating the College and also the Ghana School of Law with the Universities The Commission welcomed the suggestion made by Prof Carl Iversen, vice-chancellor of the University of Copenhagen, that the Government of Denmark might be willing to associate itself with a scheme for establishing further residential adult colleges in the various regions of Ghana

In addition to the main proposal that the Colleges at Legon and Kumasi should become independent Universities, the Commission has recommended that a University College of Cape Coast be established This College would be in some relationship with the University of Ghana, though few of the details have been worked out

As one phase of the development of higher education in Ghana ends and another begins, the Government has paid tribute to the encouragement and practical assistance given to the Colleges by the University of London, the Inter-University Council for Higher Education Overseas and the Council for Overseas Colleges of Arts, Science and Technology It records with satisfaction the high standards already achieved by the Colleges and its confidence in their future as independent Universities

OBITUARIES

Dr Otto Maass, C B.E., F R.S.

Dr Otto Maass, one of the most eminent of Canadian chemists, died in Montreal on July 3 at the age of seventy As assistant to the President of the National Research Council during the war years, director of chemical warfare in the Department of National Defence and later as scientific adviser to the Chief of General Staff, he was well known to a multitude of colleagues in both the United Kingdom and the United States As general director of the Pulp and Paper Research Institute of Canada, he had a wide acquaintance in the pulp and paper industry But he is best and most affectionately remembered by the many generations of graduate students who gained their first insight into the significance of physical chemistry and the meaning of chemical research under his stimulating guidance

Otto Maass was born in New York City on July 8, 1890, but went to Montreal with his parents at an early age He was educated in Montreal High School and McGill University, obtaining the B A degree in 1911 and the M Sc in 1912 He was awarded an 1851 Exhibition scholarship and went to Berlin in 1913 to work in the laboratory of Prof Walther Nernst Trapped in Germany by the outbreak of war in the following year, he was able to escape across the border into Switzerland and thence return home His interrupted studies were continued at Harvard under the late Prof T W Richards He submitted a thesis and received the Ph D degree from Harvard in 1919

In 1917 he was appointed lecturer in physical chemistry at McGill In 1923 he became Macdonald professor of physical chemistry and, in 1937, chairman of the Department, appointments which he occupied until his retirement in 1955

From the very outset of his teaching career, he began to be surrounded by a rapidly expanding group of enthusiastic young graduate students eager to share in the development of his ideas One very early achievement was the preparation, for the first time, of pure hydrogen peroxide , and this gave rise to a whole series of publications reporting accurate measurements of its physical properties Another early investigation involved devising a simple and accurate method of measuring densities of condensable gases This was ultimately refined to the point where reliable figures for the atomic weights of carbon and sulphur were obtained from the molecular weights of carbon dioxide and sulphur dioxide Meanwhile, similar techniques had been applied to observe density changes on mixing two gaseous components and to detect reaction between two components in the vapour phase This led on to a comparison of reaction velocities below and above the critical temperature which, in turn, gave rise to the prolonged series of investigations of critical phenomena which brought about a wholesale revision of ideas concerning the critical region

As early as 1920, Maass became aware that very few reliable physical data were available on sulphur dioxide solutions such as were used in the manufacture of sulphite pulp Work in this field was followed by a series of investigations of the factors influencing the rate of penetration of sulphite solutions into wood chips In 1928 the Pulp and Paper Research Institute of Canada was founded, and by arrangement with the Graduate Faculty of McGill, Maass and his students were enabled to pursue further researches in the laboratories of the Institute He became its general director in 1940 and was instrumental in initiating a variety of investigations of problems directly concerned with the war effort

On his retirement in 1955, Maass was appointed a principal research officer in the Division of Chemistry, National Research Council, and he spent the next three years in the laboratories of the Council in Ottawa Then he returned once more to McGill as a research associate in the Chemistry Department Much of his time was spent in his laboratory in the Chemistry Building up to the day of his death

Space does not permit even a bare enumeration of the degrees, medals, memberships of learned societies and other honours which came to him It would seem, in any event, superfluous to those who knew him His work was his life and brought with it its own reward By his work he will be remembered

J H Mennie

Prof R F Naylor

Ralph Francis Naylor, professor of chemistry in the Royal College, Nairobi, was killed in a motor accident at Mkushi in Northern Rhodesia on August 6 In this tragic accident, at the regrettably early age of thirty-nine, one who has done much for chemistry and for university education generally in East Africa was lost

Born in 1921 in London, Ralph Naylor was educated at Emanuel School and at the Imperial College of Science and Technology, London, where he graduated B Sc with honours in 1942 He then joined the British Rubber Products Research Association where he worked on olefinic reactivity and aliphatic sulphur compounds Some twelve publications on this work resulted, including one in *Nature*, over the period 1943–1949

In 1949 he went to Makerere College as a lecturer in organic chemistry and became a senior lecturer three years later In September 1953 he was given nine months leave of absence to work with Dr J H Hanks in the Department of Bacteriology and Immunology at Harvard, in which he was assisted by Smith-Mundt Research and Fulbright Travel Grants He returned to Makerere as acting head of the Chemistry Department The head of Department, Prof Tenniswood, had died shortly before, also in a motor accident He acted in this capacity for nearly two years Although one of the new chemistry buildings had just been completed, much of the design of the second building and the planning for future expansion, which he performed enthusiastically and in masterly fashion, fell to him

In spite of these increased responsibilities, Naylor continued his research on the chemotherapy of leprosy drugs He took much interest in leper colonies and villages throughout Uganda and neighbouring territories He was on the Board of Governors of the Kumi Leprosy Settlement, which he visited regularly Having decided that a radiochemical approach to this problem was likely to be fruitful he spent most of one long vacation at Harwell learning tracer techniques, so that he might design and equip a radiochemical laboratory in the Chemistry Department at Makerere He read a paper on his work at the seventh International Congress on Leprosy in Tokyo in 1958

The East Africa Section of the Royal Institute of Chemistry held its first annual general meeting early in 1956 Ralph Naylor was one of those responsible for its foundation He became the Section's first secretary, and up to the time of his death he never relaxed in arranging, organizing and reporting meetings, visits, excursions and exhibitions East African chemists owe him a particular debt in this connexion

At Makerere he always took an active personal interest in students and student affairs, particularly in his own tutorial group He was for a time a resident tutor, and later deputy warden of Mitchell Hall, one of the student halls of residence He was much sought after as a lucid lecturer, an enthusiastic committee member and an impartial chairman Ralph Naylor was a man of deep religious conviction He was the founder leader of a Boys' Crusader Bible Class in Kampala, and also for some years secretary of the Uganda auxiliary of the British and Foreign Bible Society

His interest in open-air activities was shown by his love of travel and mountain climbing He was for a time president of the Uganda Mountain Club, and had made ascents of several of the difficult Ruwenzori peaks as well as other mountains in East Africa He was also a trustee of the East Africa Outward Bound organization, which has its headquarters at Loitokitok at the base of Kilimanjaro, and carries out its activities on that mountain He had also been president of the Uganda Amateur Athletic Association at the time when Uganda was beginning to play a part in international athletics Photography was one of his hobbies

In 1955 he married Miss Dilys Rees She was slightly injured in the accident through which he died, and their son Paul, aged four, rather more seriously

In January 1961 he took up duty as first professor of chemistry at the Royal College, Nairobi, and in his short time there he was also dean of the Faculty of Science, and worked enthusiastically on the planning of the new chemistry building It is sad that he will not now see the building through to final completion and occupation

It will thus be clear that Ralph Naylor was characterized by the energy which he put into so many diverse activities, and the thoroughness with which he carried them out He took a pride in meticulous planning down to the finest detail and his plans nearly always succeeded His loss will be felt by his many friends and by all who have an interest in scientific education and development in East Africa

MALCOLM CRAWFORD

Sir Arthur Olver, C B , C M.G.

WITH the death on August 15 of Sir Arthur Olver at the age of eighty-six, the veterinary profession has lost one of its most eminent authorities on livestock development The son of a successful Cornish livestock breeder, he was early introduced to the exactions of animal husbandry After studying in London and becoming a member of the Royal College of Veterinary Surgeons, of which he later became a Fellow, he was commissioned in the Army Veterinary Department some months before the outbreak of the South African War

He saw active service with the cavalry, and later, as senior veterinary officer, Natal, first showed his flair for administration

In 1904 he obtained permission to work under Theiler at his Daspoort laboratory when that authority was so successfully competing with the prevalent epizootics, as well as demonstrating with exactness connexions between nutrition and disease In 1906, while still a captain, he was appointed principal veterinary officer, Egyptian Army and the Sudan Civil Service In his first year he stamped out rinderpest in the Nile Valley, organized the Sudan Veterinary Service and enabled the cattle trade between Central Africa, the Sudan and Egypt to be reorganized on a satisfactory basis, for which he was awarded the Order of Osmanieh, 4th class.

He then was appointed assistant director general, Army Veterinary Service, at the War Office For the greater part of the First World War he served at headquarters of the British Expeditionary Force and besides being mentioned in dispatches four times was made a C M G In 1917 he was sent to re-organize the veterinary arrangements of the Remount Commission in the United States and Canada, where equine infectious diseases were causing havoc Once again his efforts were successful and he was made a C B

In 1930, when a colonel, he retired from the Army to become animal husbandry commissioner with the Government of India, an appointment which had been created on the recommendation of the Royal Commission to investigate the state of agriculture in India During his eight years in that office he brought under centralized supervision all aspects of official livestock research and development throughout the subcontinent, drafted an all-India Act for the Control of Contagious Diseases of Animals, defined and published a description of most of the important breeds of cattle in India and organized the All-India Cattle Show Society as well as originating research and promoting veterinary education. He was knighted in 1937.

He left India in 1938 to become principal of the Royal (Dick) Veterinary College in Edinburgh, which he administered through the difficult war years that followed. He retired from Edinburgh and public life in 1946 to farm with the enthusiasm and zest with which he had pursued all his undertakings.

He had many outside interests, but he excelled at, and derived most pleasure from, field sports. Since an illness some five years ago lessened his physical activity he took up painting with the single-minded concentration so characteristic of him. Two of his paintings were accepted by the Royal Academy, and he was greatly amused that a critic predicted a promising career for the artist.

He is survived by a widow and one son.

G. WILLIAMSON

NEWS and VIEWS

The Royal Radar Establishment

Mr. W. J. Richards, C.B., C.B.E.

MR. W. J. RICHARDS resigned from the post of director of the Royal Radar Establishment in July in order to become first director of the new Staff College for Further Education recently established by the Ministry of Education (*Nature*, 190, 308; 1961). He joined the Scientific Civil Service in 1925, and was attached to the Royal Aircraft Establishment, Farnborough, where most of his active research work was carried out, his main interest being in the development of instruments for use in aircraft. He was appointed head of the Instrument Department in 1936. After a period at Ministry of Aircraft Production Headquarters in London, Mr. Richards succeeded Dr. W. B. Lewis in 1946 as chief superintendent of the Telecommunications Research Establishment, Malvern, and when this was amalgamated with the Radar Research and Development Establishment in 1953 (*Nature*, 172, 277; 1953) he was made director of the combined establishment now known as the Royal Radar Establishment. Under his directorship the amalgamation was achieved in a happy and efficient manner and the Royal Radar Establishment became a central research establishment for electronics serving both the Royal Air Force and the Army. It has played a notable part in the expansion of Britain's defence system, and, in particular, has made significant contributions to the electronic aspects of guided weapons, to the electronic equipment of the V-bomber force and to the modernized early-warning radar. The Establishment has achieved an international reputation for fundamental physics research, particularly in the physics of solids, and has pioneered in the development of many new radio techniques now commonly used in industry. One of Mr. Richards's main interests has been in education, and training of new staff members. It was largely due to his initiative that the Malvern College of Electronics was founded, in association with the Royal Radar Establishment. His new post will give him further scope for the development of these interests.

Dr. G. G. Macfarlane

DR. MACFARLANE, at present deputy director of the National Physical Laboratory, has been appointed to succeed Mr. Richards as director of the Royal Radar Establishment. Dr. Macfarlane, who is forty-five, graduated with first-class honours in electrical engineering at the University of Glasgow in 1937, and went on to do two years postgraduate research at Dresden, where he obtained the degree of Dr. Ing. in 1939. He joined the Telecommunications Research Establishment (now the Royal Radar Establishment) in 1939. Throughout the War years he concentrated on mathematical problems in radar and microwave physics. In 1945 he became head of the Mathematical Group and a year later took charge of the Theoretical Physics Division. In 1953, Dr. Macfarlane began individual research work on semiconductors in the Physics Department at the Royal Radar Establishment. In a series of fundamental studies of the optical properties of germanium and silicon he and his group obtained and explained detailed fine structure in the absorption spectra of germanium and silicon over a wide temperature-range. They demonstrated the occurrence of excitons and phonon-assisted indirect transitions in the absorption edge spectra of these semiconductors. He continued this work until early 1960 when he became deputy director at the National Physical Laboratory with special responsibilities for relations between the Laboratory and industry. Dr. Macfarlane will take up his new appointment at Malvern in March 1962.

Chemistry in the University College of Sussex:

Prof. C. Eaborn

DR. C. EABORN has been appointed professor of chemistry in the new University College of Sussex. He is best known for his publications on the chemistry of organosilicon compounds, including a book of which Prof. H. J. Emeléus recently wrote, "The outstanding impression on reading this book is that, for the first time, order has been introduced into the widely scattered literature". His major research contributions have involved mechanistic studies of reactions of silicon–hydrogen and silicon–carbon bonds, particularly cleavages of aryl–silicon bonds. He has shown that these cleavages are usually electrophilic aromatic substitutions, and with his co-workers has accumulated a large body of information on effects of substituents on the ease of such substitutions, has extended the study to include the reactions of aryl–germanium, –tin, and –lead bonds, and he has shown that these cleavages are useful in synthesis; for example, they can be used to attach a nitro- or sulpho-group at a specified position of an aromatic compound. Recently, Dr. Eaborn and his co-workers, using a simple method they devised for counting tritium, have carried out detailed studies of the rates of acid-catalysed aromatic hydrogen-exchange reactions, and the results, combined with those from the aryl–metal cleavages mentioned here, have been used to test and modify concepts of electrophilic substitution based in the past on more familiar reactions such as nitration Incidental observations in the course of Dr Eaborn's work which attracted considerable attention were the first isolation of an optically active organosilicon compound having a silicon as its single asymmetric centre, and his detection of the mesomorphism of di-isobutylsilane diol, which does not fall into any one of the three classes previously thought adequate for classification of liquid crystals

Dr Eaborn's researches involve at present, in addition to extensions of the work mentioned here, a general study of mechanisms of reactions of organometallic compounds, including the effects of high-energy radiation on such compounds, and investigation of the reactivity of fluorene derivatives Dr Eaborn is a graduate of the University of Wales, of which he holds the D Sc degree He was appointed an assistant lecturer in the (then) University College of Leicester in 1947, promoted lecturer in 1950, and in 1954 was given the title of reader in physical-organic chemistry in recognition of his distinguished work in that field He has recently been awarded a Robert A Welch visiting scholarship at the Rice University in Texas, which he expects to take up early in 1962

The Falkland Islands Dependencies Survey

The R R S *Shackleton* left Southampton on October 3 for the Falkland Islands Dependencies Survey, 1961–62, taking twenty scientists and technicians of the Survey and two meteorologists for South Georgia The R R S *Shackleton* has been extensively used since it was taken over by the Survey in 1955 Earlier this year, using the *Shackleton,* the Survey was able to collect geological specimens from the Clerke Rocks and to make landings on Visokoi Island (South Sandwich Group), Leskov Island and Vindication Island During the same voyage, a magnetic survey of the Scotia Arc (the submarine mountain range running from South Georgia through the South Sandwich Group and the South Orkneys to the South Shetlands) was carried out Mr Cameron and Mr Kennett, who were responsible for this survey, hope to take seaborne seismic soundings this season in the same area, extending their magnetic study of the rocks below the ocean floor, with the view of tracing the course of the Arc and its connexion with the Andean mountains of South America Also arranged for the R R S *Shackleton* is a series of regular observations to check the secular change in the Earth's magnetic field, this investigation is being made at the request of Prof Takesi Nagata, Tokyo University, on behalf of the International Union of Geodesy and Geophysics

The purpose of the Falkland Islands Dependencies Survey, the director of which is Sir Vivian Fuchs, is to carry out scientific research in the Antarctic, under the administrative control of the Governor of the Falkland Islands For nearly seventeen years it has maintained permanent bases on the mainland of Graham Land and on nearby islands At these bases between ninety and one hundred scientists and technicians spend periods of up to two years carrying out a systematic series of investigations which, so far, have been producing valuable information about the weather and structure of the south polar continent In addition, numerous investigations are being made in the fields of geophysics and human physiology in Antarctic conditions Each Antarctic summer (November–March) the sea ice clears sufficiently for specially strengthened ships, such as the R R S *Shackleton,* to make voyages to these bases with relief staff and stores

Russian Space Research

Recent cosmic flights in the U S S R found their reflexion in the popular scientific journal, *Priroda,* for 1961 Thus, N M Sisakyan (1, 7) discusses the biological factors, while space–time problems are discussed by D A Frank-Kamenetsky (1, 16) and by A S Kompaneets (5, 17); V G Kurt (2, 23) discusses the upper atmosphere and the interplanetary space V D Davydov (3, 3) concerns himself with the Earth–Venus flight, and finally in No 5 (May) of *Priroda* there is a number of articles dealing with the flight of Y A Gagarin on April 12 (pp 3, 5, 6, 8, 14, 16) The space ship which circumnavigated the Moon on October 7, 1959, automatically photographed the other side of the Moon, and an account of these photographs and of the map constructed of the other side of the Moon are given by Y N Lipsky (*Priroda,* 1, 35, 1961), a map on the scale of 1 10,000,000 is appended

Atomic Energy and its Applications

Atoomenergie en haar Toepassingen (Atomic Energy and its Applications), the scientific and technical periodical published under the auspices of The Netherlands Reactor Centre (Reactor Centrum Nederland), normally contains articles in Dutch dealing with various aspects of nuclear energy, but the June issue (3, No 6), which is devoted entirely to the activities and achievements of the nuclear industry in The Netherlands, is printed in English for the benefit of a wider public The introductory article, which is written by Prof M Bogaardt, adviser to the Centre, gives a general picture of the industrial activity in the nuclear field in The Netherlands, and is followed by a brief illustrated description of the Centre and a number of short articles contributed by leading Dutch firms The comprehensive buyers' guide of nuclear and allied products, materials and services—the first of its kind—which concludes the issue, has been compiled from answers received to a questionnaire circulated among a large number of Dutch firms which are actively engaged in the nuclear field The guide consists of two sections, the first being an alphabetically arranged classified list of products with the names of the respective manufacturers, and the second an alphabetical index of firms and their addresses with brief details of their chief products

Society for Folk-Life Studies

A very successful conference to inaugurate the Society for Folk-Life Studies was held at University College, London, during September 6–8, 1961 More than eighty people from many parts of Great Britain and Ireland attended and, at a general meeting held on September 8, the Society was formally established The following officers were elected *President,* Dr Iorwerth C Peate (Welsh Folk Museum), *Vice-President,* Mr Andrew Jewell (University of Reading), *Secretary,* Mr J Geraint Jenkins (Welsh Folk Museum), *Treasurer,* Mr Stewart F Sanderson (University of Leeds) The following members were elected to serve on the Council Mr Frank Atkinson (Barnard Castle, Durham), Mr G Ewart Evans (Ipswich), Mr A T Lucas (National Museum of Ireland), Mr B R S Megaw (University of Edinburgh), Mr C S Mundy (University of London), Dr Melville Richards (University of Liverpool), Mr G B Thompson (Ulster Folk Museum), Mr D M Wilson (British Museum)

Membership is open to all individuals and institutions interested in the study of the traditional patterns of life both in rural and urban communities Although various bodies such as the Folklore Society, the British Agricultural History Society and several other societies are each interested in particular aspects of folk-life and social history, this new Society to cover the complete field fulfils a long-felt need It can serve as a focus for the activities of those individuals, amateur and professional alike, who take an interest in their native culture In addition to holding an annual conference at various centres in Great Britain and Ireland in turn, the Society will also publish its own journal Further information can be obtained from the Secretary, c/o Welsh Folk Museum, St Fagans, Cardiff

The Mankind Quarterly

A RECENTLY published new journal seems to be devoted to the promotion of the idea of the basic significance of the biological endowment of human races in determining psychological and cultural characteristics (*The Mankind Quarterly An International Journal dealing with Race and Inheritance in the Fields of Ethnology Ethno- and Human Genetics Ethno-Psychology Racial History Demography and Anthropo-Geography* Vol 1, No 4, April 1961, Vol 2, No 1, July–September 1961 Edinburgh 6*s* each) It presents some descriptive articles, for example, on the Kurumbas and Todas of the Nilgiri Hills of South India (by Ruggles Gates) and on Bantu facial conformation (by J D J Hofmeyr and H Hitzeroth), and some cautiously analytical ones, for example, on "the cultural hypothesis" of mental types (by Gutorm Gjessing) But most of the contributions argue strongly that there is a close relation between innate physical and mental racial characteristics In various contexts it is stated· that there is a genetic predilection for certain cultural forms, that there is a significant correlation between the occurrence in ancient times of "the Aryan speech" and the peoples of largely Nordic strain that within any society an intellectually *élite* group tends to maintain its genetic superiority through selective mating The "equalitarian dogma" of Negro aptitudes—as illustrated by Gunnar Myrdal's work—is attacked, and it is implied that social segregation of the Negro in the United States would be a proper course The material in this journal is of very uneven quality and it is unreliable as a guide for students

The South Australian Museum

MUSEUMS in many places are, at the present time, undertaking extensive re-organization schemes The report of the Board of the South Australian Museum for 1959–60 is yet another example of this world-wide movement, and it is only labour and finance which dictate the speed of these transformations (Pp 16 Adelaide Government Printer, 1961) The activities of the Museum include television broadcasts, a display of Australian wild flowers at the Adelaide Festival of Arts and an extensive programme of field work both in archæology and natural history A rather unusual feature is the formation of an underwater research group, the members of which have collected marine invertebrates and have been especially successful in stupefying fish by means of the distribution of rotenone in the wreckage of a ship lying in 50 ft of water The fish are collected in nets by the aqualung divers Members were fortunate in being able to observe and capture two specimens of the rare leafy sea-dragon, *Phycodurus eques*

Neurology of Language

IN a wide-ranging review of the neurology of language, Sir Russell Brain shows how difficult it is to infer from cases of aphasia the physiological organizations underlying speech (*Brain*, 84, Part 2, 1961) He also shows the inadequacy of purely psychological classifications of aphasia and of the attempt to correlate disturbances which can be classified psychologically with lesions in particular situations The physiological organizations on which speech depends are of great complexity, extending over considerable areas of the brain, and are organized in time as well as in space These serve psychological functions, but the breakdown of a physiological schema does not necessarily, or indeed usually, disturb speech in a way that corresponds to a single type of psychological defect Moreover, the anatomical organization of the schemas means that only rarely will a single type of schema be disturbed in isolation from others What the clinician calls aphasia is almost always the product of a mixture of physiological disturbances resulting in a complex medley of psychological disabilities For the present, the classification of the aphasias must be largely empirical The task for the future is to use all the available modern methods of psychological testing, linguistics, phonetics and communication theory, to correlate psychological with physiological functions Already the directions in which progress is likely to be made can be seen From a number of investigations it is hoped to arrive at an analysis of speech disturbances at both the psychological and physiological levels, and of their interrelationships, which will provide a scientific basis for the recognition of the type of disorder of function present in each individual aphasic patient

Immunization against Disease

THE subject for discussion at the Thirteenth World Health Assembly in 1960 was "The Role of Immunization in Communicable Disease Control" A recently published volume contains the introductory address of the chairman, Prof Zhdanov of Moscow, papers on the background of immunization, by Prof Cruickshank of Edinburgh, and the efficiency of immunization procedures used in public health practice by Prof Edsall of Harvard, there is also a paper on compulsory or voluntary vaccination, by Dr J de Moerloose of the World Health Organization, and finally, the report of the technical discussion (*The Role of Immunization in Communicable Disease Control* Various authors World Health Organization Public Health Papers No 8, 1961 Pp 118 Price 6*s* 8*d* Also published in French Available through H M S O) There are nowadays so many vaccines and immunization schedules that the average practitioner is understandably bewildered and uncertain which to use and when This small volume contains authoritative and up-to-date views on the value of the various vaccines, and the pros and cons of immunization as well as the most suitable times for vaccination

Producing Transplantable Human Tissues in the Laboratory

The July issue of *Discovery* contains an article by Dr. D. Petrucci, Medical Biological Studios, Bologna, entitled "Producing Transplantable Human Tissue in the Laboratory". The article resulted from Dr. Petrucci's original work on building tissues which, in contrast to adult tissues, would be transplantable and acceptable by adult organisms. The idea was that the chances of a glandular transplant surviving would be improved if the gland were obtained from an embryo that is first cultivated outside the maternal body. The technique was to fertilize the ovum between two chamois disks, in what Dr. Petrucci calls his "biological cradle". Various 'cradles' have been used in which the developing zygote and embryo could be photographed continuously. The article is extensively illustrated, and the 48 illustrations show the fertilization and development of the embryo covering a period of 58 days of growth from fertilization; they also demonstrate the 'cradle'. In a field so sparse in workers, who themselves tend to be highly sceptical of all and everybody's work in that field, it is a pleasant relief to find a general article. The illustrations will be of great use to science teachers.

Geology and Ceramics

To mark the occasion of the Seventh International Ceramic Congress, held in London during May 1960, a special exhibition of rocks and minerals used by the British ceramic industry was shown at the Geological Museum in South Kensington. A booklet (*Geology and Ceramics*. Pp. ii+28. London: H.M.S.O., 1961. 3*s*. 6*d*. net), prepared by Dr. P. J. Adams, was issued to give a brief account of the rocks and minerals used in the industry, describing their nature and geological occurrence. The many ways in which the raw materials are used by the ceramist, potter and engineer are also described. The booklet is well illustrated and the subject-matter is dealt with under the headings of pottery and whitewares, structural clay products and refractories and technical ceramics.

Announcements

The first award of the Herbert E. Gregory Medal for distinguished service to science in the Pacific, established by the Trustees of the Bernice P. Bishop Museum, Honolulu, Hawaii, in memory of Prof. Herbert E. Gregory, director of Bishop Museum during 1919–36 and Silliman professor of geology in Yale University, was recently made at the tenth Pacific Science Congress to A. P. Elkin, emeritus professor of anthropology at the University of Sydney. Prof. Elkin is distinguished by his contribution to anthropological research in Australia and Melanesia and by his active role in the development of institutions supporting Pacific research.

Prof. Seymour S. Kety, professor of psychiatry, Johns Hopkins University, has been awarded the Alvarenga Prize by the College of Physicians of Philadelphia for his work on cerebral blood flow and the effect of drugs on the central nervous system.

Oscar A. Quihillalt, of Argentina, has been elected president of the International Atomic Energy Agency at its recent meeting in Vienna.

Dr. J. W. Menter has been appointed director of the Tube Investments Research Laboratory at Hinxton Hall, near Cambridge. The Laboratory carries out fundamental research in metal physics and physical metallurgy. During seven years at Hinxton, Dr. Menter has directed research teams studying the microstructure of metals and has promoted notable advances in the techniques of electron microscopy and X-ray microanalysis.

Mr. J. C. Hanbury, chairman of Allen and Hanburys, Ltd., has been elected chairman of the British Pharmaceutical Conference for 1961–62. Mr. Hanbury took the Natural Science Tripos at Cambridge and later obtained the degree of bachelor of pharmacy of the University of London. He has spent the whole of his working life with Allen and Hanburys, Ltd.

Dr. W. J. Bates, lecturer in physics at the University of Bristol, has been appointed chief engineer at R. and J. Beck, Ltd. Dr. Bates will lead Beck's design team at the Lister Works, Dickenson Street, London, N.W.5. Later he and his team will move on to the Company's new factory at Watford when it is completed.

The Basic Science Section of the British Ceramic Society is holding a meeting on "Ceramics in Nuclear Science" at the Atomic Energy Research Establishment, Harwell, during November 28–30. Further information can be obtained from Dr. J. P. Roberts (honorary section secretary), Houldsworth School of Applied Science, University, Leeds, 2.

The Institute of Biology is organizing a symposium on "The Better Use of the World's Fauna for Food", which will be held in the Lecture Hall, Royal Geographical Society, Kensington Gore, London, S.W.7, during October 27–28. Attendance at the symposium is open, and further information can be obtained from the Institute of Biology, 41 Queen's Gate, London, S.W.7.

A short course on "Chemical Applications of Diffraction Methods" is being organized by the Department of Chemical Technology, Bradford Institute of Technology, and will be held at the Institute during November 24–25. The fee for the course is 45*s*., and further information can be obtained from the Registrar, Institute of Technology, Bradford, 7.

The autumn meeting of the Parasitology Group of the Institute of Biology is to be held at the National Institute for Medical Research, Mill Hill, London, N.W.7, on November 10. The meeting will be devoted to a series of papers under the general title of "Parasites of World Importance". Further information can be obtained from the acting secretary of the Group, L. P. Joyner, Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food, New Haw, Weybridge, Surrey.

The South London Branch of the British Institute of Management is organizing a second conference on the preparation and use of case studies. This is to be held at the British European Airways Staff College, East Burnham, Slough, Bucks., during October 13–15. Inquiries should be addressed to Mr. B. E. Baldrey, Conference Secretary, Palmer's Travelling Cradle and Scaffold, Ltd., 3 Woodside Green, London, S.E.25.

Erratum. In the article entitled "A New Test for detecting Anaphylactic Sensitivity: the Basophil Reaction", appearing on p. 1056 of the September 9 issue of *Nature*, the fixative solution consisted of ethyl alcohol 6 ml., chloroform 2 ml., and acetic acid 2 ml. The solution did not contain acetone as stated.

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UNIVERSAL LANGUAGE FOR COMPUTING MACHINES

THE traditional pattern of Section A* (Mathematics) meetings at the British Association, consisting of a division into pure and applied mathematical sessions, was this year preserved with a difference

On September 1 at Norwich the 'pure' session opened with a paper by Prof R L Goodstein having the title "Truth Tables" Starting with a simple account of formal mathematical logic Prof Goodstein showed how the apparently 'basic' operations *and*, *or*, *not*, etc , could successively be reduced to a single pair and, eventually, to the single 'Sheffer stroke' function The talk was a model of systematic presentation and took the audience from the elements to up-to-date research in the field of multi-valued logics

In the discussion which followed it was made clear that this very 'pure' subject had considerable practical application, particularly as the Sheffer function gives a means for constructing the most complex digital computer from a single type of building block

The second paper, by Prof P J Hilton, was concerned with group theory and showed how the basic ideas of elementary arithmetic might better be taught via this fundamental discipline than by the present rote-learning methods

To conclude this session, a paper by Dr P F Scott described the characteristics of the various kinds of radio telescope After showing that any large increase in size over the instrument at Jodrell Bank was impossible, he went on to show that, by using relatively small movable aerials of simple geometry, and by making the rotation of the Earth act as a scanning mechanism the effect of receiver areas of several acres could be simulated The catch was, that to obtain a picture of the heavens of the type used by astronomers, it was necessary to use a digital computer to process the 250,000 or so observations Thus the first session ended with a distinct flavour of computers

On September 5, there was a symposium on artificial computer languages Mr P V Ellis, of International Computers and Tabulators, Ltd , opened the meeting by explaining how, in the early computers, a programme was set into the machine almost by the individual operation of switches which controlled the electronic elements He pointed out that an operation which took only a few human words to mention might take as many thousands of the code symbols understandable by a computer From this basis he explained how the notion of a human/machine language for communication arose and described the early attempts at formulating such systems for both mathematical and business use These were, he said, now tending to merge, but difficulties existed because, while the language of mathematics was relatively precise, that of commerce was not. so that the same words might mean quite different things to different people

To show the three stages of interpretation which are involved, the example of developing a simple statement like "We wish to take Smith's pay and compute the income tax", into a detailed procedure expressed in words and appropriate mathematical symbols and thence into a detailed machine coding, was analysed After rejecting the simple solution that accountants and business men should learn mathematics—"because they will not"—current progress in the development of a commercial language 'Cobol' was described The value of such a language, if accepted, was shown by the fact that, over the life of a machine, the cost of programming was about 1½ times that of the machine itself

The theme of commercial computer languages was then taken up by Mr R M Paine, of the Corporation for Economic and Industrial Research After explaining that about 6,000 electronic computers of various sizes were now in operation, he went on to remark that a computer code such as

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          60     6     31
    25    72     5     34
         400     4     17
              etc
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would be difficult to explain to people unfamiliar with the particular machine concerned—and possibly even to those who were On the other hand, a statement in 'Cobol'

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Overtime
    If month-number is not equal to 3,
    go to print
    If overtime rate is equal to 0,
    multiply salary by 0 015 giving overtime,
    otherwise multiply salary by overtime
    rate giving overtime,
    go to print.
```

could be understood by all

'Cobol' was a beginning of an agreed language and was accepted by the producers of some 90 per cent of the world's output of computers Some other attempts at artificial languages such as the Ferranti 'Nebula', were mentioned and the paper ended with an account of a modern development whereby computers could directly understand selected words of ordinary language presented to them

The scientific side of computer language was then taken up by Mr C Strachey, who suggested that programming was now emerging from its adolescent phase in which an intimate knowledge of the machine, *ad hoc* tricks, and infinite patience on the part of the programmer were of supreme importance He suggested that the two 'new techniques of greatest future promise were a function language, and the replacement of the idea of linear sequences of instructions by branched chains in which no attempt was made to maintain natural order, but in which each constituent gave explicit reference to its successors Having dismissed these topics Mr Strachey then developed an attack on British computer designers and research workers in the universities and elsewhere The burden of his discontent was that, whereas the American approach was to use a machine of any cost, however high, to save human effort, the British machines required the use of human genius to help the machine He ended by claiming that this trouble was caused by the absence of very large random access stores on the British machines

The last of the formal papers was read by Mr R G Goodman, of the Brighton College of Technology

The basic structure of the mathematical 'language' 'Algol' was explained and developed to the state in which a complete problem was coded for illustration.

The discussion was opened by Dr A. D. Booth, of Birkbeck College, London, who, after commenting on the implication of Mr Ellis's figure of 1½ for programming/machine cost, pointed out that Mr Strachey's strictures were, to a large extent, unjust. The ideas of function languages were well known in Great Britain and much work has been done in them. The branched chain technique had been in use at Birkbeck College for at least three years and was described in several papers so that a claim that "no one in Britain had experience of it" was patently false. So far as lack of large, random-access, stores on British machines was concerned, Dr Booth maintained that far from being due to lack of imagination the situation was actually caused by lack of Government support for British machines.

A vigorous discussion in which the topics ranged from qualifications and training of programmers to the various kinds of 'meta-language' in machine translation, ended the session. A. D. BOOTH

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY

By Dr W. P. D. WIGHTMAN

Department of History and Philosophy of Science, King's College, University of Aberdeen

THE spate of publications relating to the Royal Society of London during the past two years seems to have increased rather than diminished the already existing misunderstanding of the relation between the Society and the famous periodical which it encouraged, but for which on several occasions the Society published or caused to be published disclaimers for any responsibility. Recently, references have been made to volumes of the *Philosophical Transactions of the Royal Society* (sometimes with '*of London*' added) in the seventeenth century, when no periodical with this title existed, statements have been made (or implied) that there was only one (or no) interruption in its publication, and, worst of all, that it was the 'official' journal of the Royal Society. The precise title would perhaps not be of any serious import but for the fact that the inclusion of the words 'of the Royal Society' suggests that it was a record of the 'Transactions' of that body and hence gives plausibility to the term 'official'. Since statements about the *Philosophical Transactions* (a few incorrect, and others by condensation definitely misleading) are scattered about over a large number of recent articles, it seemed desirable to assemble a sufficient number of 'advertisements' by the Society or its officers to establish the facts relating to the *Philosophical Transactions*. The purpose of this article is thus primarily bibliographical in the broad sense: it is not concerned with the purpose or quality of the *Transactions*. It is based on an examination (though, of course, far from exhaustive) of every volume of the *Transactions* (and *Philosophical Collections* to be referred to below) in the first two centuries of its existence.

The title on the first number issued and on the title page of every subsequent volume until Vol. 66 of 1776 was "Philosophical Transactions giving some accompt of the present undertakings, studies, and labours of the Ingenious in many considerable parts of the world".

There is no mention of the Royal Society other than in the imprint of its printers, John Martyn and James Allestry, and in Oldenburg's dedicatory epistle. Moreover, there is a clear indication that the editor and publisher had cast his net widely to get a representative review: the result was in fact a great deal more like *Nature* than a mere organ of publication of individual researches. But, despite this clear indication, confusion must have arisen at so early a date as to require the following disclaimer to be published in No. 12 of May 1666: "Whereas 'tis taken notice of that several persons perswade themselves that these Philosophical Transactions are publish't by the Royal Society, notwithstanding many circumstances to be met with in the already publish't ones that import the contrary: the Writer thereof hath thought fit, expressly here to declare that that perswasion, if there be any such indeed, is a mere mistake. Though he denies not, but that having the honour and advantage of being a Fellow of the said Society, he inserts at times some of the Particulars that are presented to them".

The last number of the *Transactions* in the first run was dated February 1679. From some time in the same year (colophon of first number) to April 1682 their place was taken by Robert Hooke's *Philosophical Collections* "containing an account of such physical, anatomical, . . . or other mathematical and philosophical experiments and observations as have lately come to the publisher's hands. As also an account of some books of this kind lately published". A similar 'advertisement' was printed in the number of December 1681. The *Philosophical Transactions* recommenced in January 1683 with the preface: "Although the writing of these *Transactions* is not to be looked upon as the Business of the Royal Society, yet in regard they are a Specimen of many things which lie before them . . . They have thought fit to take care for the revival thereof . . . ." Vol. 16 contained the numbers issued during 1686 and 1687 and bore the imprint "London 1688" (some previous volumes were printed at Oxford "at the [Sheldonian] Theater"). Thereafter, publication was suspended until early in 1691 "chiefly by reason that the unsettled posture of publick affairs did divert the thoughts of the curious towards matters of more immediate concern than are physical and mathematical enquiries . . . ". These were, of course, the years of the 'Glorious Revolution', and this gap in the *Transactions* has been overlooked in some accounts.

It is not possible, nor indeed necessary, to cite every declaration of editorial policy: all call the same tune with variations—a review of what is going on in the world of 'philosophy', including "an exact account of such experiments as having been made before the illustrious Royal Society they shall be pleased to order or permit the publication of" (Halley, 1714).

Then in the Volume 47 (London 1753, but as usual containing the numbers issued during the years 1751 and 1752) came the announcement of a fundamental change "The Committee appointed by the Royal Society to direct the publication of the *Philosophical Transactions*, take this opportunity to acquaint the public, that it fully appears, as well from the Council-books and journals of the Society as from the repeated declarations which have been made in several former *Transactions*, that the printing of them was always, from time to time, the single act of the respective Secretaries, till this present XLVII volume And this information was thought the more necessary, not only as it has been the common opinion, that they were published by the authority, and under the direction, of the Society itself, but also, because several authors, both at home and abroad, have in their writings called them the *Transactions of the Royal Society* [italic in original] .

"But the Society being of late years greatly inlarged it was thought adviseable that a Committee of their Members should be appointed to reconsider the papers read before them, and select out of them such, as they should judge most proper for publication in the future *Transactions*, which was accordingly done upon the 26 of March 1752 "

In 1752, then, for the first time the *Philosophical Transactions* were in fact, but not yet in name, "of the Royal Society" the old, and by then quite unsuitable, title was retained until Vol 66, dated 1776

That the announcement of this change of policy was not a mere formality is shown by reference to the Council minutes for January and February 1752, when a number of 'questions' put by Lord Macclesfield (not yet president) were considered The only one relevant to our purpose was whether there was any evidence of "intermeddling" by the Society with the *Transactions*, to decide which Thomas Birch, secretary, was requested to take the relevant literature home and examine it Within a week he had made a sufficient investigation to satisfy the Council, and the changes were made as already described

It remains only to add that the announcement made in 1752 appeared regularly for the next 123 years it appeared for the last time in 1875, by which time it was no longer true that "in late years" the Society had been "greatly inlarged", it had, on the contrary, in pursuance of the 'reforms' of 1847, been greatly reduced

DEOXYRIBONUCLEIC ACID: STRUCTURE, SYNTHESIS AND FUNCTION

THE eleventh Annual Reunion of the Société de Chimie Physique was held at the Col de Voza during June 26–30 and was devoted to papers and discussions on the structure, synthesis and function of deoxyribonucleic acid (DNA) There were some thirty contributors to the symposium and the total number of participants was about 100 The full texts of the contributions, including the discussions, are to be published in the October and November issues of *Journal de Chimie Physique* and will be republished afterwards in the form of a book

The papers were divided into groups according to their subject-matter and the meeting commenced with a session on "Macromolecular Structure of DNA" Ch Sadron (Strasbourg) presented results pointing to the existence of sub-units in DNA particles in solution and this was followed by a paper from C Levinthal (Cambridge, Mass) on the hydrodynamic shear degradation of *T*2 bacteriophage DNA in solution This degradation of DNA can be brought about by normal laboratory procedures, and this had led to the conclusion that the molecular weight of DNA in cells is probably very much greater than the 6–10 million usually quoted and might be as much as 100 million M H F Wilkins (London) presented new data on the *A*, *B* and *C* configurations of DNA and evidence against the existence of base pairing other than that of Watson and Crick In addition, the structure of denatured DNA and the relation of such structures to RNA was discussed X-ray diffraction studies on DNA in solution were reported by V Luzzati (Strasbourg), who concluded that the form and molecular weight of DNA were the same in dilute solution and in gels

The second session dealt with "Electronic Aspects of Nucleic Acid Structure" and started with a paper by B Pullman (Paris), who dealt with the electronic structure of nucleic acid constituents and the influence of this on the chemical and physico-chemical properties of DNA This was followed by a paper from M Kasha (Tallahassee), who was concerned with $n \rightarrow \pi^*$ optical transitions in polynucleotides, the exciton splitting phenomenon in hydrogen-bonded *N*-heterocyclic base pairs, and energy transfer and hypochromism in polynucleotides L A Blumenfeld (Moscow), who unfortunately was not present, submitted an abstract on the magnetic and dielectric properties of DNA, and P Douzou (Paris) presented results of experiments on the excitation and emission spectra of purine and pyrimidine bases, the corresponding mononucleotides, polyadenylic acid, DNA and nucleoproteins

The third session was entitled "Chemical Sequence and Heterogeneity", and commenced with a presentation by B. Horecker (New York) of some recent results of Hurwitz and his collaborators on the DNA-requiring system for polyribonucleotide biosynthesis In this system, the base composition of the newly formed polyribonucleotide appears to be determined by that of the DNA primer J Marmur (Brandeis University) described in some detail work on strand separation in DNA, on the recombination of strands and on the formation of hybrid chains A system capable of the non-enzymic synthesis of polydeoxyribonucleotides from mono- and oligo-nucleotides present in deoxyribonuclease digests of DNA was described by A Bendich (New York)

"Enzymic Synthesis of DNA" was the title of the fourth session, which was opened by a paper from R M. S Smellie (Glasgow) on the properties and intracellular location of the DNA polymerase in certain mammalian systems and on some possible mechanisms of control of the biosynthesis of DNA This was followed by a presentation by J A V Butler (London), who described some experiments on the phosphorylation of thymidine by tissue extracts and on the incorporation of labelled thymidine into DNA S S Cohen (University of Pennsylvania) discussed the changes in enzyme complement of bacteria infected with *T*-even bacteriophage, and referred in particular to the appearance of an active system which converts ribonucleotides to deoxyribonucleotides Some recent work on the mechanism of replication of bacteriophage φ*X*174 were described by R. L Sinsheimer (Pasadena), from which it seems that on infection a replicative form of DNA is produced which has characteristics of a double-stranded DNA

The fifth and sixth sessions dealt with "Induced Changes in DNA" R Litman (University of Colorado) discussed the effects of ultra-violet irradiation and nitrous acid on pneumococcal-transforming DNA, and the chemical and biological effects of nitrous acid and hydroxylamine on DNA from tobacco mosaic virus and *T*-even bacteriophages were described by H. Schuster and W Vielmetter (Tubingen) P D Lawley (London) reported on the effects of alkylating agents on DNA The relative reactivities of the different bases in DNA were described and the possibilities of cross-linking in the DNA molecule were discussed Experiments directed towards elucidating the mechanism of action of succinic peroxide on DNA by chemical, physico-chemical and biological procedures were discussed by D Luzzati (Strasbourg) G Stent (Berkeley) discussed the susceptibility to mutagenesis by 5-bromodeoxyuridine and the stabilization to phosphorus-32 decay-inactivation of *T*4 bacteriophage-infected bacteria D Shugar (Warsaw) was unable to attend the meeting, but submitted an abstract dealing with the primary effects of ultra-violet radiation on nucleic acids; these include hydration and dimerization of pyrimidine residues An additional paper on the dimerization of thymine by ultra-violet radiations was presented by W Berends (Pays Bas) R Latarjet (Paris) described recent investigations on the action of X-rays on pyrimidine bases in aqueous solution with the formation of hydroperoxides, and some work on the lethal effects of radioactive decay in bacteria after labelling of the DNA in different sites with phosphorus-32, carbon-14 and tritium W Harm (Cologne) discussed the reactivation of bacteriophage following inactivation by exposure to ultra-violet light and divided the reactions into two principal classes, namely, 'recombinative reactivation' and 'true repair mechanisms'

The seventh session was concerned with "Code and Replication" A Garen (Philadelphia) described alterations in alkaline phosphatase resulting from mutations, and J Weigle and F Stahl (Pasadena) discussed chromosome breakage and genetic recombination in bacteriophage λ and the role of duplication in recombination

"Genetic Aspects" provided the subject of the final session of the symposium L Grossman (Brandeis University) presented some work on the chemistry and antigenecity of DNA from *T*2, *T*4 and *T*6 bacteriophage Thermally denatured DNA from these bacteriophages will react with antibodies in the antisera of rabbits immunized with the respective ruptured bacteriophage, but native or renatured DNA shows little reactivity H Ephrussi-Taylor (Paris) described a study of recombination at the molecular level in bacterial transformations and F Jacob and F Gros (Paris) discussed the regulation and mode of action of genes The role of messenger RNA in these reactions was also discussed The final paper of the session was delivered by R Herriott (Baltimore), who described experiments on the annealing of mixtures of transforming DNAs

The timing of the meeting was such as to allow for plenty of discussion during each session, and advantage was taken of this by many of the participants with the result that lively and valuable discussions followed most of the presentations In every respect this was a highly successful meeting and the organizing committee of the Société de Chimie Physique is to be congratulated on the arrangements

R M S SMELLIE

QUANTITATIVE BIOLOGY

AN informal symposium on "Quantitative Biology", at which sixty biochemists, biophysicists and biologists met to discuss their common problems, was held at the Dominion Physical Laboratory, Lower Hutt, New Zealand, on May 12 The meeting was arranged by the biophysicists, who felt that New Zealand workers in these fields had insufficient opportunity to get together The enthusiastic response and the success of the meeting showed how general this feeling must have been

In the morning session of the symposium the hosts introduced their own work Dr M C Probine, who is measuring the physical properties of the primary wall of plant cells, showed how changes in the shape of growing *Nitella* cells could be predicted from the initial orientation of the reinforcing microfibrils and the elastic and plastic properties of the wall Dr K J McCree discussed his programme for measuring the photoconductivity of oriented monolayers of organic materials, produced by the Langmuir technique Mr M A Collins described his electron-spin-resonance spectrometer, and demonstrated the signals of free radicals occurring naturally in coal and those produced by the γ-irradiation of cellulose The spectrometer will also be used to study the physics of photosynthesis, in conjunction with the photoconductivity experiments

Some striking electron micrographs of the wax on the surfaces of clover leaves were exhibited by Mr D M Hall They showed how the pattern varied with the species of clover, and how the wax could be removed by the rubbing together of leaves in a high wind Mr W S Bertraud described the changes in the components of the spermatid cells of the cave weta (*Pachyrhamma fascifer*) which took place during spermiogenesis

In the afternoon, Dr E G Bollard, of the Fruit Research Division, Department of Scientific and Industrial Research, described the utilization of various organic nitrogen compounds as sole sources of nitrogen, in the sterile culture of *Neurospora*, *Chlorella*, *Spirodela* and carrot root tissue He was followed by Dr R E F Matthews, of the Plant Diseases Division, who had found (working with

Dr R I B Francki) that the uracil analogue 2-thiouracil had very different effects on the rod-shaped tobacco mosaic virus and on the spherical turnip yellow mosaic virus After tea, Dr G A Nicholls, of New Zealand Forest Products, Ltd , took his audience on an illustrated tour of his firm's plant, and discussed the work of his laboratory in the fields of bark utilization, pulping, bleaching and turpentine refining Dr A T Wilson, of Victoria University of Wellington, then wound up the proceedings with a highly entertaining exposition of his theories on the physical system in which life may have originated

In the discussion of the way in which future meetings should be organized, there was general agreement that they should be kept as informal as possible, so that ideas could be aired and discussed before they had reached the stage of publication It was also agreed that the meetings should be held at various laboratories in turn, so that in time everyone would have a first-hand knowledge of what everyone else was doing Of the various time-intervals which were suggested, a year was most favoured, and Drs McCree and Probine were asked to handle the arrangements for the next meeting

K J McCREE

MEDICAL RESEARCH IN THE BRITISH CARIBBEAN

THE sixth scientific meeting of the Standing Advisory Committee for Medical Research in the British Caribbean was held at the Marine Hotel, Bridgetown, Barbados, during April 10–13. Thirty-eight communications were delivered, and six were read by title only Chairmen for the five sessions were Sir Harold Himsworth, Prof T H Weller, Prof. A C Frazer, Dr E T C Spooner and Prof. E K Cruickshank

The meeting was remarkable for the high standard of presentation, the diversity of subject-matter, and for the evidence which it offered of the stimulating effect of a research interest on all fields of medicine and its ancillary professions The theme of the meeting—the impact of research on public health—was chosen to be as nearly all-embracing as possible

R A Irvine, C. Miller and L S Grant (Univ Coll , West Indies) reviewed typhoid infection in Jamaica During the period 1952–59, typhoid fever had affected chiefly the younger age groups, with a relapse-rate of 8 per cent and a case fatality of 5 8 per cent M T Ashcroft (British Guiana) reported a steady decline in fatality of typhoid fever in British Guiana, partly due to improved water supply and partly to the introduction of chloramphenicol treatment C C Nicholson and J M Ritchie (British Guiana) directed attention to imperfections still remaining in British Guiana water supplies E H Back and S E Brooks (Univ Coll , West Indies) analysed 134 cases of gastroenteritis in infants admitted to University College Hospital, Jamaica, and discussed prognosis F C Ramsey (Barbados) described scarlet fever in Barbados, suggesting that the disease is less rare than it is said to have been G Bras, D Watler and S E Brooks (Univ Coll , West Indies) quoted figures from the Cancer Registry at University College Hospital to show that carcinoma of the cervix in Jamaica is responsible for more than 30 per cent of all malignancy in females J D Arneaud (Trinidad) discussed drug resistance of tubercle bacilli isolated in Trinidad K D B Charles (Jamaica) and L S Grant (Univ. Coll , West Indies) reviewed poliomyelitis in Jamaica with special reference to the epidemics of 1954, 1957 and 1960 J S R Golding (Univ Coll , West Indies) described the management of those disabled in a moderately severe epidemic of poliomyelitis, and demonstrated the necessity for a planned programme L Spence (Trinidad) gave an account of the distribution of Eastern equine encephalitis virus in Trinidad and British Guiana In Trinidad, the virus has been isolated on six occasions from culicine mosquitoes, and in British Guiana from horses during an outbreak In Trinidad, immunity has been demonstrated in humans, birds and poultry L S. Grant (Univ Coll , West Indies) reported a survey of *Q* fever antibodies in Jamaica, showing that this infection is not at present prevalent W G Downs, L H Turner and A E Green (Trinidad) found 10 per cent of human sera from cases of undiagnosed fever in Trinidad agglutinated leptospiræ, and reported 17 isolations of leptospiræ in culture They directed attention to the lack of knowledge of the local ecology of leptospirosis A V. Wells (St Lucia) described the successful eradication of malaria from St Lucia G Giglioli (British Guiana) discussed the effect of malaria eradication in British Guiana on mortality from chronic nephritis F R S Kellett (Trinidad), as part of the malaria eradication plan in Trinidad, had used 'Baytex' (*S* 29493) as a spray, and showed that this insecticide is effective on various household pests including culicine mosquitoes T H G Aitken (Trinidad) discussed the ecology of *Culex quinquefasciatus* and its importance for public health in the Caribbean area as a vector of filaria, St. Louis encephalitis and Cache Valley fever L M Burke (Jamaica) gave an account of the clinical features of poliomyelitis in Jamaica, as seen in a study of 309 cases admitted to Kingston Public Hospital during 1957–60 J A. S Hall (Jamaica) discussed the role of infection in a series of twenty cases of myelomatosis E H Back and E E DePass (Univ Coll , West Indies) considered rheumatic fever as seen in University College Hospital, Jamaica, during 1952–60, and described the beneficial effect of maintenance benzathine penicillin. F M. McWatt (British Guiana) read an interesting paper on liver cirrhosis in cattle on the savannahs of British Guiana, where three species of *Crotalaria* have been identified The cause of the disease has not been ascertained, but liver biopsies have shown that the fibrosis regresses at certain times of year or when the animals are moved to improved pastures E E Ward and E. H Back (Univ Coll , West Indies) reported work on electrolyte disturbance in infantile gastroenteritis. T Poon-King (Trinidad) gave an important account of electrocardiographic changes observed in patients stung by the Trinidad scorpion, *Tityus trinitatis* The sting of this scorpion produces severe physiological disturbance and sometimes kills. Electrocardiographic abnormalities were found in 39 of 45 patients studied E E Ward (Univ Coll , West Indies) discussed γ-globulin-levels in Jamaicans; he found no racial differences, but levels were higher in those with chronic infections than in the healthy R A Webb (Barbados) analysed 330 first admissions to the Barbados Mental Hospital, in which records of mental ill-health for the whole island have been kept for many years Heredity was revealed as a more important etiological factor than environment K L Standard (Barbados) presented an analysis of child mortality in Barbados, reporting a marked reduction in infant mortality over the past six years Miss G Shaw, L S Grant and A A Peat (Jamaica) gave an account of the statistics of communicable disease in Jamaica up to 1956 K H Uttley (Antigua) discussed mortality from tuberculosis in the negro population of Antigua over the past 100 years B Weinstein (British Guiana) reported a survey of diabetes among residents on the sugar estates of British Guiana He found an overall incidence of 2 04 per cent and discussed etiological factors J Parker-Williams (Univ Coll, West Indies) described a survey of abnormal hæmoglobin in Barbados, with special reference to the sickling gene He compared Barbados with Jamaica in this respect, and discussed genetical implications R J Gourlay (Univ Coll, West Indies) described the incidence of certain worm infestations in Jamaica Miss A Walters and Mrs G Jordan (Barbados), two public health nurses in Barbados, presented a most interesting time-study of the work of public health nurses at three health centres in Barbados, showing that more time was spent on maintenance and records than on the treatment and care of patients ' H M. Beadnell (British Guiana) recounted eight years experience with injuries and workmen's compensation in the sugar estates of British Guiana D O Gore (Univ Coll, West Indies) gave a clinical and pathological account of the portal hypertension produced by 'veno-occlusive disease', and reported on six patients treated by establishing porto-caval shunts F F Laband (Trinidad) gave an account, illustrated with colour slides, of four years experience of oral surgery in the San Fernando General Hospital, Trinidad A G. Leacock and R K Rowley (Barbados) reported the results of repairing inguinal hernias by the 'nylon darn' technique J. A B Jones (Univ Coll., West Indies) discussed some anatomical and pathological abnormalities of the alimentary tract as seen in Jamaica. Winston Adams (British Guiana) gave an account of the control of two malaria outbreaks in the interior of British Guiana, discussed the difficulties of control of *Anopheles darlingi*, the local malaria vector, and mentioned the programme of chloroquinized salt prophylaxis now in operation in this area E L S. Robertson (Trinidad) showed a film demonstrating the surgical treatment of filaria lymphœdema.

Excellent arrangements were made for the meeting by Dr Maurice Byer, director of Medical Services, Barbados, and his staff

HISTORY OF SCIENCES IN ANCIENT AND MEDIEVAL INDIA

A SYMPOSIUM on the "History of Sciences in Ancient and Medieval India" was held during August 4–5 at the Bose Institute, Calcutta, under the auspices of the National Institute of Sciences of India The symposium was inaugurated by Dr D M Bose, director of the Bose Institute In his opening remarks, Dr A C Ukil, convener of the symposium, referred to the importance the study of the history of sciences in India had assumed during recent years and the problems which the workers in the field have to face, especially in connexion with the source materials The discrepancy of knowledge regarding chronology, he observed, constituted a serious difficulty which should engage the attention of the research workers in the subject Regarding the investigations already made, which he termed secondary sources, the attitude taken in the past and points of view expressed required in many instances to be re-examined and re-evaluated

Thirty-five papers were received for the symposium of which twenty-three were read and discussed In his paper on the social and international relations in the development of science, Dr R. C Majumdar pointed out that there is direct evidence to show that scholars from India visited Western countries and taught the people there medical science, astrology, astronomy, algebra and arithmetic as known to India and also indirect evidence of international exchange of scientific knowledge between India and foreign countries European scholars have generally held that in scientific matters it was India that must have borrowed from Western countries But with the advance in knowledge of history, he observed, it was difficult to subscribe to this view of one-way traffic This observation was further substantiated in the paper by Sri S N Sen, who furnished archæological and epigraphic examples and also case-histories such as the transmission of the knowledge of the decimal place-value notation, and some medical and astronomical ideas.

In biology, health and medicine, Dr N. H. Keswani discussed the concepts of generation, reproduction, evolution and human development as found in the writings of Indian scholars of the ancient and medieval period He noted that the results of their observations, unaided by microscopes and modern appliances, approached in accuracy those of modern work, especially in the field of embryology Zoological knowledge in the works of Kālidāsa formed the subject of two papers by Sri C S. Gupta and Sri B Rama Rao, while Dr S S. Misra gave a review of the development of medical sciences in India.

On Indian chemistry, Dr B V. Subbarayappa's paper dealing with Indian atomism showed independent development of the atomic concept of matter in the Hindu, Buddhist and Jaina literature and the difference between the Indian ideas and those propounded by the Greek scholars headed by Democritus and Leucippus Dr S P Raychaudhuri and Dr A K Bhattacharyya dealt with the agricultural practices and soil science in ancient India

The geographical ideas of the Hindus were presented by Dr S M Ali. Besides these were the discussions on the development of mathematical series in India after Bhāskara II, Bhāskara's works on differential and integral calculus, science and society in the Arthaśāstra and the Rāmāyana, and the general scientific contents in the Vedic literature S N S

TELEVISION FOR SCHOOL SCIENCE

THE economic strength of Western Europe depends increasingly on the application of science and technology and, consequently, on a growing supply of scientific and technical manpower. Unfortunately, there is a growing shortage of teachers and schools are already overburdened. School television could perhaps be of considerable assistance in overcoming such deficiencies in educational facilities. The medium has proved effective in various countries, including France, Italy, the United Kingdom and the United States of America, while its scope has been considerably extended by recent technical developments such as the use of closed-circuit systems. A year ago, the Organization for European Economic Co-operation, believing that much could be gained from the experience of countries which had been exploiting the new medium for teaching science subjects, conducted a survey on the present status of school television in Western Europe, and convened a forum of international experts in this field, "Teaching through Television", during June 1960. The report of this meeting is now available*.

The seminar was held at Ashridge (Nr Berkhamsted), with three main objectives: to give countries beginning educational television an opportunity to learn from the experience of those where educational television has been in operation for some years, to encourage the development of international contacts, on a personal basis, between programme producers and to seek ways and means to secure improved co-ordination in securing sources of information on programme material. Two different tendencies emerged during the conference: the use of school television as a complete substitute for traditional teaching (this is the case with the 'Telescuola' in Italy, where there is a severe shortage of teachers and schools) or as a complementary broadcast for use in the framework of normal school activities. School television is also used for teachers' training, as, for example, in the 'continental classroom' of the United States.

Other topics included technical aspects of programme-production problems, the use of films, of consultants, advisers and specialists, of economical visual techniques and devices. Future developments were concerned chiefly with the use of closed-circuit television. Great progress has been made in the development of inexpensive systems which can be used to link any number of classrooms (or even schools) to a central school studio from which live or recorded lessons are 'piped' to pupil audiences. During the seminar it became clear that great possibilities exist for the exchange of information on films and other material and the international exchange of production facilities and personnel.

The report also provides information about the Tele-Lille mathematics experimental project, lists of films available for educational television, examples of science programmes, and a list of scientific film associations and their addresses. A small group has been set up to carry out the recommendations (improved information, training school science producers, research, experimental projects, co-operation with industries) of the seminar. It will report progress to the Organization for European Economic Co-operation and ensure liaison with organizations concerned with science teaching, films and television.

* Organization for European Economic Co-operation. Office for Scientific and Technical Personnel. Television for School Science. Report on an O.E.E.C. Seminar, Ashridge (England), July 1960. Pp. v+181. (Paris: Organization for European Economic Co-operation, 1961.)

THE INSTITUTE FOR THE ENCOURAGEMENT OF SCIENTIFIC RESEARCH IN INDUSTRY AND AGRICULTURE, BRUSSELS

THE annual report of the Institute for the Encouragement of Scientific Research in Industry and Agriculture, Brussels, for 1960* records sixty subsidies, totalling 264,772,837 francs, during the year, including 3 million francs to the Committee for the Study and Exploitation of Electronic Computers, and estimates that 267 research workers and 529 technicians are thereby employed. The National Centre for Metallurgical Research received 29,275,000 francs for analytical research, investigations on the physical metallurgy of steel, analytical research on the physical metallurgy of zinc, research on the agglomeration of minerals, etc., while the Belgian Society for Nitrogen and Chemical Products, of Marly, received 25,898,000 for research on the pyrolysis of hydrocarbons, on the synthesis of vinyl chloride and other investigations in the field of heterogeneous catalysis.

The Committee for Mapping the Soils and Vegetation of Belgium received a grant of 20,155,000 francs for its systematic study of the pedological character of the soils and towards the pedological map of Belgium, while grants of 10,000,000 francs and 8,560,000 francs went to the Centre for the Study of High Polymers for its work on high polymers and their degradation, on photopolymerization initiated by silver salts and the stability of the polymers and for some industrial work on the extrusion of crystallizable polymers. The Centre of Applied Electronics received 12,450,000 francs for work on passive circuits, on amplifiers, modulators and oscillators of transistors and on industrial circuits, 11,505,000 francs went to the Committee for the Study of Diseases and Feeding of Farm Animals, 9,950,000 francs to the Centre for Scientific and Technical Research of the Metal Manufacturing Industry for investigations on the adaptation of numerical techniques to other machine tools and in automation, as well as 2,980,000 francs for research on resistance to fatigue of cooled alloys, etc., 8,317,590 francs to the National Centre of Metallurgical Research for low-temperature furnace research, and 7,474,273 francs to the Scientific and Technical Centre of the Belgian Textile Industry for research on wool, cotton,

* Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Rapport Annuel Exercice, 1960. Pp. 255. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1961.)

jute, linen and rayon, as well as 1,534,000 francs for investigations of the utilization of acrylic fibres.

A grant of 7,050,000 francs went to the Committee for the Study of the Solid State for continuation of work on the mechanism of photographic processes and of sensitization; 6,520,000 francs to the National Committee for the Study of Fruit Cultivation, 5,360,000 francs to the Committee for the Scientific and Technical Study of Milk and its Derivatives, 4,300,000 francs to the National Centre for Research on Herbage and Forage for botanical, phytosociological and ecological studies on herbage and on the control of adventitious plants of the prairies, 4,050,000 francs to the Centre for Scientific and Technical Research on the Conservation of Vegetables, and 3,817,000 francs to the Belgian Centre for the Study and Documentation of Water, for studies on water supply, corrosion, residual water, and on atmospheric corrosion

The Belgian Institute of High Polymers received a grant of 3,710,000 francs towards its experimental thermodynamic work at high pressures on the inhibition of the explosiveness of acetylene, the Centre for Studies and Research in Agriculture, 3,590,000 francs for research on cultivation in mineral substrates and on the mineral requirements of vegetables, the Scientific and Technical Centre of the Brewing, Malt and Related Industries, 3,316,000 francs, and the Committee for the Study of the Structure of the Soil, 3,000,000 francs Among other grants may be mentioned 2,770,000 francs for studies on diseases of cereals, 2,280,000 francs for ceramic research, 2,125,000 francs for research on the synthesis of saturated heterocyclic amines substituted in position 3 or 4; 2,065,000 francs for phytovirological research, 2,049,500 francs for research on paints, varnishes and printing inks, 2,040,000 francs for studies on the creep of metals at high temperatures, 2,000,000 francs for work on new penicillins and the new antibiotic, staphylomycin, and 1,840,000 francs for research on the culture of medicinal plants

HELICOPTER STATIONS IN LONDON

IN July 1959, the Minister of Transport and Civil Aviation formed a committee to consider the setting up of helicopter stations in the London area, assuming (1) that the helicopter is developed to the point of offering sufficiently low fares on regular services to attract large numbers of passengers for inter-city flights on international routes and possibly internal ones as well, (2) that the helicopter will not offer fares sufficiently low for mass travel, but will meet the needs of small numbers of travellers for whom the extra time saved is more important than the money spent

In its recommendations*, the Committee assumes that regular services between London and the near Continental cities with helicopters carrying twenty-five passengers or more will begin in 1965, or soon after, that fares will be offered approximating those of first-class 'fixed-wing' services, and that services to provincial cities in the United Kingdom will follow if fares can be reduced to compete with those of the other forms of transport

Allowing for executive and charter flights with passengers, freight and mail as well as regular services, the total traffic could amount to 20–25 helicopter movements in a peak hour, with a mixture of single- and multi-engined types by 1975, when the heliport would have to handle 1½ million passengers a year These estimates, the committee suggests, represent a reasonable basis for planning the area required for the first permanent heliport

In order to provide enough space for helicopters to land and take-off and for working stands (for loading, unloading, minor maintenance, etc) at the heliport, a level area of about 8 acres would be required to handle 25 movements an hour In addition, an area of about 4 acres would be needed for terminal buildings and car parks, which, if the landing area were on a raised platform, could be provided on a lower deck or at ground-level

For reasons of air traffic control, the capacity of a heliport could not be increased much beyond 25 movements an hour even by providing an additional landing area, which would involve a disproportionate amount of extra space

The heliport should satisfy the following requirements

(1) Since the appeal of the helicopter depends on saving time, the heliport should be brought as close as possible to the main source and destination of a substantial proportion of the traffic More specifically, it should not be much more than a quarter of an hour from Grosvenor Square, and ideally, between the West End and the City Because of the helicopter's potentialities for freight and mail, nearness to one of the sorting offices of the General Post Office would also be an advantage

(2) It should be conveniently placed for all forms of surface transport, good road connexions and local traffic access being especially important in view of the high proportion of passengers who will travel to the heliport in private cars and taxicabs

(3) It should have sufficient unobstructed approaches to enable operations to continue safely in all weathers, allowing for a climb and descent gradient not much steeper than 1 in 4 This may entail some restriction on building heights in the neighbourhood of the site

(4) To be usable at all by single-engined helicopters it should be beside the River Thames

(5) In order to avoid conflict between helicopter and fixed-wing traffic to and from London Airport, it should not be located farther west than Battersea For effective control of air traffic, it is unlikely that two sites on the Thames within easy reach of the West End could be simultaneously used to capacity

(6) It should be compatible with the proposals of the County of London development plan

(7) It should be so placed as to avoid interference with the normal life and work of the neighbourhood

Measurements of the noise of the various types of helicopters indicate that noise in level flight at 1,000 ft would be no greater than traffic noises in a busy street, but, since it will be more widespread, routes should be chosen which, so far as possible, reduce flights over areas particularly sensitive to noise The greatest noise will be in the immediate locality of the

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[*] Ministry of Aviation Report of the Committee on the Planning of Helicopter Stations in the London Area Pp 105 (10 plans) (London H M S O , 1961) 7s net

heliport, and within a radius of half a mile of it under the take-off and landing paths

Provided the site is carefully chosen, that is, on the Thames and in an area where the activities are mainly industrial and commercial, the quieter types of turbine-driven helicopter, as well as existing piston-engined types, should be able to use it at moderate frequencies without causing a serious problem Whether the same would apply to high-frequency or night operations remains to be seen Furthermore, tests carried out in open country and over London suggest that interference with television and very-high-frequency broadcasting is unlikely to be serious

A continued effort will be needed to clarify the effects of the noise of helicopters and means of abating it. Research into noise abatement at source is being carried out by Government establishments and manufacturers Meanwhile, proposals for heliport development should be expedited on the basis that noise can and will be brought down to reasonable levels.

After a preliminary survey, nine sites near the River within reasonable range of the West End have been examined and the following locations are considered the most promising Nine Elms, Cannon Street Station, St Katherine Docks

No attempt has been made to assess precisely the cost, since this is a matter for the promoters. The capital cost is likely to run into several millions of pounds, although it may vary considerably between one site and another A single heliport of 12 acres should be capable of handling traffic well into the 1970's, but will not be able to handle all the traffic indefinitely if the helicopter becomes accepted as a means of mass travel The question of a second heliport must be left for a decision until nearer the time when the requirement is seen to emerge

THE RED DEER IN SWEDEN

FROM information furnished by Å Sjostrom, in a bulletin from the Royal School of Forestry in Stockholm, it appears that the red deer in Sweden is now reduced to some 150 animals confined to three relatively isolated localities in the southern province of Skåne*. The paper gives the results of a study conducted over an area of about 740 acres within the Sovdeborg locality, which extends to some 4,300 acres and contains a herd of 30 deer The annual hunting licence allowed the shooting of 5 animals but might be extended to equal the annual increase, which is 8 The area studied contains some 376 acres of Norway spruce plantations and 179 acres of beech forest, to which the deer do a considerable amount of damage, said to be due to a deficiency of certain nutrient elements in their diet A careful assessment is made of the damage, which is caused mainly to trees from 15 to 45 years old, and leads to the conclusion that the cost of producing one red deer is 4,000 kr (£280) In a Danish deer-forest of some 7,200 acres with a head of 60–100 animals, Prof Grøn has estimated that the cost of production of one animal is about 6,700 kr (£465). Among the suggestions for dealing with these remnants of Sweden's original fauna are fencing of plantations—regarded as too expensive, change of tree-species—thought to be a possible solution, use of chemical repellents—regarded as of doubtful and uncertain efficiency and too expensive, heavier shooting—inapplicable in view of the already small number of animals, formation of red deer reserves and, finally, transfer to more suitable localities in natural spruce forests in west and central Sweden One such transfer has already started The interesting proposal is made that a charge of 10 kr (14*s*) per head on every roe deer, for which a shooting licence is required, should be levied. This would bring in about 300,000 kr (£20,800) a year, which would be used to purchase and maintain an area of suitable land where a new and vigorous stock of the Swedish red deer could be built up

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* Kungl Skogshögskolans Skrifter (Bulletin of the Royal School of Forestry, Stockholm) Nr 85 *Kronhjortens Skadegörelse på Granskog Esamensarbete vid Skogshogskolan i amnet Skogszoologi med Viltvård Utfört ar* 1959 Av Åke Sjöström Pp 36 (Stockholm Skogsbiblioteket, Skogshogskolan, 1961) 5 kr

O'NYONG-NYONG FEVER: AN EPIDEMIC OF VIRUS DISEASE IN EAST AFRICA

EARLY in 1959 an outbreak of unfamiliar disease appeared in human populations in the north-west corner of Uganda, and spread across the northern half of the country in a south-easterly direction The disease was usually characterized by headache, fever, adenitis and crippling joint pains, frequently followed on about the fourth day by an eruption of an itching rash, mainly on the trunk and arms These symptoms usually persisted for 5–7 days and were generally followed by quick and complete recovery

The clinical, and some of the epidemiological, aspects of the disease were investigated by Dr H Shore, the acting provincial medical officer in the Northern Province of Uganda, who reported that a dengue-like disease was affecting "vast numbers of people, old, young, men, women and children", and provisionally estimated from the rate of attack that at least 25–50 per cent of the population was at risk That all ages were affected led him to suspect a causative agent new to the region This view is supported by the fact that the local African population have coined a new name for the disease in each successive language group in which it has appeared, one of the first of these being 'o'nyong-nyong'

The epidemic advanced steadily on about a 100-mile front for ten months, crossing the Uganda–Kenya border some 300 miles south-east of the original site of the disease in Uganda, in November, 1959 Between December 1959 and February 1960, the epidemic fanned out in the Kano Plains near Kisumu, Kenya It is still active at discontinuous points east, south and west of Lake Victoria From June, the epidemic has been under detailed study by members of the East African Virus Research Institute Staff, in close collaboration with members of the Uganda, Kenya and Tanganyika Medical Departments

In a report*, prepared by J D Gillet, M. C Williams, J. P Woodall, P S. Corbet and J M Ellice, it is shown that the isolation of o'nyong-nyong virus allowed field epidemic studies and attempted transmission work to be followed virologically, and opened the way for quantitative investigations. These support the general epidemiological and vector findings that *Anopheles* species and, in particular, *A. funestus* are responsible for the transmission of o'nyong-nyong fever, but they do not exclude the possibility of transmission by *A. gambiae*. It is thought that this is the first time that a patchy alopecia has been used as an indicator in mice of arthropod-borne virus infection. An attempt has been made to measure three of the variable factors involved in order to gain an understanding of the epidemic. These are the mosquito infection-rate, the inapparent human infection-rate and the period of viræmia. Considerable work will be required before the success or failure of this attempt can be assessed.

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* East Africa High Commission East African Virus Research Institute Report, July 1959–June 1960 Pp i + 57 (Entebbe East African Virus Research Institute, 1960)

A WARNING SYSTEM FOR NUCLEAR ACCIDENTS

RADIOACTIVITY, which was once the practical interest of no more than a few scientists and medical men, is now the daily concern of a great many people in most industrialized communities. Moreover, as events at Windscale demonstrated in Great Britain, radioactivity can be released accidentally in quantities sufficient to affect the life and livelihood of considerable numbers of the general population. With the increasing use of nuclear energy for peaceful purposes, nuclear installations will become more numerous, and, in the event of a nuclear accident, radioactive material might be transmitted over wide areas and across national boundaries.

The decision of the Council of the Organization for European Economic Co-operation* to establish "a supervision and emergency warning system in cases of increase in environmental radioactivity" is therefore an important and encouraging step in international collaboration in the field of nuclear energy. The main purpose of the supervision and emergency warning system is to facilitate and improve health protection measures in the event of a substantial increase of radioactivity in any member country of the Organization for European Economic Co-operation by immediately notifying it to the responsible authorities in the other member countries. The transmission of information is to be governed by three criteria: (1) health protection in the event of a nuclear incident; (2) health aspects of an increase in environmental radioactivity of undefined origin; and (3) scientific interest of any case of abnormal increase in environmental radioactivity. As monitoring stations for measuring airborne radioactive material are already in existence in all member countries of the Organization the proposed system is to be based at first on air monitoring, although the importance of water and dietary contamination is recognized, and extension of the system to these fields of monitoring is envisaged at a later stage.

The three levels of artificial total β-radioactivity in air, given as a basis for determining the categories of increase in radioactivity, are: (1) an 'emergency' level of the order of 1,000 $\mu\mu$c./m.^{3}; (2) a 'pre-emergency' level of the order of 100 $\mu\mu$c./m.^{3}; and (3) a level of 'scientific interest', set at ten times higher than the previous monthly average and exceeding the value of 5 $\mu\mu$c./m.^{3}. The levels are averages over one day, the 'emergency' level being determined in relation to possible nuclear incidents and regarded as valid for emergencies of short duration. Very approximately in terms of the Windscale reactor accident, the 'emergency' level corresponds to that observed within 50 miles (downwind) of Windscale itself, the 'pre-emergency' level to radioactivities observed at that time in Devon, and the level of 'scientific interest' to observations made at some places on continental Europe. It should be noted that the warning-levels are those of artificial β-radioactivity, and that average levels of natural β-activity in the air in Great Britain are typically of the order of 100 $\mu\mu$c./m.^{3}.

An administrative organization is laid down, and reports are to be made to the European Nuclear Energy Agency as well as to the "designated authorities" in the member countries. After an initial period of two years operation, the Steering Committee for Nuclear Energy is to make a report to the Council of the Organization for European Economic Co-operation on the way the system has worked.

Perhaps the most important aspect of the scheme is that it represents the beginning of collective action on a problem which transcends national boundaries, and that, as the Steering Committee itself suggests, "when the supervision and warning system is set up, the possibilities of more extensive international collaboration should be considered". There would appear to be no insuperable difficulties to complete international collaboration on this aspect of the peaceful uses of nuclear energy.

F. W. SPIERS

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* Report of the Steering Committee for Nuclear Energy and Council Decision on the Establishment of a Supervision and Emergency Warning System in Cases of Increase in Environmental Radioactivity. Organization for European Economic Co-operation. Paris. July 7, 1961.

MOLECULAR DISARRAY IN A CRYSTAL LATTICE PRODUCED BY A FISSION FRAGMENT

By DR. F. P. BOWDEN, C.B.E., F.R.S., and L. T. CHADDERTON

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IT should be possible, if we choose an appropriate crystal, to observe directly in the electron microscope the lattice disarray caused by the passage of a fission fragment, and an attempt has been made to do this. The first observations of crystal lattices and associated defects were made by Menter[1] with phthalocyanine and its metal derivatives. These are polycyclic hydrocarbons with large flat molecules and the shape of the molecule influences the mode of crystallization. Platinum phthalocyanine, for example, crystallizes in the monoclinic form[2] as long flat needles and with a large unit cell ($a_0 = 23{\cdot}9$ Å., $b_0 = 3{\cdot}81$ Å., $c_0 = 16{\cdot}9$ Å., $\beta = 129{\cdot}6$ Å.). It is this property, in addition to the fact that the platinum atom, a heavy scatterer of electron waves, lies in the centre of the molecule, which facilitates the direct observation of (20$\bar{1}$) lattice planes, separated by 11·94 Å., in the electron microscope. In a carefully aligned electron microscope with a nominal resolving power of 'better than 10 Å.', transmission electron micrographs of thin unstrained crystals of platinum phthalocyanine possess parallel fringes the separation[3,4] and direction[5] of which correspond to (20$\bar{1}$) lattice planes in the crystal structure. The experimental detail so far is in full accord with the only extensive theoretical analyses published[6,7].

Platinum phthalocyanine was prepared from platinous chloride and phthalonitrile[8] and purified by repeated vacuum sublimation. Crystalline specimens suitable for the electron microscope examination were obtained in two ways. The first method involved simple mechanical grinding of the pure crystals, a drop of a suspension in ethyl alcohol being allowed to evaporate from a perforated carbon film on a copper electron microscope specimen grid. The second method, by far the more successful, consisted of condensing the crystals directly on to the support films from the vapour phase[9]. Crystals varying in thickness from some 40 Å. upwards could thus be prepared at will. Specimen grids were placed in contact with thin foils of natural uranium and irradiated with thermal neutrons in the experimental reactor *Bepo* at the Atomic Energy Research Establishment, Harwell, for periods of 5 min.–20 hr. at a pile factor of 12. After a few weeks the specimens irradiated with fission fragments and thermal neutrons were safe enough to handle and were examined in transmission at high magnification in a Siemens Elmiskop I electron microscope. Crystals which were not placed in contact with natural uranium foil, and which consequently suffered neutron irradiation only, were also examined.

In electron micrographs taken in transmission at low beam intensities with exposure times of up to 30 sec., the platinum phthalocyanine crystals show tracks and spots about 100 Å. in diameter. These can be attributed unambiguously to individual fission fragments, since they do not appear in crystals irradiated with thermal neutrons only and since their number correlates with that calculated from dose considerations. The tracks, which are sometimes dashed and which sometimes have an alternating light and dark contrast, would appear to result from fission fragments which are travelling at an oblique angle, or almost parallel, to the plane of the crystal. The spots, however, have a light contrast on a dark background and these may be attributed to fission fragments travelling at a high angle or perpendicular to the plane of the crystal. We shall consider here the damage due to particles such as these which pass normally through crystals about 100 Å. thick in a direction roughly parallel with the (20$\bar{1}$) planes.

Figs. 1 and 2 show what can happen to the (20$\bar{1}$) fringes when the damage is in the main body of the crystal. In Fig. 1, for example, the regular lattice is disordered and there is a roughly circular diffuse region which extends over about 80 Å. Close to the edge of the diffuse region the (20$\bar{1}$) fringes curve inwards towards the disordered region : this is shown in the adjoining diagram. In Fig. 2, however, the

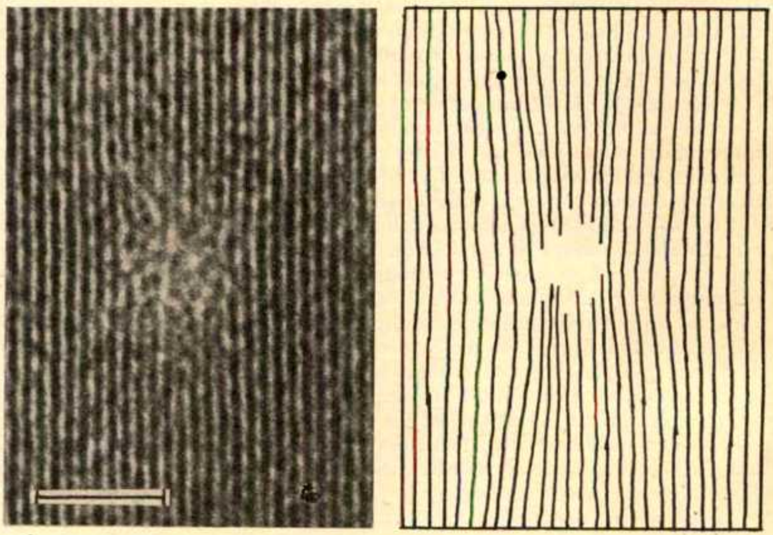

Fig. 1. Electron micrograph of a platinum phthalocyanine crystal showing the molecular disarray when a fission fragment travels perpendicular to the projection of the (20$\bar{1}$) planes and within the main body of the crystal. The drawing illustrates the situation more clearly. Scale, 100 Å.

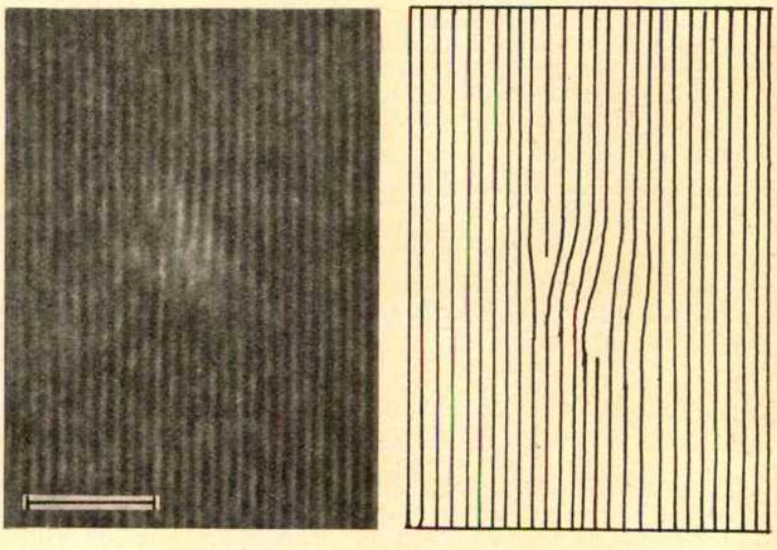

Fig. 2. Electron micrograph of a platinum phthalocyanine crystal showing damage sustained when a fission fragment travels perpendicular to the projection of the (20$\bar{1}$) planes and within the main body of the crystal. A dislocation dipole results. Scale, 100 Å.

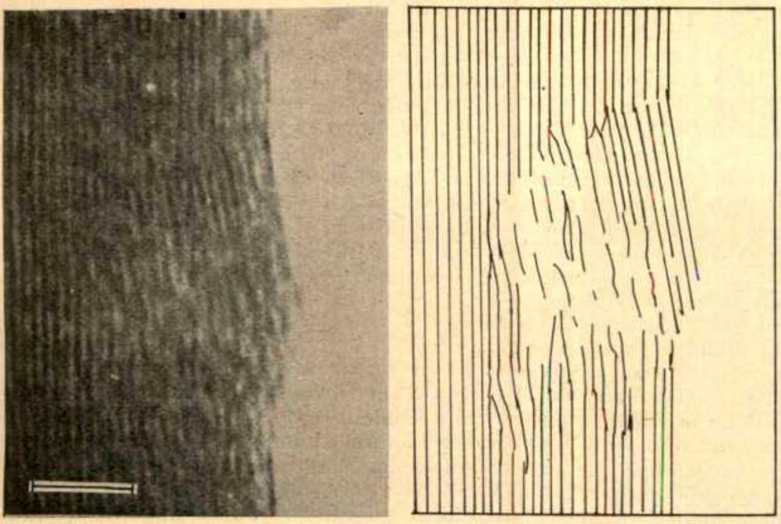

Fig. 3. Electron micrograph of a platinum phthalocyanine crystal showing damage sustained when a fission fragment strikes a crystal edge. Scale, 100 Å.

picture is quite different. Instead of a diffuse region the fringes are resolved clearly over the damaged area. Although those bordering this area are again curved, the reason for this is apparent ; it will be seen that there are two fringes which terminate abruptly within the area of damage.

When the fission fragment penetrates the crystal close to one of its parallel edges, the damage is very much greater in extent. This is illustrated in Fig. 3.

If we assume that fringe patterns obtained with platinum phthalocyanine crystals in the electron microscope represent in some measure the behaviour of the $(20\bar{1})$ molecular planes (and for thin crystals the approximation is a good one[6]), then certain conclusions can be drawn from the above observations. In the first instance, a measure of the extent of the damage due to a fast-moving fission fragment is obtained. In the body of the bombarded crystal the diameter of the damaged region caused by one nuclear fragment varies between 80 and 120 Å. (that is, between 7 and 10 molecular planes). In Fig. 3, however, at a crystal edge, the appropriate figure is nearer 250 Å. The perfection of the $(20\bar{1})$ lattice planes is destroyed over this region and close to it there is evidence for a curvature of the molecular planes. An interesting conclusion may be derived from Fig. 2. It has been emphasized that a terminating half-fringe is not to be interpreted as a terminating half-plane, that is, an edge dislocation of Burgers vector $(20\bar{1})$[10]. On the contrary, we can only infer that a dislocation of quite general character, with both screw and edge components, passes from the top to the bottom surface of the crystal. In Fig. 2, however, a Burgers circuit traced around the damaged region closes completely, and indeed we might have expected this since we started with a perfect lattice. It would seem that the effect of the fission fragment on the lattice has been the introduction of two dislocations of quite general character but of opposite polarity. The concept of the dislocation dipole is not entirely new—Bollman[11] has recently shown, using moiré fringe techniques, that dislocation dipoles may be present in neutron-irradiated graphite.

The energy of a fission fragment at birth is about 100 MeV. and the atomic weight may be anywhere between 72 and 161. Thus we are dealing with very fast moving particles ($\sim 10^9$ cm./sec.) and so the fragments have high charges ($\sim 18e$). It can be deduced, using a value of 1·70 eV. (Chadderton, L. T., unpublished work) for the low-energy limit of the main optical absorption band, that loss of energy by

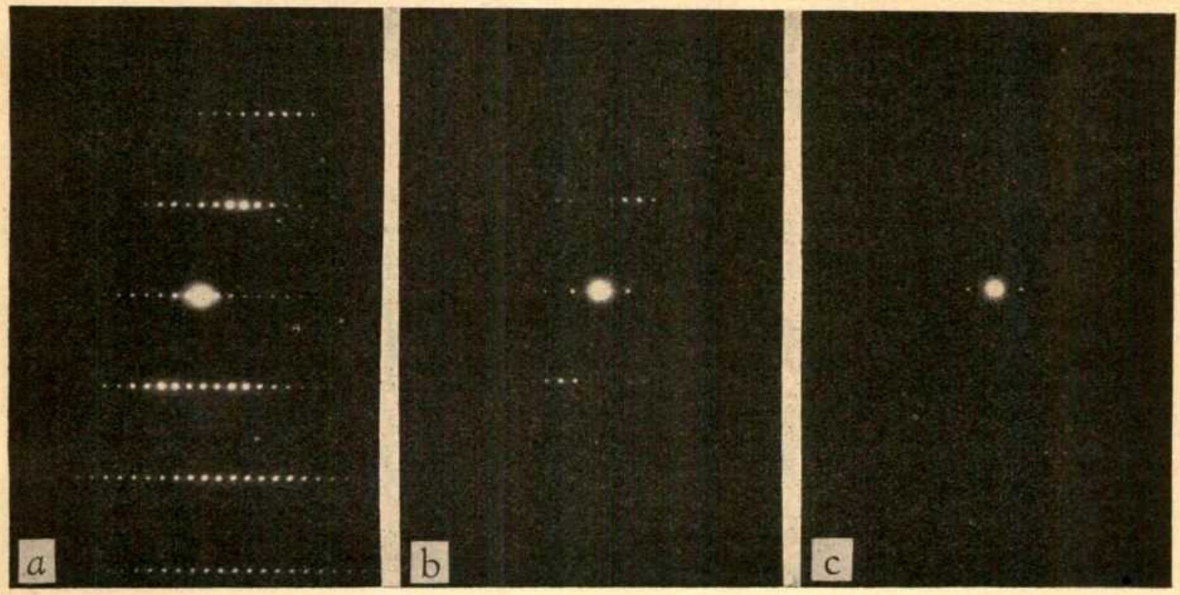

Fig. 4. Changes in the electron diffraction pattern from a thin single crystal of platinum phthalocyanine after irradiation with electrons at high magnification and low beam intensity : (*a*) after 40 sec.; (*b*) after 80 sec. ; (*c*) after 120 sec. Apparently the platinum atoms remain roughly in their original positions

ionization[13] exceeds that due to elastic collisions by a large factor. The production of primary knock-ons by Rutherford collisions accounts for only about one part in a thousand of the total loss of energy. In such circumstances we may consider the resulting excitation to be a purely thermal temperature spike; the damage arises from the rapid delivery of heat to the lattice by the moving particle. Temperatures exceeding 1,000° C. endure for times in the neighbourhood of 10^{-11} sec., exact values depending on the thermal properties of the lattice. Since platinum phthalocyanine sublimes in air at temperatures above 500° C., it becomes evident that 'evaporation' will occur along the length of the cylindrical track of the moving fragment and that recrystallization, if it takes place, is unlikely to be in perfect register with the undamaged lattice. Bombardment of platinum phthalocyanine with heavy nuclear particles of high velocity and charge may therefore introduce dislocation arrangements into an initially perfect crystal. Other authors have examined the damage produced by fission fragments in mica[13-14], uranium oxide[15,16], and graphite[17], but without the assistance of direct lattice observations. Bowden and Montagu-Pollock have described the damage produced by a fission fragment in an explosive crystal[18].

Effect of Electron Bombardment

One important factor has been so far omitted, namely, the effect of the electron waves on the crystal while it is being examined and photographed in the electron microscope. The electrons themselves constitute a radiation capable of producing considerable damage to the lattice since they are accelerated to relativistic velocities by a high voltage of 80 kV. Their effect has been investigated with the assistance of the electron micro-diffraction device, whereby changes in crystal structure can be examined as irradiation with electrons proceeds. Fig. 4 shows what happens to the diffraction pattern from a single crystal of platinum phthalocyanine while it is being irradiated with electrons at a low beam intensity. A fringe pattern is obtainable from the crystal throughout the irradiation, but its nature and origin change. In the first instance the pattern is derived from the $(20\bar{1})$ molecular planes, but as the cumulative electron dose on the specimen builds up the crystal structure changes. All order in the lattice (except that which gives rise to the $(20\bar{1})$ and $(\bar{2}01)$ spots in the diffraction pattern) disappears and the resulting fringe pattern observed in bright-field electron micrographs becomes much smoother. It would appear that although the large organo-metallic molecules are being destroyed, the platinum atoms remain roughly in their original positions in the $(20\bar{1})$ planes, embedded in a matrix of carbonaceous matter. The information derived from fission fragment bombarded crystals is, however, not affected by these considerations, since the electron micrographs were taken before decomposition by electron irradiation had proceeded to any marked extent.

Conclusion

We see that it is possible to make a direct observation of the molecular disarray produced in a crystal lattice when a single fission fragment passes through it. The extent of the damage can be measured to within one molecular plane and in platinum phthalocyanine varies between seven and twenty $(20\bar{1})$ planes, being dependent on where, and at what angle, the fragment strikes the crystal. In some cases dislocation dipoles are formed. The method appears to be a perceptive one for studying the detailed mechanisms of radiation damage.

One of us (L.T.C.) is indebted to the Department of Scientific and Industrial Research for the award of a research studentship.

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ELECTRON-SPIN RESONANCE SIGNALS FROM LYOPHILIZED BACTERIAL CELLS EXPOSED TO OXYGEN

By DR. M. B. LION*, DR. J. S. KIRBY-SMITH and DR. M. L. RANDOLPH

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ESCHERICHIA COLI cells, lyophilized from distilled water, lose their viability very rapidly when exposed to oxygen, but not when kept in vacuum[1]. Certain aspects of these observations suggested that free radicals might be involved in this phenomenon. An investigation of electron-spin resonance was therefore undertaken. The details of the spectrometer have been described[2,3]. An associated analogue computer enabled the determination of the number of magnetic centres in the sample as compared with the diphenylpicrylhydrazyl (DPPH) standard[4].

Cells of *E. coli* strain *B/r* were grown on nutrient agar and, after collection, were washed and resuspended in distilled water. About 2 × 10^{11} cells were lyophilized in each ampoule. An electron-spin resonance sample tube (diameter 5 mm.) was sealed to each ampoule as a side arm. After completion of the lyophilization the samples were either sealed off under vacuum or filled with dried oxygen and then sealed The dry bacterial powder was pulverized by some large-diameter glass beads which had been introduced into the ampoules before lyophilization The powder was then poured into the side arm, which was introduced into the cavity of the spectrometer Fig 1 shows the spectra of two samples of dried cells One had been exposed to oxygen for 2 days while the other remained in vacuum The signal produced in presence of oxygen was apparent 30 min after the beginning of exposure, when the earliest measurement was made, and reached its final height after some hours After reaching a peak no change either in height or shape of the signal occurred for at least 4 months Contrary to experiments performed on lyophilized tissue by Miyagawa *et al*[5], the signal remained stable even after removal of oxygen by re-evacuation, as shown in Fig 2 While heating the lyophilized cells to 100° C for 75 min after exposure to oxygen did not affect the signal obtained (Fig 3, II), the cells heated at 100° C for 5 min before freeze-drying produced a much smaller signal after exposure to oxygen (Fig 3, III)

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* On leave of absence from the Israel Institute for Biological Research, Nes-Ziona, Israel, which is Dr. Lion's present address.

This behaviour of free-radical signals present after lyophilization with respect to heat and exposure to oxygen differed from radiation-induced resonances Dried bacteria irradiated with 750 kr of X-rays showed a broad, weak, and double-peaked signal which became higher and sharper after heat treatment and rapidly decayed on exposure to oxygen (Fig 4) This change in pattern after heating suggests a heat-induced transition from one radical species to another, and a fundamental difference between radiation-induced radicals and those occurring after lyophilization alone

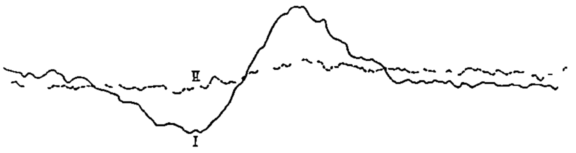

Fig 1 First derivative of electron-spin resonance absorption curve of *E coli* cells lyophilized from distilled water I, 18 mgm exposed to oxygen for 2 days II, 21 mgm kept in vacuum

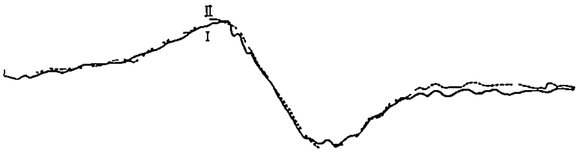

Fig 2 First derivative of electron-spin resonance absorption curve of *E coli* cells lyophilized in distilled water and exposed to oxygen I, before removal of oxygen by re-evacuation, II, after re-evacuation

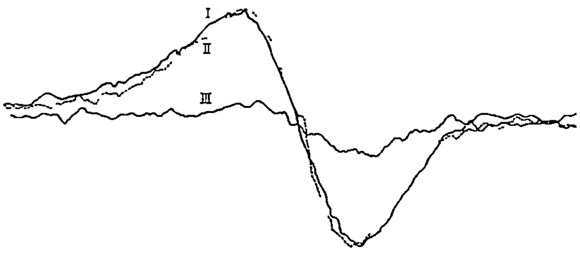

Fig 3 First derivative of absorption curve of *E coli* cells lyophilized from distilled water and exposed to oxygen I, normal cells (60 mgm) II, cells heated to 100° C for 75 min after exposure to oxygen (60 mgm), III, cells heated to 100° C for 5 min before lyophilization (70 mgm)

Dried *E coli* cells may be protected against the lethal effect of oxygen by different substances, as, for example, by thiourea or even more effectively by sodium iodide[6], but cysteine (as hydrochloride partially neutralized to pH 6 0 with sodium hydroxide) is completely ineffective in this respect[6] In a typical experiment the viability of dried cells after exposure to oxygen for 4 hr at 28° C was found to be control, 0 05 per cent, in presence of cysteine, 0 001 per cent, in presence of thiourea, 42 per cent; in presence of sodium iodide, 80 per cent[6]

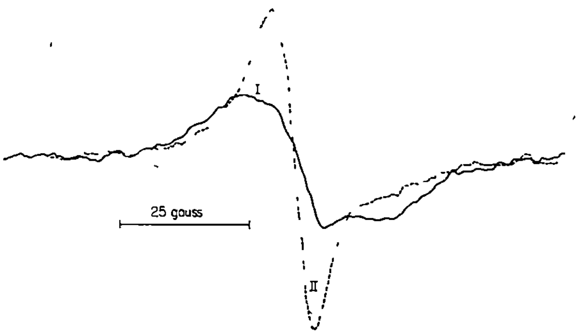

Fig 4 First derivative of absorption curve of *E coli* cells lyophilized and irradiated in vacuum by 750 kr X-rays I, before heating, II, after heating to 100° C for 20 min Spectrometer sensitivity about one-sixth that for other figures All rates of scanning were equal

Measurements of electron-spin resonance were therefore made on dried cells exposed for 2 days to oxygen in the presence of these substances The ampoules were then opened in relative humidity less than 35 per cent, and the fluffy material was rapidly compressed with a glass rod into sample tubes used in measuring electron-spin resonance, thus enabling greater amounts of material to be introduced into the sensitive volume of the cavity The sample tubes were sealed immediately Fig 5 shows the signals produced when dried cells are exposed to oxygen in the presence of cysteine, thiourea, sodium iodide, and no additive Each sample contained 50 mgm of bacterial cells, the additives comprising a further 16–18 mgm A qualitative relationship seems to exist between the ability of these additives to suppress the signal from electron-spin resonance of oxygen-exposed, dried bacterial cells, and their ability to protect these cells against the lethal effect of oxygen

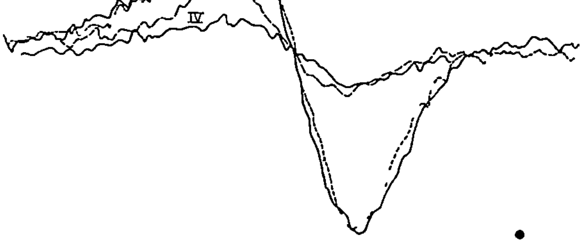

Fig 5 First derivative of absorption curve of *E coli* cells lyophilized and exposed to oxygen for 2 days in presence of I, cysteine, II, no additive (control), III, thiourea, IV, sodium iodide

The approximate number of magnetic centres produced by oxygen in dried bacteria is about 2×10^{16} spins/gm This figure is probably correct only within a factor of two owing to the high gain needed in the detection system of the apparatus with consequent drift and noise when the signals of these materials were determined Since the number of bacterial cells in 1 gm of dry material is roughly 4×10^{12}, the number of free radicals produced for each dry cell reacting with oxygen is about 5,000 Possibly the oxygen reaction is quite specific and involves only few molecular species the normal state of which might be vital to the survival of the cell It also seems that the 'oxygen acceptor' in this reaction is quite heat-sensitive while the reaction product is not

The experiments of Miyagawa *et al*[5] were done on ground, non-viable animal tissues and are therefore not directly comparable with our results They show that molecular species of biological origin which in the dry state are able to react quickly with oxygen are not restricted to bacteria Our results differ in that the reaction between oxygen and the sensitive site of bacteria seems to be irreversible

This last observation would exclude a normal metabolic role for the reaction, for otherwise one might expect a resonance signal in dried bacteria (which are intact and viable cells) kept in vacuum. One might rather imagine that the site sensitive to oxygen is protected in a wet-cell system by the action of normal metabolic machinery in removing excess oxygen. Once this normal machinery is stopped in the dry cell, the site becomes easily accessible to oxygen Unless other protecting materials are present, there follow consequent inactivation and loss of the capability of the cell to recover when it is re-introduced into its normal environment This latter property of the bacterial cell makes it an interesting model for investigating biological phenomena by means of electron-spin resonance

The Oak Ridge National Laboratory is operated by the Union Carbide Corporation for the U S Atomic Energy Commission

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ANGULAR MOMENTUM OF CONTINUA

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When applied to the continuum model of matter, the principle of conservation of linear momentum becomes Cauchy's law of motion, which in spatial co-ordinates is the familiar equation of change of linear momentum In Cartesian tensor notation, with D/Dt the substantial time derivative and other symbols having their usual meanings

$$\rho \frac{\mathrm{D} V_j}{\mathrm{D}t} = \rho F_j + T_{ij,i} \qquad (1)$$

The stress-tensor can be resolved into symmetrical and anti-symmetrical parts $T^{(s)}_{ij} + T^{(a)}_{ij}$, the second is given by (1/2) $e_{ijk}\ A_k$, where the pseudo-vector $A_k \equiv e_{kmn}\ T_{mn}$ With this resolution, the first moment of equation (1) about an arbitrarily chosen origin is found to be

$$\rho \frac{\mathrm{D}}{\mathrm{D}_t}(e_{kij}\, R_i\ V_j) = \rho\, e_{kij}\, R_i\ F_j + (e_{kij}\, R_i\ T_{lj})_{,l} - A_k \qquad (2)$$

This equation of change of moment of momentum, or external angular momentum, $e_{kij}\, R_i\ V_j$ (per unit mass), shows that quantity to be conserved in the usual sense only when the stress-tensor happens to be symmetrical, for otherwise A_k does not vanish, and the last term represents local consumption (or production) of moment of momentum throughout the system What is the fate of this external angular momentum ?

A system may exchange with its surroundings not only linear but also angular momentum The two classes of interaction need not be wholly equivalent in the case of real materials, although they are so regarded in most traditional continuum models But just as the distant material surroundings may exert locally a vector body force F_j, they may also exert locally on the system a pseudovector body-couple G_j per unit mass and just as the contiguous material surroundings may exert a vector surface traction $T_j = n_i\ T_{ij}$, they may also exert on the surface of the system a pseudovector couple $C_j = n_i\ C_{ij}$ per unit area, where C_{ij} is the couple stress-tensor If the pseudovector L_k denotes the local density of angular momentum per unit mass, the principle of conservation of angular momentum for the constant mass system associated with an arbitrary, convected volume is

$$\frac{\mathrm{d}}{\mathrm{d}t}\int_V \rho L_k \mathrm{d}V = \int_V \rho(e_{kij}R_i F_j + G_k)\mathrm{d}V + \int_S n_l(e_{kij}R_i T_{lj} + C_{lk})\mathrm{d}S \qquad (3)$$

In the usual way this leads directly to the equation of change of total angular momentum

$$\rho \frac{\mathrm{D}L_k}{\mathrm{D}t} = \rho\, e_{kij}\, R_i\ F_j + \rho\, G_k + (e_{kij}\, R_i\ T_{lj})_{,l} + C_{lk,l} \qquad (4)$$

Even more important than this equation is the following, obtained by subtracting equation (2) from equation (4) and denoting as M_k the difference $L_k - e_{kij}\, R_i\ V_j$ between total angular momentum and moment of momentum

$$\rho \frac{\mathrm{D}M_k}{\mathrm{D}t} = \rho\, G_k + C_{lk,l} + A_k \qquad (5)$$

This equation of change of internal angular momentum makes clear the fate of the external angular momenturn it is transmuted to the internal form by the alchemy of an unsymmetrical state of stress After transformation it may be accumulated within the system or removed to the surroundings Just as the body-force is the rate of arrival of linear momentum from distant material surroundings, the body-couple G_k is the rate of arrival of internal angular momentum from the same source, and in a similar analogy the net surface couple $C_{ik,j}$ is the rate of arrival, by diffusion, of internal angular momentum from contiguous material surroundings

So far as we know, equation (5) was first given in complete form in 1959 by Dahler[1], who derived it as a general hydrodynamical equation in the context of a statistical mechanical theory of transport processes in fluids composed of polyatomic molecules In his developments for classical and quantum cases, patterned after the methods of Irving and Kirkwood and of Irving and Zwanzig, a key step (as in the continuum development here) consists of distinguishing interactions of a system with distant from those with contiguous surroundings Somewhat earlier Grad[2] had argued in favour of a version lacking only the body-couple term Much earlier the Cosserats[3] had given another version lacking the accumulation term, long neglected, theirs has been revived in the modern theory of rheology (cf refs 4 and 5) and re-discovered in the theory of elasticity[6] Other incomplete versions have appeared in various contexts, for example, the theories of liquid crystals and of what are called anisotropic fluids[7,8]

The derivation sketched here confirms that equation (5) is a basic law of continuum mechanics fully as general as Cauchy's What are some of its consequences ? In the first place, equations (2) and (5) indicate that moment of momentum and internal angular momentum are separately conserved only when the local state of stress is symmetrical Therefore the usual proof of symmetry fails when there are couple stresses and accumulation of internal angular momentum, as Grad[2] has observed, as well as when there are body couples to be accounted for This implies that whenever the adoption of a continuum model requires averaging over the centres of mass of sub-continuum units having rotational degrees of freedom, the stress-tensor is in general asymmetrical, its asymmetry stemming from the interaction of the centres-of-mass system with an interpenetrating twist or spin system[1] An example is provided by Toupin's theory of the elastic dielectric[4], a farther-reaching one is provided by the new Laval–LeCorre–Raman theory of elasticity[6,9,10] Indeed, equation (5) is a means of joining that theory to extensive researches on nuclear and molecular spin systems in the solid state It suggests, incidentally, a need for directing attention to the angular momentum aspect of magnetism in quantitative descriptions of macroscopic behaviour of not only solids but also fluids and less-well-defined forms of matter As yet, in the physics of fluids there are few examples of antisymmetrical states of stress predicted for polyatomic fluids in general by the statistical mechanical theory, they seem to have been first considered in detail only recently by Ericksen in a theory of certain non-Newtonian fluids[7,8] The complete equation of internal angular momentum, which is basic to any full consideration of such states of stress, merits further attention in rheology, particularly as an aid to understanding non-linear behaviour There might be interesting consequences for the hydrodynamic theory of helium II The possible existence of asymmetrical stress seems to have been overlooked, and perhaps the peculiar system of spin-excitations in that liquid might be more conveniently described in terms of intrinsic angular momentum density and equation (5) than in terms of vorticity singularities The equation does suggest the existence of unexplored effects of interchange between moment of momentum and internal angular momentum in macroscopic systems rotated at high angular velocities

Another consequence of the angular momentum equation is the need it shows for discovering the constitutive relations giving the dependences of antisymmetrical stress, couple stress, and body-couples on a set of independent variables that completely describes the dynamical state This need compels one to recognize that continuum models of real matter must be endowed with point structure, a notion that goes back to Duhem and the Cosserats[3–5] This structure must then be characterized quantitatively Further work on energy and entropy accounting, on constitutive relations, on certain applications to polar fluids, and on consequents for turbulent flow is in progress

We are grateful to Prof W. H Schwartz, of Stanford University, for bringing the most recent papers by Ericksen to our attention

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APPLICATION OF PALÆOMAGNETISM TO GEOLOGICAL CORRELATION

By A LAROCHELLE

Geological Survey of Canada

THE Monteregian Hills are formed from the erosional remnants of eight igneous plugs which intrude the Palæozoic sedimentary beds of south-eastern Quebec (Fig 1). Work on these intrusive rocks[1] by a number of geologists has shown that they were derived from a common magmatic source of basic composition

Suggestions have been made[2] to include in the Monteregian Hills series a number of alkaline intrusives which are located outside the immediate area

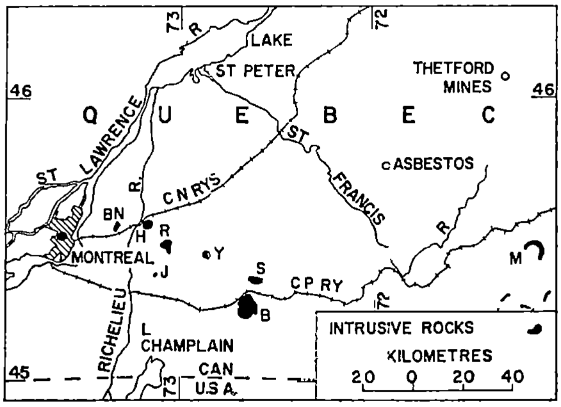

Fig. 1. Areal distribution of Monteregian hills (*BN*, *H*, *R*, *J*, *Y*, *S*, *B*) and Mount Megantic (*M*)

of the Hills. One of these bodies forms the collar of basic rocks on the outer rim of Mount Megantic, some seventy-five miles east of the main group. This body (labelled *M* in Fig. 1) was recently shown (Reid, A. M., unpublished M.Sc. thesis, University of Western Ontario, 1961) to bear petrological affinities with the Monteregian Hills series. The contemporaneity of the Mount Megantic rocks and the Monteregian Hills is suggested in this article on the basis of palæomagnetic data.

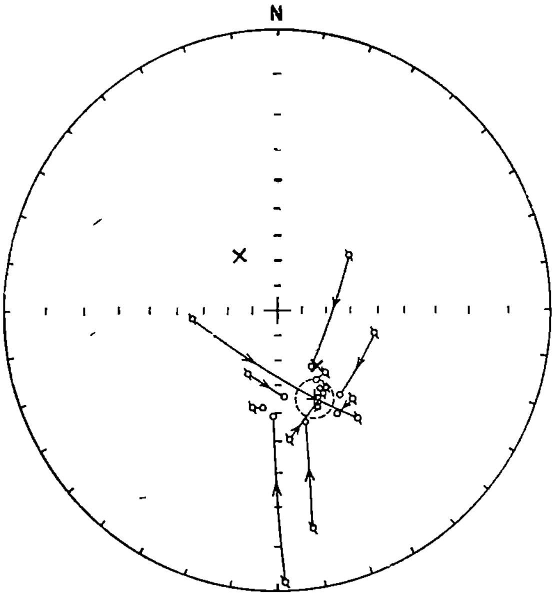

Fig. 2. Directions of magnetization of Mount Megantic rocks. ɸ, original readings; ×, mean direction for other Monteregian hills; ○, after magnetic cleaning; ⊕, circle of confidence and mean direction for Mount Megantic rocks

A suite of twelve oriented specimens was collected from different sections of the basic part of Mount Megantic. For the sampling, a solar compass was used to eliminate the possible error that could be introduced by the interaction of the magnetic needle of an ordinary compass with the relatively strongly magnetic rocks dealt with. Two 1-in. cubes were cut from each specimen and their magnetization directions were measured. The mean direction of magnetization of the two cubes was taken as representative of the specimen. For the purpose of eliminating unstable components of magnetization in the rocks, the latter were then submitted to the action of a decreasing amplitude, alternating field in a magnetic-field-free space. The maximum initial field amplitude used was 250 œrsteds. This treatment improved the clustering of the magnetization directions a considerable amount, as illustrated in Fig. 2.

The mean direction of magnetization for the group is represented by a cross centring the dotted line circle of confidence, the radius of which was computed using Fisher's method.

The fact that the parameter $\hat{K}$ of the group is relatively high, coupled with the fact that the mean direction of magnetization is almost diametrically opposed to the direction of the present field of Earth at the collecting site, suggest strongly that the mean direction given in Fig. 1 is that of a stable component of magnetization in the rocks and that it reflects the attitude of the ambient magnetic field of Earth at the time the rocks cooled from magmatic temperatures.

In a previous study[3], I found that the mean directions of magnetization of the Monteregian Hills lie along an axis represented by two *X*'s in Fig. 2. The attitude of this axis was computed from the mean directions of magnetization of two groups of rocks which were respectively polarized downward in the north-west quadrant and upward in the south-east quadrant. Although the magnetization direction of the Mount Megantic rocks is almost parallel to this axis, it is more accurate, for the sake of geological correlation, to compare the pole positions, which may be inferred from the Mount Megantic and the Monteregian Hills palæomagnetic directions respectively. The reason for this is the remoteness of the two groups of collecting sites. These pole positions are represented stereographically in Fig. 3, and they are labelled '*m*' and '*M*' respectively. Their proximity and the overlapping of their respective ellipse of confidence strongly suggest that the Mount Megantic

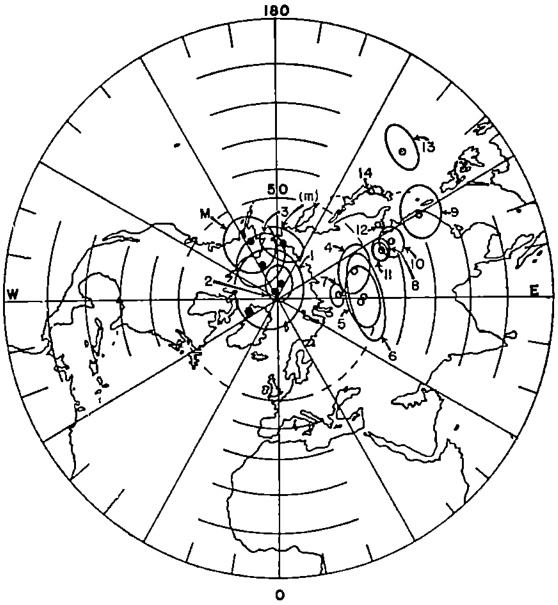

Fig. 3. Palæomagnetic pole positions inferred from North American rocks. *M*, Monteregian Hills, Mount Megantic excluded; *m*, Mount Megantic; 1, Tertiary rocks; 2, Pliocene and Miocene rocks; 3, Cretaceous rocks; 4, 5, 6, 7, Triassic rocks; 8, 9, 10, Permian rocks; 11, 12, Carboniferous rocks; 13, Silurian rocks

Table 1 DIRECTIONS OF MAGNETIZATION OF BASIC INTRUSIVE ROCKS

N, Number of samples, θ, radii of circles of confidence, $\hat{K}$, dispersion parameter, D, declination, I, inclination

	N	θ	$\hat{K}$	D	I
Mount Megantic	12	6 5	44	157	−52
Other Monteregian Hills	10	18°	7 9	340	+57
Other Monteregian Hills	39	9°	7 5	142	−62

Table 2 PALÆO POLE POSITIONS

	Latitude	Longitude
Monteregian Hills	65° N	157° W
Mount Megantic	68 5 N	171 5 E
Dakota sandstones*	74 N	168 W

* Pole position as computed by me from directions of magnetization and collecting sites given by Runcorn (1956)

Table 3

	Mineral in parent rock	Age in 10^6 yr	Laboratory
Mount Royal	Pyroxene in Tinguaite	100	M I T
Mount Johnson	Biotite in Essexite	110	M I T
Mount Brome	Biotite in Nordmarkite	122	G S C
Mount Megantic	Biotite in Gabbro	115	G S C

Potassium/argon age determinations by Geological Survey of Canada (G S C) and the Massachusetts Institute of Technology (M I T)

basic rocks and the Monteregian Hills were intruded at the same time

Pole positions derived from other North American rocks of known ages[4] are also plotted in Fig 3 It appears from these data that a marked shift of the pole occurred between Cretaceous and Triassic times It would seem, therefore, that pre- and post-Triassic rocks could be distinguished on the basis of their palæomagnetic directions If this line of reasoning were legitimate, the '*M*' and '*m*' poles belong to the post-Triassic group and their proximity to the Cretaceous pole would suggest further a Cretaceous age for both the Mount Megantic and the Monteregian Hills rocks This conjecture was recently supported by potassium/argon age determination data (Table 3) released by two independent laboratories on samples collected from three of the Monteregian Hills and from Mount Megantic Thus, palæomagnetic and radiogenic data support independently the synchronous intrusions of these two rock units and place them in Cretaceous times

I thank the Director of the Geological Survey of Canada for permission to publish this work

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DIFFERENTIAL SECONDARY MAGNETIZATION IN SOME BRITISH CAMBRIAN ROCKS

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TWELVE orientated rock specimens were collected from three horizons in the Hartshill Quartzites and the Hyolithus Limestones from two localities near Nuneaton Geologically[1] the quartzites include true quartzites and ferruginous sandstones coloured red or pink, while the limestones consist of organic material in a fine-grained matrix of calcite, iron minerals and other material, the whole strongly red stained The rocks dip to the south-west at about 40 deg

Magnetic results were obtained for eleven rocks and are plotted on the equal area stereonet (Fig 1) in their field orientations, the twelfth was too weak to give significant results with the existing magnetometer Combining the vectors for the four specimens from the Hyolithus Limestones by taking the mean of the direction cosines and making a statistical estimate of the dispersion after Fisher[2] gives

$$D\ 017° \quad I\ 23° \quad \alpha\ 16° \quad \varkappa\ 33$$

The following abbreviations are used D, declination measured from true north, I, inclination to the horizontal, positive downwards, α, semi-angle of the cone of confidence drawn at the 95 per cent probability-level, $\varkappa$, estimate of precision In contrast, the mean direction obtained from the seven specimens from the quartzites is

$$D\ 132° \quad I\ 64° \quad \alpha\ 25° \quad \varkappa\ 6$$

Correcting for the dip of the rocks moves both the mean directions towards the south-west by about 20 deg , so that when these directions are compared with those found in Cambrian rocks from Wales[3] the quartzites give a similar direction, but the mean vector for the limestones is about 90 deg removed

All the rocks were demagnetized in a c fields of up to 100 œrsteds using apparatus similar to that described by Creer[4] The new vectors obtained on re-

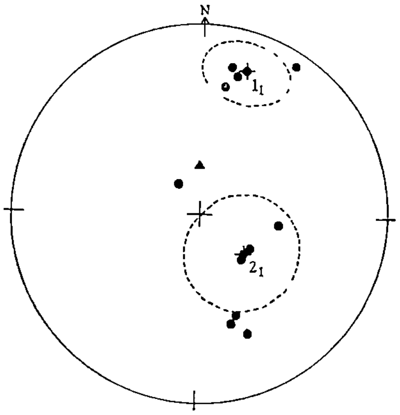

Fig 1 Equal area stereonet showing the positions of the magnetic vectors for eleven rocks from the British Cambrian together with the group mean vectors All vectors are in field orientation ●, Vector plotting in lower hemisphere, 1_1, mean vector for limestones, 1_2, mean vector for limestones after a c washing, 2_1, mean vector for quartzites, 2_2, mean vector for quartzites after a c washing, 2_3, mean vector for quartzites after a c washing and correction for dip, •, vector for Trias rocks, ■, vector for Cambrian rocks, ▲, direction of present dipole field The broken lines show the circles of confidence drawn at the 95 per cent level

measuring (Fig. 2) give mean directions for the quartzites

$$D\ 161° \quad I\ 55° \quad \alpha\ 15° \quad \kappa\ 14$$

and for the limestones.

$$D\ 015° \quad I\ 44° \quad \alpha\ 9° \quad \kappa\ 115$$

where the rocks are in their field orientations Both groups show a reduction in the amount of scatter and both show a displacement of the direction of the mean towards the south-west which cannot be attributed to the removal of a component of secondary magnetization parallel with the present geomagnetic field

The displacement of both mean vectors in a similar direction would also rule out the possibility that the limestones were originally magnetized in a reverse direction to the quartzites and have both taken on a component of secondary magnetization to give their present positions The presence of a reversal is also unlikely since the limestones occur stratigraphically within the quartzite series, samples from above and below the limestone horizon give similar directions of magnetization and there is no evidence of any marked break in deposition

Comparing the change in the intensities of magnetization produced by the a c washing there is a marked difference between the limestones, which lose about 85 per cent, and the quartzites, which show little change in intensity This suggests that the two rock types have taken on very different components of secondary magnetization

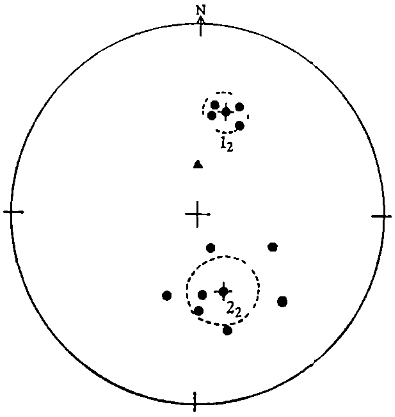

Fig 2 Equal area stereonet showing the positions of the magnetic vectors after a c washing, together with the group mean vectors All vectors in field orientation Symbols as in Fig 1

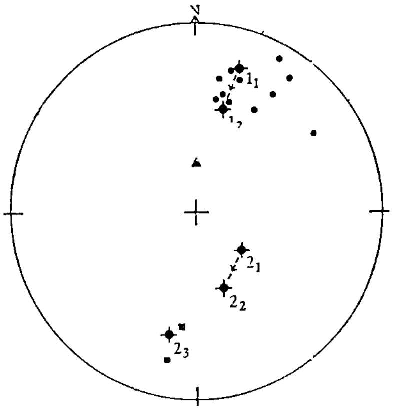

Fig 3 Equal area stereonet showing the relation of the mean positions for these Cambrian rocks to directions determined by other workers from Trias and Cambrian rocks Symbols as in Fig 1

The mean vectors for the two groups in field orientation are given in Fig 3 both before and after a c washing together with mean vectors for rocks of Trias age from Europe[5,6] and for the Caerbwdy Sandstones of Cambrian age from Wales[3] The mean direction for the Hyolithus Limestones before washing lies within the group of Trias results and accords well with those from rocks of Bunter age, the effect of the washing is to shift the mean direction towards the Cambrian direction For the Hartshill Quartzites the mean before washing lies close to the Cambrian direction, and the washing moves the vector still closer so that if this mean direction is corrected for the observed dip of the rocks the resultant vector

$$D\ 193° \quad I\ 34° \quad \alpha\ 16° \quad \kappa\ 12$$

is in good accord with the Cambrian direction

Combining the magnetic and geological evidence these observations may be explained as follows The Hartshill Quartzites and the Hyolithus Limestones were weakly magnetized parallel to the geomagnetic field in Cambrian times Uplift and erosion having removed any overlying rocks, these Cambrian rocks lay at or near the surface in the Trias During the deposition of the thick series of red beds of Trias age which are now exposed to the north-west of the Cambrian rocks, these were penetrated by iron-bearing ground waters which, in depositing new iron minerals and oxidizing existing minerals to new forms, imposed a chemical remanent magnetization[7,8] The degree to which the Cambrian rocks were affected by this process depended on their permeability, this was slight in the quartzites, but greater in the more permeable limestones That this new magnetization of the limestones could not be removed completely by washing in a c fields up to 100 œrsteds is in agreement with Creer's work[4] on unstable Keuper Marls which in some cases still retained components of secondary magnetization after washing in fields of up to 850 œrsteds

If it is correct to assume that the quartzites after a c washing give a true record of the direction of the Cambrian geomagnetic field the position of the magnetic pole at that time can be calculated as

$$\text{Lat } 18°\text{ N} \quad \text{Long. } 165°\text{ E} \quad d\psi\ 10° \quad d\chi\ 18°$$

where $d\psi$ and $d\chi$ are the lengths of the semi-axes of the elipses of confidence drawn at the 95 per cent probability-level This pole position is in good agreement with those determined from the British Cambrian and from rocks of similar age from Norway[9]

This work was carried out while I was a student at the Sedgwick Museum, Cambridge, and I take this opportunity to thank W B Harland and Dr R Hey of that department for their help and encouragement

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AUTO-IMMUNIZATION AND LIVER CIRRHOSIS

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RECENT progress of work on auto-immunization is remarkable, and the occurrence of this unusual condition has been reported or suggested in various sorts of diseases, one of which is liver cirrhosis Gajdusek[1] applied the complement-fixation reaction widely and revealed the existence of auto-antibodies against the liver as well as several other tissue homogenates in cases of some chronic diseases, including liver cirrhosis Tsujino[2] succeeded in producing by means of an immunological method some experimental condition which he called cirrhosis-like liver Recently, King[3] reported a case of liver cirrhosis in which many *LE*-cells were revealed, and with a review of similar conditions reported, he concluded that some kinds of liver cirrhosis should be a manifestation of auto-immune reaction of the liver

In our recent work, we tried to reveal the presence of an auto-antibody in serum against liver tissue obtained from the same individual, which we thought to be the real auto-immunization As shown in Table 1, fifteen cases have been tested so far, most of which at autopsy revealed various kinds of liver alterations such as cirrhosis or fibrosis, liver cell necrosis, primary liver cell carcinoma, etc Age of the patients varied between 8 and 74 yr , and all these were Japanese males The time of autopsy after death varied from 2 25–15 hr , most of them were rather fresh and less than 4 hr , during which period the bodies were not kept under refrigeration In one case of primary liver cell carcinoma, both the tumorous and non-tumorous parts of the liver were examined As for the cases with metastatic tumours in the liver, special care was taken to avoid contamination of the liver specimen with the tumour tissue

A small piece (20–30 gm) of the liver tissue and a cup of blood were collected from each case of autopsy Within 24 hr , the blood was centrifuged, and the separated serum was incubated in a water bath kept at 56° C , for 30 min Sodium azide was added in 0 1 per cent as a preservative On the other hand, the tissue specimen was weighed and cut with a razor blade into small pieces , four times by weight of saline solution was added This crude suspension was homogenized thoroughly, first with a Waring blender, and next with tissue grinder. After sodium azide (0 1 per cent) was added the tissue homogenate was centrifuged (5,000 r p m , 20 min) and the supernatant was preserved for the serological test A small segment of tissue was put into formalin solution for histological study

To reveal auto-antibodies, Ouchterlony's agar reaction method[4], modified by Wilson and Pringle[5], was used Interactions between matched serum and liver tissue were chiefly observed

Among fifteen cases examined, two showed a positive reaction, as shown in Table 1 Incubation for 4 days at 38° C was necessary to develop a definite line on the agar plate in one case (No 2), and it took 10 days in the other (No 14) Table 1 also shows the histological feature and weight of the liver of each case None of the five cases, including one each of hæmolytic anæmia and chronic nephritis,

Table 1 RESULTS OF AUTOPSY EXAMINATION

Case No	Age (yr)	Sex	Autopsy (hr after death)	Pathological diagnosis (brief histopathological findings of the liver)	Liver weight (gm)	Serological reaction
1	74	M	15	Stomach carcinoma (marked, periportal fibrosis and moderate congestion)	720	(—)
2	60	M	2⅓	Œsophagus carcinoma (fine-nodular cirrhosis and three nodes of metastasis)	1,540	(+)
3	71	M	3	Acquired hæmolytic anæmia (extra-medullary hæmatopoiesis in periportal areas and sinusoids)	940	(—)
4	19	M	2¼	Stenosis of inferior vena cava (irregular fibrosis and granular pseudolobuli formation)	1,950	(—)
5	72	M	2¾	Stomach carcinoma (meso-nodular, atrophic cirrhosis with necrosis and tumour metastasis)		(—)
6	45	M	3¾	Iatrogenic massive liver necrosis (extensive, total necrosis and fatty degeneration of liver cells)	985	(—)
7	14	M	2¾	Primary liver cell carcinoma (hepatocellular carcinoma in non-cirrhotic, icteric liver)	2,260	(—)
8	68	M	5¼	Stomach carcinoma (multiple, nodular metastases in congestive, atrophic liver)	1,390	(—)
9	30	M	3½	Chronic glomerulonephritis (cloudy swelling of liver cells, marked)	1,680	(—)
10	8	M	6½	Acute bulbar paralysis and poliomyelopathia (slight congestion of liver)	625	(—)
11	51	M	2½	Accessory sinus carcinoma (two, small nodular-metastases and slight lymphocytic infiltration)	1,404	(—)
12	70	M	2⅓	Hæmochromatosis (hæmochromatotic liver cirrhosis with degenerative change)	900	(—)
13	68	M	3	Aortic aneurysma, liver cirrhosis (macro-nodular cirrhosis with marked, nodular hyperplasia)	1,040	(—)
14	36	M	6	Fatty cirrhosis of the liver (fine-nodular cirrhosis with marked, fatty metamorphosis)	1,500	(+)
15	67	M	5½	Liver cirrhosis (fine-nodular, atrophic, irregular cirrhosis with scarring)	780	(—)

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which showed no remarkable primary liver change at autopsy, gave positive results in the serological test. Actually, there were ten cases with more or less altered liver tested in our work, among which none of the cases of acute liver necrosis, primary liver cell carcinoma, and liver fibrosis showed the development of auto-immunization. A total of six cases of cirrhosis of the liver were tested, and both the cases which showed serologically positive results were in this last group.

Grossly, no atrophy was noticed in either of those two cirrhotic livers which showed positive serological reaction, one weighed 1,540 gm. and the other 1,500 gm. Three metastatic tumour nodes were found in the liver of case No. 2, but they were too small to influence the weight of the liver.

Microscopical investigation of those cases revealed an interesting fact that the two were quite similar also in histological character. Sections of the liver in those two cases showed rather small, uniform pseudolobules surrounded with thin trabeculæ of fibrous tissue, without any marked scar formation. Lymphocytic infiltration and bile-duct proliferation in portal areas were seen, but quite slight. Neither nodular hyperplasia nor adenomatous growth of liver cells was noticed. Elastosis was not marked. Fatty metamorphosis of the liver cells was especially marked in case No. 14. As shown in Table 1, the cirrhotic or fibrotic livers tested in this work showed various histological features, and the only two cases of those which had quite the same gross and histological characters gave a positive auto-immune reaction. This fact was considered to be too serious to be neglected as a matter of accidental coincidence. Moreover, those two cases had one common feature in regard to previous histories, namely, alcoholism. The liver of those two cases showed the feature of mild, slowly-progressing cirrhosis of a rather portal type which could be classified as nutritional when both clinical and pathological results were considered.

The definite serological interactions were found only in cases of liver cirrhosis so far as examined, and they could be exclusive for certain types of this disease. Even so, the reason why the auto-antibodies against the liver tissues were found only in cases of a so-called nutritional type of cirrhosis are still unknown. However, the fact that numerous fat droplets were revealed histologically in the lung capillaries of those cases apparently suggests the possibility of an outflow of the fat extracts of the liver cell components into the blood stream in the case of severe fatty metamorphosis of the liver, which might cause developing auto-immunization.

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COUNTERACTION BY CYSTEINE OF GLYCOGENOLYSIS AND INHIBITION OF MICROSOMAL PROTEIN SYNTHESIS PRODUCED BY DIMETHYLNITROSAMINE

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WITHIN a few hours of administration of dimethylnitrosamine (DMNA) and before any other lesion can be observed, there is found a severe inhibition of amino-acid incorporation into the proteins of the isolated microsomal-soluble fraction of rat liver[1,2]. Indirect evidence has been presented[1] that DMNA is not inhibitory in itself, but that the compound is first converted by an oxygen- and reduced triphosphopyridine nucleotide-dependent *N*-demethylase, located in the microsomal membranes, to the *N*-hydroxymethyl (methylol) derivative. The latter is very reactive and unstable so that either this derivative, or the formaldehyde released therefrom, may act as the toxic agent *in situ*. Moreover, the monomethylol derivative might also give rise to diazomethane[2]. Any one of these derivatives may react with susceptible groups of the enzymes, cofactors or ribonucleic acid (RNA) templates of the protein synthetic process[1]. Although it is not known which of the possible derivatives, $O=N-N=(CH_2OH)_2$, H_2CO or CH_2N_2, is in fact responsible for the toxic action of DMNA, all three may react with sulphydryl groups which have been recognized[3] as cofactors of protein synthesis at the microsomal level. It is of interest that the lesion, in incorporation of amino-acid produced by DMNA, is located in the microsomes[1,2] and not in the soluble (*s*) fraction which catalyses amino-acid activation and the transfer of the latter to *s*-RNA. Next to its effect on protein synthesis, DMNA has also been found to cause the disappearance of glycogen from rat liver[4]. Three hours after the administration of DMNA (50 mgm./kgm. body-weight), when changes in the endoplasmic reticulum[4] and an inhibition of microsomal protein synthesis were observed, the glycogen content appeared, both by electron microscopy and chemical analysis, to be normal, whereas 17 hr. later almost all the glycogen had disappeared. Three hours after a two-fold greater dose of DMNA, 20 per cent (average) of the glycogen had disappeared, the bulk of the latter being lost in the period 3–10 hr. after DMNA injection.

In the present experiments we have been able to show that administration of cysteine to rats markedly counteracts both the percentage inhibition of microsomal protein synthesis and the glycogenolysis which result from the injection of DMNA. Cysteine administration according to the régime listed in the legend of Table 1 (or slightly varied) caused a marked increase in the *in vitro* protein labelling of the later isolated microsomal-soluble fraction of liver by leucine-1-^{14}C (50–150 per cent increase in 8 experiments). A relatively greater effect (150–350 per cent increase) of cysteine administration was always

Table 1 *In vitro* INCORPORATION OF LEUCINE-1-^{14}C INTO THE PROTEINS OF THE MICROSOMAL-SOLUBLE FRACTION AND THE GLYCOGEN CONTENT OF LIVERS FROM NORMAL AND DMNA-TREATED RATS AS AFFECTED BY CYSTEINE ADMINISTRATION

DMNA	Cysteine	Protein radioactivity of microsomal-soluble fraction			Glycogen content of liver slices
		(counts/min at 'infinite' thickness)	(per cent change)		(per cent of wet weight of liver)
—	—	685			8 1
—	+	1409	+106		6 1
+	—	201		—71	2 2
+	+	832	+314	—41	5 5

150 mgm DL-cysteine was administered subcutaneously twice daily for 2 days to 300 gm rats of the inbred strain *U* Early next morning the rats received saline (0 1 ml) or DMNA (100 mgm /kgm body-wt) dissolved in saline intravenously and another 150 mgm of cysteine subcutaneously These, and similarly treated rats which had not received the cysteine, were killed 5 hr later The incorporation of leucine-1-^{14}C into the proteins of the isolated microsomal-soluble fractions (10,000*g* supernatant) was studied as described in ref 1 Glycogen was measured in slices prepared from the same livers by the anthrone method

observed on the decreased incorporation of amino-acid of the corresponding fractions prepared from the livers of rats 3–10 hr after injection of DMNA The experimental results in Table 1 illustrate a case in which the rats had been subjected to the toxic effect of DMNA for 5 hr Experiments of this kind can only be interpreted in terms of an increase in amino-acid incorporation, or of a counteraction by cysteine of the DMNA-inhibited amino-acid incorporation, if the radioactivity of the immediate precursor is known If cysteine in some way or another had produced a decrease in the concentration of the endogenous leucine of the soluble fraction, a smaller dilution of the added ^{14}C-leucine and an increase in labelling of protein would have resulted The following results, however, make it likely that this was not the case

Experiments on the incorporation of amino-acid into the proteins of liver slices of the various rats, treated according to Table 1, gave results identical to those of Table 1 Since the increase in the incorporation of amino-acid into the proteins of the liver slices of rats which had received cysteine (controls 40 and 66 per cent, DMNA-treated 62 and 112 per cent increase in two experiments) was not accompanied by an increase in carbon-14 content of the respiratory carbon dioxide derived from leucine-1-^{14}C, as compared with the corresponding data obtained with rats which had not received cysteine, it followed that the increase in protein labelling was not due to a decrease in the amount of endogenous leucine The results of four experiments in which the 105,000*g* supernatants of the livers from four rats, treated according to Table 1, were incubated with leucine-1-^{14}C and adenosine triphosphate, followed by isolation (phenol method) and purification (through 'Sephadex') of the ^{14}C-leucyl-*s*–RNA formed, supported the latter conclusion The radioactivity of the control- and DMNA-treated preparations (± cysteine) was identical This suggests that both the stimulatory effect of cysteine on the incorporation of amino-acid system of the normal rats and the counteraction by cysteine of the percentage inhibition by DMNA is exerted at the microsomal level

It is shown in Table 1 that cysteine administration also counteracted the glycogenolysis produced by DMNA, some measure of variation in this and the above effect of cysteine being noted with individual rats Cysteine administration (150 mgm twice daily for 2 days) also resulted in, or markedly prolonged, the survival of rats which had received a lethal dose of DMNA (22 5 mgm /300 gm rat, given immediately after the fourth cysteine administration , all controls dying within 40 hr)

It may thus appear that cysteine, although not being tolerated too well by some of the rats (especially the younger ones), and apart from its more specific effect on microsomal protein synthesis (compare the controls), prevents or counteracts DMNA toxicity in general It is of interest that cysteine and cysteamine (cysteamine *per se* shows also some measure of toxicity) also protect against a lethal dose of nitrogen mustard[5] Although not permitting a definite choice, the correspondence in protective effect of cysteine against DMNA and the alkylating agent nitrogen mustard, might indicate that the methylating agent diazomethane is the actual toxic derivative of DMNA The protective effect of cysteine against DMNA toxicity may be due to a direct interaction of the sulphydryl groups of the former with the highly reactive and toxic DMNA derivative, or to a transitory blockage of tissue SH-groups which are thus unable to react with the DMNA derivative

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DETECTION OF A POSSIBLE INTERMEDIATE IN OXIDATIVE PHOSPHORYLATION

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A STRIKING feature of oxidative phosphorylation is that although occurrence of the process has been recognized for some twenty years, no phosphorylated intermediates have been demonstrated The formation of three or more molecules of adenosine triphosphate (ATP) for each reduced nicotinamide or flavin-containing co-enzyme oxidized by oxygen suggests participation of one or more intermediates Several investigations[1–3] have indicated that mitochondria, in the absence of an added phosphoryl acceptor, may accumulate material capable of allowing rapid formation of ATP Whether the observations reflect the presence of a phosphorylated intermediate or some other form of energy storage is uncertain Data on ADP ⇌ ATP exchange have been interpreted as indicating a phospho-enzyme intermediate[4] Various findings, in particular the demonstration that oxygen between the β- and γ-phosphorus atoms of ATP is furnished by ADP[5], strongly infer that any phosphorylated intermediates formed are derived from orthophosphate (P_i), followed by reaction with ADP to give ATP Present information does not exclude, however, the possibility that the first covalent compound formed from both P_i and ADP in one or more steps of oxidative phosphorylation is ATP itself

The importance of detection and isolation of any intermediates in oxidative phosphorylation to the understanding of the reaction mechanism served as the motivation for our work As reported in preliminary communications[6], these investigations have led to the demonstration of the rapid uptake of $^{32}P_i$ into a protein-bound form Conditions affecting the labelling of this unidentified phosphate suggest its participation in oxidative phosphorylation, and presentation of some details in this regard, together with some properties of the substance is the principal purpose of this article

Rapid Formation of an Unidentified Phosphate

Conditions which might be expected to favour accumulation of an intermediate of oxidative phosphorylation in mitochondria are incubation in the presence of excess substrate, oxygen and P_i, but without added ADP In our experiments, mitochondria have been incubated under such conditions but in the absence of added P_i, then exposed for short periods to a trace of high specific activity $^{32}P_i$ The trace of added $^{32}P_i$ to the medium was avoided because such addition would reduce the possibility of detecting a small fraction of the total added phosphorus-32 that might be present in any intermediate Stopping of the reactions and nearly complete removal of $^{32}P_i$ and AT^{32}P from the protein-bound phosphorus-32 was achieved by adding a concentrated urea solution containing a non-ionic detergent to give apparent solubilization of the mitochondria[7], followed by passage through a column of anion exchange resin. After incubation for a few sec or more with $^{32}P_i$ presence of ^{32}P in this unidentified material and in AT^{32}P are shown in Fig 1 In this and other comparable experiments, the average amount of ^{32}P in the unidentified fraction has been slightly greater at 10 sec than at 1 min. The tendency for the decrease in 1 min sometimes noted might reflect decrease in specific activity of $^{32}P_i$ resulting from interchange with P_i and ATP pools of mitochondria Evidence exists for the presence of intramitochondrial P_i which does not readily mix with medium P_i (refs 10, 5)

The rate of labelling of the unidentified phosphate is much more rapid than that of phospho-proteins or phospho-lipids, as reported by Conover *et al* [11] and by Burnett and Kennedy[12], and thus does not appear to represent the initial uptake into a large pool of phosphorylated compounds A time-course as given in Fig 1 is in harmony with participation of the unidentified phosphate as an intermediate A rate of labelling comparable with or slower than that of ATP would not, however, preclude participation of a compound as an intermediate in view of possible effects of relative reaction-rates as well as compartmentation in mitochondria An effect of relative reaction-rates arises from the demonstrated dynamic reversal of ATP formation accompanying oxidative phosphorylation[13,14] The pathway of phosphorus-32 may be simply represented as follows

$$P_i \rightleftharpoons \text{phosphorylated intermediate} \rightleftharpoons \text{ATP}$$

After addition of $^{32}P_i$, the specific activity of the intermediate would be expected to be similar to that of the P_i if the interchange in the first step were rapid compared with that in the second step Conversely, the specific activity of the intermediate would approximate that of the ATP if the interchange in the second step were more rapid Indeed, in some of our experiments with small variations in conditions, the rate of appearance of ^{32}P in ATP and in the unidentified phosphate has been similar.

The absolute amount of phosphorus-32 appearing in the unidentified fraction is only about 0 2–0 3 per cent of the added $^{32}P_i$ It became important, therefore, to assess the possibility that the small activity found might arise from experimental artefact and not represent a metabolically derived phosphate compound Controls in which $^{32}P_i$ or AT^{32}P was added with or after the concentrated urea demonstrated that appearance of the product depends on incubation for the short periods with mitochondria That the product very likely is a derivative of P_i is shown by the liberation on short heat treatment at neutral *p*H of the radioactivity in a form extractable with added P_i by isobutanol-benzene from acid molybdate[8] and precipitable with P_i as magnesium ammonium phosphate In addition, the product had a radioactivity with penetration characteristics and half-life similar to ^{32}P

The experiments have been hampered considerably by the presence of varying amounts of impurities in $^{32}P_i$ as received which appear in the column eluates of controls Of various purification techniques, that used at present, and which is successful with most samples, is as follows The sample is made alkaline with ammonia, adsorbed on to a small column of 'Dow-1-bicarbonate' anion exchange resin, and the

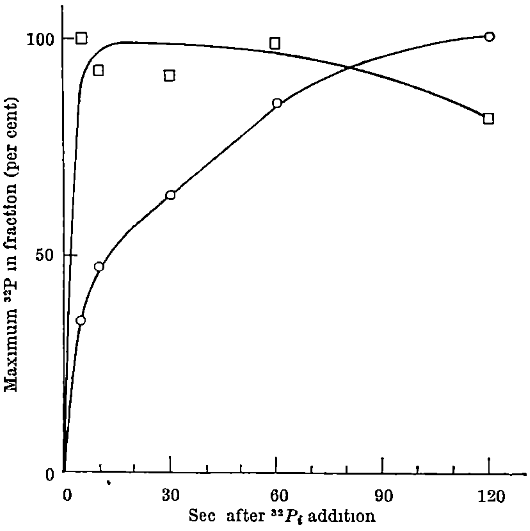


Fig 1 Time-course of appearance of $^{32}P_i$ in ATP and in the unidentified phosphate A mixture containing 11 m*M* sodium glutamate, 6 m*M* KCl, 240 m*M* sucrose, 1 m*M* ethylenediamine tetra-acetate (EDTA) and mitochondria from roughly one-fourth of a rat liver in a total volume of 2 ml was incubated with rapid stirring under oxygen at *p*H near 7 and 15° C for 1 min A drop of high specific activity $^{32}P_i$ was then added, and the reaction stopped at the desired time by additions of 6 vol of cold 6 *M* urea and 4 per cent detergent ('Triton X-100') The sample was passed through a column (about 0 8 × 8 cm) of 'Dow-1-Cl' and ^{32}P in the eluate measured The incorporation of $^{32}P_i$ into ATP was estimated as the ^{32}P not extracted by isobutanolbenzene in the Berenblum and Chain assay (ref 8), performed on the supernatant solution after precipitation of protein by cold 0 3 *M* perchloric acid The urea–'Triton' and the sucrose solutions were purified by passage through a mixed ion-exchange resin The mitochondria were prepared essentially as described by Schneider and Hogeboom (ref 9) with 1 m*M* EDTA, *p*H 7 4, added to the 0 25 *M* sucrose

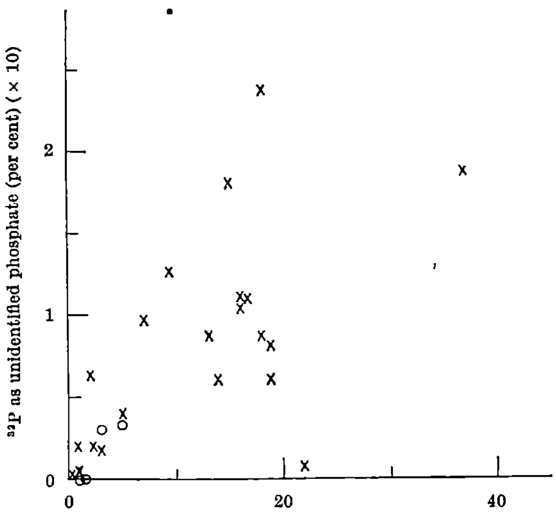


Fig 2 Comparison of the percentage of the total $^{32}P_i$ incorporated into the unidentified phosphate and ATP Experimental conditions were as given in Fig 1, with incubation for 30 sec after $^{32}P_i$ addition, for results designated by crosses For results designated by circles, 10^{-3} M 2,4-dinitrophenol was present

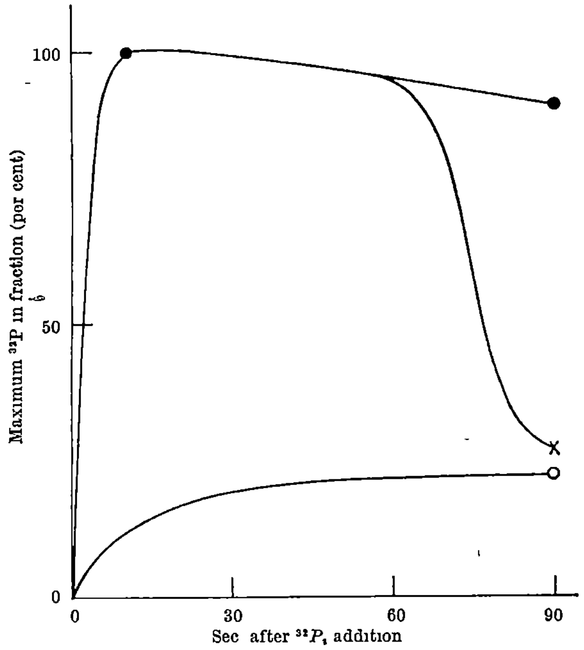


Fig 3 Effect of added P_i and ATP on the percentage of $^{32}P_i$ appearing in the unidentified phosphate Experimental conditions were as given in Fig 1, with addition of P_i and ATP to give final concentrations of 1 mM each at the times indicated ●, No added P_i and ATP, ×, P_i and ATP added at 60 sec, ○, P_i and ATP added before $^{32}P_i$

$^{32}P_i$ eluted with 0 15 M ammonium bicarbonate–0 013 M ammonia Mixing of the column eluate with 'Dow-50(H$^+$)' removes ammonia and liberates carbon dioxide to give a $^{32}P_i$ solution low in total salt concentration This procedure has given $^{32}P_i$ in which controls showed less than 1 per cent as much phosphorus-32 in column eluates as did incubated samples

The amount of the unidentified phosphate detected, although representing only a small fraction of the $^{32}P_i$ added, is about comparable on a molar basis with the level of cytochromes present, as estimated from the data of Chance and Williams[1] If one assumes that the specific activity of the unidentified phosphate is equal to that of the P_i of mitochondria, the maximum amount detected is roughly 0 05 µmole per gm of rat liver mitochondrial protein. The possibility exists that this amount represents only partial phosphorylation of the precursor of the unidentified phosphate.

Production of material from a given preparation of mitochondria is fairly reproducible, but different preparations show considerable variation in their ability to incorporate $^{32}P_i$ into both the unidentified phosphate and ATP A summary of results collected over a period of several months with liver mitochondria is given in Fig 2 These show that mitochondrial preparations with ability to accumulate a higher per cent of the added trace amount of $^{32}P_i$ as ATP show a tendency for formation of more of the unidentified substance On storage for a few hours at 0°, ability to form both intramitochondrial AT^{32}P and the unidentified substance declines

A similar labelling of the unidentified phosphate is obtained with glutamate, α-ketoglutarate, β-hydroxybutyrate or succinate as substrates or without added substrate The material has also been detected in mitochondria from rat liver and heart, as well as ox liver and heart and rabbit heart

Effect of Added P_i and ATP and of Inhibitors on the Unidentified Phosphate

Any substance which was an intermediate in the formation of ATP from P_i would be expected to undergo rapid interchange with added P_i or ATP or both The results given in Fig 3 show that addition of unlabelled P_i and ATP after incorporation of ^{32}P into the unidentified phosphate readily results in loss of ^{32}P from the fraction Clearly, a dynamic interchange between the fraction and P_i–ATP exists. Further, addition of unlabelled P_i and ATP before

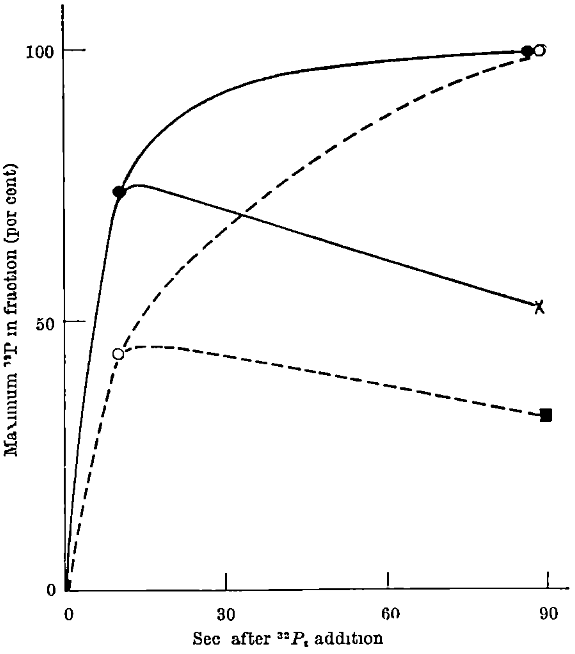


Fig 4 Effect of addition of 2,4-dinitrophenol on the labelling of the unidentified phosphate and ATP Experimental conditions were as noted with Fig 1, except that the added urea was at pH about 9 5 and no detergent was present 2,4-Dinitrophenol additions were made as noted The solid lines indicate the ^{32}P in the unidentified phosphate and the dashed lines the ^{32}P in the ATP ●, ○, No DNP, ■, ×, 10^{-3} M DNP at 10 sec

Table 1 REPRESENTATIVE EFFECTS OF INHIBITORS ON LABELLING OF UNIDENTIFIED PHOSPHATE AND ATP

Exp	Inhibitor	Sec after $^{32}P_i$ addn	Percentage added as $^{32}P_i$ as Unidentified phosphate	ATP
1	None	10	0 07	9
	2,4-Dinitrophenol, 10^{-4} *M*	10	0 06	6
	2,4-Dinitrophenol, 10^{-3} *M*	10	0 01	0
	None	30	0 10	14
	2,4-Dinitrophenol, 10^{-4} *M*	30	0 04	5
	2,4-Dinitrophenol, 10^{-3} *M*	30	0 00	1
	None	90	0 14	34
	2,4-Dinitrophenol, 10^{-4} *M*	90	0 04	11
	2,4-Dinitrophenol, 10^{-3} *M*	90	0 02	3
2	None	30	0 11	20
	Cd^{++}, 10^{-5} *M*	30	0 02	1
3	None	30	0 14	27
	Oligomycin, 12 μgm /ml	30	0 13	6
4	None	30	0 07	30
	Oligomycin, 50 μgm /ml	30	0 06	9
	2,4-Dinitrophenol, 10^{-3} *M*	30	0 02	5
5	None	30	0 08	18
	2,4-Dinitrophenol, 10^{-4} *M* Oligomycin, 100 μgm /ml	30	0 01	2
6	None	30	0 17	6
	KCN, 10^{-3} *M*	30	0 05	3
	Amytal, 3 × 10^{-3} *M*	30	0 04	4
	p-Mercuribenzoate, 10^{-3} *M*	30	0 00	0

Conditions were essentially as given with Fig 1 with rat liver mitochondria unless otherwise specified In some experiments, urea solutions made alkaline with NH_3 were used instead of urea-detergent solutions 2,4-Dinitrophenol was added 1 min before $^{32}P_i$ Cd^{++} were added 5 min before $^{32}P_i$ Oligomycin, KCN, amytal and *p*-mercuribenzoate were present during 5–10 min at 0° and 1 min at 15° before $^{32}P_i$ addition In experiment 6, ox liver mitochondria were used Mitochondria from about 1 gm of liver were used in experiments 1, 3, 4 and 6, and from about 0 2 gm of liver in experiments 2 and 5

the trace of $^{32}P_i$ nearly prevents incorporation of phosphorus-32 into the unidentified phosphate This is the expected result from marked diminution of the specific activity of $^{32}P_i$

The effect of various inhibitors of oxidative phosphorylation establishes a close relationship between the labelling of the unidentified phosphate and oxidative phosphorylation Presence of 2,4-dinitrophenol, a well-recognized 'uncoupler' of oxidative phosphorylation, both reduced the formation and accelerated the disappearance of the fraction, as shown by the results in Fig 4 and Table 1 The effectiveness of a given concentration of 2,4-dinitrophenol was not as great as might at first be anticipated from its known action on net oxidative phosphorylation and on the P_i–ATP exchange reaction, 10^{-4} *M* 2,4-dinitrophenol nearly completely abolishes these processes[13,15] As noted from Table 1, considerable phosphorus-32 was incorporated into the unidentified phosphate even in the initial presence of 10^{-3} *M* dinitrophenol Two factors may be responsible for this First, with decrease in the concentration of mitochondria, lower concentrations of 2,4-dinitrophenol are more effective, and secondly, the inability of 2,4-dinitrophenol, when initially present, sharply to reduce the labelling of the unidentified phosphate was paralleled by continued ability to label intra-mitochondrial ATP This could reflect a resistance of intra-mitochondrial ATP to breakdown in presence of 2,4-dinitrophenol as noted by Azzone and Ernster[16]

Effects of other inhibitors are also summarized in Table 1 Of particular interest are the effects of oligomycin This antibiotic has been shown by Lardy, Johnson and McMurray[17] to prevent ATP formation without an 'uncoupling' action Uptake of $^{32}P_i$ into the unidentified phosphate fraction is not affected or actually slightly increased when mitochondria are exposed to oligomycin Under these conditions, formation of $AT^{32}P$ is markedly decreased This gives strong evidence that the unidentified phosphate ^{32}P arises from $^{32}P_i$ and not from $AT^{32}P$ Further, as shown in Table 1, presence of 2,4-dinitrophenol decreases formation of the unidentified phosphate in presence of oligomycin

Cadmium ions, which Jacobs and Jacobs have shown to be potent inhibitors of oxidative phosphorylation[18], also prevent formation of the unidentified phosphate Azide, which has effects like both 2,4-dinitrophenol and oligomycin[19], as well as an inhibitory action on electron transport, also is effective in reducing formation of the unidentified phosphate Cyanide and presence of a nitrogen atmosphere cause some, but not marked, decreases in incorporation of $^{32}P_i$ into the unidentified phosphate These effects of inhibitors on formation of the unidentified phosphate are similar to the effects noted on the $^{32}P_i$–ATP exchange reaction[13,16]

Properties and Fractionation of the Unidentified Phosphate

The unidentified phosphate as present in column eluates is non-dialysable, retained on charcoal, and is separable from P_i by passage through 'Sephadex *G*-50' It is partly cleaved to P_i but mostly precipitated by brief exposure to dialysed eluates to cold 0 3 *M* trichloracetic acid, and is precipitated by acidic or neutral ammonium sulphate, ammonium sulphate plus dodecyl sulphate or ethanol It is not extracted from lyophilized fractions by ethanol, 3 to 1 ethanol diethyl ether, or acetone Treatment of the unidentified material with a bacterial proteinase has yielded a radioactive product which is dialysable and not precipitated as magnesium ammonium phosphate Dialysis of column eluates in presence of crude alkaline phosphatase markedly accelerates the appearance of $^{32}P_i$ in the dialysing medium These various findings suggest that the phosphoryl group of the unidentified phosphate is attached to a co-factor firmly bound to the protein or lipo-protein of mitochondria or to an amino-acid side-chain

After heating the dialysed eluates in a boiling water bath for 5 min at neutral *p*H, the phosphorus-32 readily passes through charcoal and is identifiable as P_i A *p*H-stability curve as based on the charcoal absorption test is shown in Fig 5 The half-life at *p*H 7 and 77° is 22 min First-order rate plots for the decomposition at *p*H 7 are essentially linear, giving strong indication that only one phosphorus-32 compound is present The rate of decomposition is not markedly modified by the presence of hydrogen

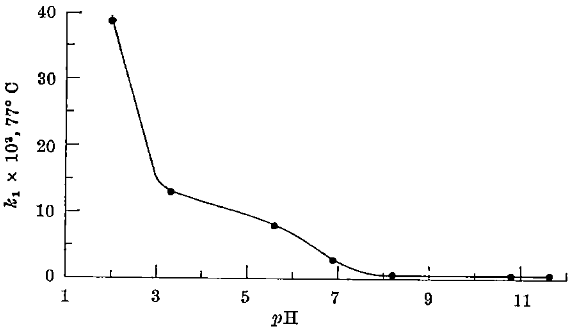
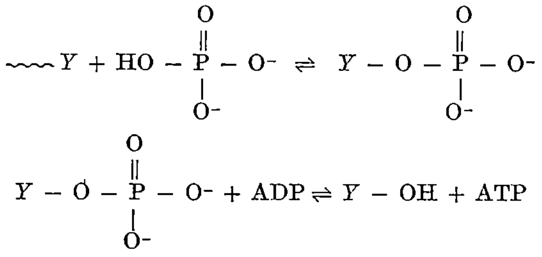


Fig 5 Effect of *p*H on the stability of bound $^{32}P_i$ The first-order rate constant, k_1, was determined as follows the eluates from columns after extensive dialysis against 0 02 *M* P_i, *p*H 6 8, were used directly in cleavage measurements The *p*H was varied by addition of HCl or NaOH, the sample heated at 77°, cooled, and the *p*H adjusted to near 7 The amount of bound fraction remaining was approximated by the amount retained on charcoal, $^{32}P_i$ appears in the eluate

peroxide, hydrosulphite, molybdate, 2,4-dinitrophenol or *p*-mercuribenzoate, by ionic strength variation, or by replacement of air by nitrogen, but is about doubled in the presence of bromine The data are in agreement with attachment to a nitrogen, sulphur or oxygen atom in a structure not susceptible to hydroxyl ion attack but modifiable by bromine The stability work appears to eliminate the possibility of attachment of the phosphoryl residue to a seryl side-chain The unidentified phosphate is considerably more stable and shows a different dependence of stability on *p*H than 2,3-dimethyl-1,4-naphthaquinol 1-phosphate[20] Ubiquinone monophosphate is, however, more stable than the methyl substituted naphthaquinol phosphate[20]

Relation of the Unidentified Phosphate to Oxidative Phosphorylation

If the unidentified phosphate is an intermediate of oxidative phosphorylation, certain suggestions concerning the mechanism of formation can be offered Data from experiments with oligomycin, which is known to inhibit phosphate oxygen exchange associated with oxidative phosphorylation[21], indicates that the P_i molecule is incorporated intact into an intermediate The phosphoryl group is then transferred to ADP and the remaining oxygen exchanges with water oxygens as depicted in the following scheme



$$\sim\sim Y + \mathrm{HO} - \underset{\underset{\mathrm{O^-}}{|}}{\overset{\overset{\mathrm{O}}{\|}}{\mathrm{P}}} - \mathrm{O^-} \rightleftharpoons Y - \mathrm{O} - \underset{\underset{\mathrm{O^-}}{|}}{\overset{\overset{\mathrm{O}}{\|}}{\mathrm{P}}} - \mathrm{O^-}$$

$$Y - \mathrm{O} - \underset{\underset{\mathrm{O^-}}{|}}{\overset{\overset{\mathrm{O}}{\|}}{\mathrm{P}}} - \mathrm{O^-} + \mathrm{ADP} \rightleftharpoons Y - \mathrm{OH} + \mathrm{ATP}$$

followed by a rapid oxygen exchange,

$$Y - \mathrm{OH} + \mathrm{H^{18}OH} \rightleftharpoons Y - {}^{18}\mathrm{OH} + \mathrm{HOH}$$

Reversal of the above reactions would give rise to an exchange of P_i oxygens Such a possibility has been suggested by Boyer *et al* [6,13] and by Drysdale and Cohn[22] Furthermore, even though the search for possible phosphorylated intermediates is by no means complete, the finding at this time of only one apparent candidate for an intermediate together with other data suggests consideration of mechanisms by which the various oxidation steps are in some manner coupled to formation of a common phosphorylated precursor to ATP

In summary, the conditions governing the formation and disappearance of the unidentified phosphate fraction are consistent with, but by no means prove that, the substance is an intermediate in oxidative phosphorylation

This work was supported in part by U S Public Health Service grant *RG*-4930, by U S Atomic Energy Commission Contract *AT*(11-1)-341, and by the Hill Family Foundation Thanks are extended by Drs C H Suelter and J B Peter for support as U S Public Health Service Postdoctoral Fellows

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MECHANISM OF ACTION OF LIVER ALCOHOL DEHYDROGENASE

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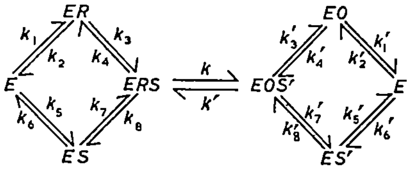
SEVERAL lines of inquiry have now shown that liver alcohol dehydrogenase (LADH) acts by forming binary and ternary complexes with coenzymes and substrates[1].



There are two reaction phases with interconversion between them (LADH and YADH represent liver and yeast alcohol dehydrogenase, respectively *E* represents 1/2 LADH, since each molecule has two independent binding sites *R* and *O* represent reduced (DPNH) and oxidized (DPN) diphosphopyridine nucleotide, respectively *S* and *S′* represent aldehyde and alcohol, respectively) It has also been confirmed that this complicated mechanism can, from a kinetic point of view, be described by the Theorell–Chance mechanism[2]

$$E + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ER$$

$$ER + S \underset{k_3'}{\overset{k_3}{\rightleftharpoons}} EO + S'$$

$$EO \underset{k_1'}{\overset{k_2'}{\rightleftharpoons}} E + O$$

However, among previous attempts at experimental confirmation[3-5], there had been indications that at lower *p*H the ternary complexes affected the rate[4,5]

Kinetics without Inhibitors

For the general two-substrate equation:

$$e/v = \varphi_0 + \varphi_1/[S_1] + \varphi_2/[S_2] + \varphi_{12}/[S_1][S_2]$$

(primed symbols represent the reverse reaction), Dalziel[6] has derived the relations $\varphi_0 = \varphi_0'\varphi_2'/\varphi_{12}'$, $\varphi_0' = \varphi_1\varphi_2/\varphi_{12}$ and $\bar{K}_{eq}$ (0 9 × 10^{-11} *M*) (ref 7) = $\varphi_{12}/\varphi_{12}' = \varphi_0\varphi_1\varphi_2/\varphi_0'\varphi_1'\varphi_2'$, which distinguish the Theorell–Chance mechanism from other possible alternatives Consequently, Lineweaver–Burk plots were determined for various concentrations of each constant partner and the intercepts and slopes plotted against these concentrations to give the desired φ-values[6] Initial rates were measured on a sensitive fluorimeter with pen recorder[8], by following the DPNH fluorescence as DPN does not fluoresce Table 1 shows the experimental φ-values The relationship between them fulfils very well the sensitive requirements of the Theorell–Chance mechanism The rate constants calculated on the basis of that mechanism and the binary dissociation constants, $K_{E,\text{ald}}$, $K_{E,\text{alc}}$, $K_{E,R}$ and $K_{E,O}$, calculated from the rate constants are also given The agreement of the binary enzyme–coenzyme dissociation constants $K_{E,R}$ and $K_{E,O}$, with the values determined spectrophotofluorimetrically in equilibrium experiments in the absence of substrates (below), underlines further the operation of the Theorell–Chance mechanism There are several reasons for the simplifications in the overall mechanism which lead to Theorell–Chance kinetics

Table 1 0 1μ PHOSPHATE, 23 5° C, *p*H 7

(*a*) φ-Values and Theorell-Chance mechanism rate constants (1/φ)

φ_0 = 0 0135 sec
φ_1 = 0 090 sec μ*M*
φ_2 = 3 2 sec μ*M*
φ_{12} = 0 9 sec μ*M* (ref 2)
φ_0' = 0 32 sec
φ_1' = 1 9 sec μ*M*
φ_2' = 82 sec μ*M*
φ_{12}' = 10 500 sec μ*M* (ref 2)

k_2' = 74 sec^{-1}
k_1 = 11 1 sec^{-1} μM^{-1}
k_3 = 0 31 sec^{-1} μM^{-1}
k_2 = 3 12 sec^{-1}
k_1' = 0 525 sec^{-1} μM^{-1}
k_3' = 0 012 sec^{-1} μM^{-1}

(*b*) Calculated equilibrium constants for binary complexes

$K_{E,R}(k_2/k_1)$ = 0 28 μ*M*
$K_{E,\text{alc}}(k_2'/k_3')$ = 1060 μ*M*
$K_{E,O}(k_2'/k_1')$ = 141 μ*M*
$K_{E,\text{ald}}(k_2/k_3)$ = 10 0 μ*M*

(1) $k_1 \approx k_7$ and $k_1' \approx k_7'$ The rate constants for the binding of the coenzyme are nearly independent whether the enzyme is free or already combined with the substrate, (2) *k* and *k'* are both high, the ternary complexes being in rapid equilibrium, (3) $k_4 \gg k_8$ and $k_4' \gg k_8'$ The ternary complexes prefer to liberate the substrates first, these being less tightly bound than the coenzymes Thus, the dissociation velocity of the binary complexes in the last phase of the reaction becomes rate-limiting when both substrate and coenzyme are present in high concentration in the first phase and the maximum velocities are k_2 and k_2' rather than k_6 and k_6' k_1 and k_1' determined in the presence of high concentrations of aldehyde or alcohol really reflect the 'on' velocity constants k_7 and k'_7, which, since the equilibrium and kinetic values of $K_{E,R}$ and $K_{E,O}$ are practically the same, means that $k_1 \approx k_7$ and $k_1' \approx k_7'$

Spectrophotofluorimetric Dissociation Constant Determinations

Previously, fatty acids were found to form *EI* and *EOI*, but no *ERI* complexes, while the amides formed *EI* and *ERI*, but no *EOI* complexes[9] Many nitrogen compounds have now been found to form ternary complexes with both *R* and *O*, and imidazole has proved particularly interesting, as it forms complexes with zinc ions[10] and LADH contains zinc[3] like YADH[11], while the ternary imidazole complex *ERI* also had an even greater fluorescence than *R* and *ER* (Fig 1) This increased fluorescence may be due to interaction between the pyridine ring and zinc, although greater rigidity[12,13] and most probably energy transfer[14] have also been suggested The formation of the *ER* and *ERI* complexes is also shown by the different maximum wave-lengths of their emission spectra

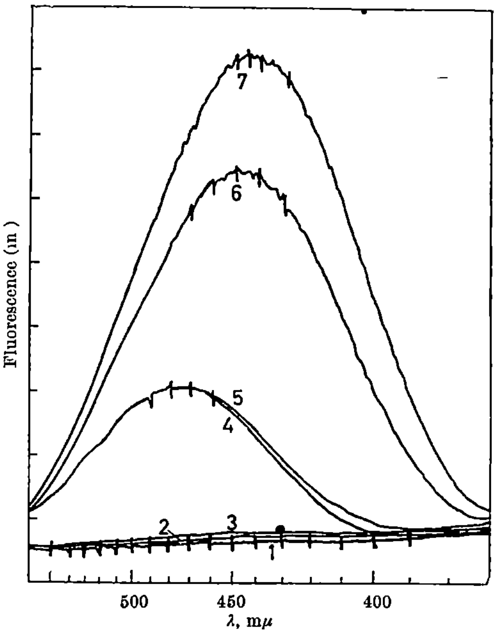


Fig 1 Fluorescence emission curves for LADH, DPNH and imidazole Excitation = 330 mμ
(1) Buffer (0 1μ phosphate, *p*H 7, 23 5° C), (2) buffer + 0 1 *M* imidazole, (3) buffer + 0 1 *M* imidazole + 2 2 μ*M* LADH, (4) buffer + 2 1 μ*M* DPNH, (5) buffer + 2 1 μ*M* DPNH + 0 1 *M* imidazole, (6) buffer + 2 1 μ*M* DPNH + 2 2 μ*M* LADH (*ER*), (7) buffer + 2 1 μ*M* DPNH + 2 2 μ*M* LADH + 0 1 *M* imidazole (*ERI*)

Table 2 summarizes the dissociation constants determined using the recording spectrophotofluorimeter (a Bausch and Lomb grating monochrometer to isolate the activating wave-lengths was coupled to a Beckman *DU* spectrophotometer with pen recorder to analyse the emitted fluorescence[5]) Except for $K_{E,O}$ and $K_{EO,I}$ the dissociation constants are essentially invariant with *p*H However, $K_{E,R}$ increases rapidly above *p*H 9 (ref 5), suggesting that SH-groups join the protein to the DPNH pyridine ring[15] The $K_{E,O}$ values were now higher than before, which resulted in the disappearance of previous disagreements between the equilibrium and kinetic values at lower *p*H (ref 5), the results now agreeing throughout with the Theorell–Chance mechanism They also show that with LADH–DPNH, the fluorescent and active sites are identical, which is apparently not the case with glutamic dehydrogenase[16] It is noticeable that at *p*H 6, *ER* is a thousand-fold more stable than *EO*, at *p*H 9 only twenty-fold, the change being caused largely by the increase in the stability of *EO* The variation of $K_{E,O}$ with *p*H was found to fit a monovalent dissociation curve which indicated the presence of a titratable group with a *pK* of 8 6

Table 2 DISSOCIATION CONSTANTS FOR LADH, COENZYME AND IMIDAZOLE COMPLEXES $\mu = 0\ 1$, 23 5° C

(The values are calculated using the concentration of un-ionized imidazole)

pH	$K_{E,R}$ μM	$K_{ER,I}$ mM	$K_{E,I}$ mM	$K_{EI,R}$ μM	$K_{E,O}$ μM	$K_{EO,I}$ mM	$K_{EI,O}$ μM
6	0 23	4 2	0 47	2 01	266	0 80	448
7	0 31	3 5	0 55	2 03	160	1 45	430
8	0 41	3 6	0 68	2 20	51	5 9	440
9	0 65	3 0	0 67	2 91	12	24 2	435

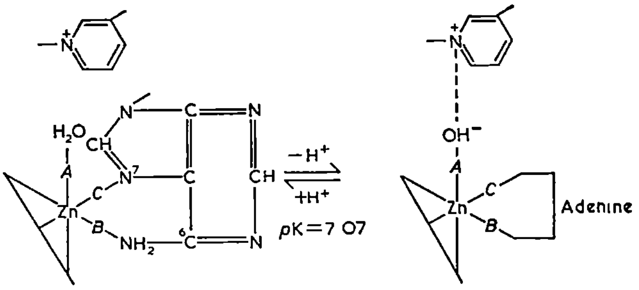
in the free enzyme, and of 7 1 in the binary *EO* (DPN–LADH) complex

Preliminary experiments with YADH indicated complex formation similar to that with LADH, while lactic dehydrogenase gave no such indication, which supports the hypothesis that imidazole and certain nitrogen compounds are joined to zinc and lactic-dehydrogenase does not contain zinc[17]

Kinetics with Imidazole and Inhibitors

Whereas with fatty acids and amides the ternary complexes were more stable than the binary[9], the opposite is the case with imidazole (Table 2) The influence on the kinetics of caprate, *iso*butyramide (IB) and imidazole was therefore studied For the general mechanism described here, $k_1 \approx k_7$ and $k_1' \approx k_7'$ This was found to be an example of a general rule that partners in a ternary complex do not seem to interfere appreciably with each other in regard to 'on' velocity constants, but mainly with the 'off' velocity constants, these increasing or decreasing to the same extent as there is mutual repulsion or stabilization in the complex While caprate and IB had only inhibitory actions, imidazole was interesting as at high concentrations of substrate and coenzyme it gave a stimulation of reaction velocity due to the formation of labile ternary complexes in the second phase of the reaction (Fig 2), but, when either concentration was low enough, inhibition occurred

Imidazole, which forms both the ternary complexes *ERI* and *EOI*, also competed with both aldehyde and alcohol This behaviour of imidazole, in view of its complexing properties with zinc, strongly suggests that zinc binds the substrates. That the binding of both coenzymes is weakened or disrupted by imidazole favours the idea that the adenine moiety which, unlike the pyridine ring, is the same in the coenzymes, forms a bidentate chelate to zinc[18] Inhibition experiments with the bidentate ligands orthophenanthroline and $\alpha\alpha'$-dipyridyl[19], and equilibrium experiments with glycine, all of which do not form ternary complexes and are strictly competitive with the coenzymes, as would be expected when only three bonds are free and the coenzyme–adenine forms a bidentate itself, support this They also indicate that the zinc bonds are octahedral[20], that zinc plays a central part in the binding of coenzymes and substrates[18,21], and that it is bound by three bonds to the protein In the natural β-isomer of DPNH the pyridine ring is so close to the adenine that energy transfer takes place between them[22], and, since adenine is presumably bound to zinc, the pyridine ring must also lie close to zinc In the free enzyme the free zinc bonds presumably hold water molecules, one of which at high pH (pH 8 6) loses a proton, the resulting attraction between ZnOH⁻ and the positively charged pyridinium ring of DPN accounting for the increase in the stability of the DPN–LADH complex with increasing pH



This does not operate with DPNH–LADH, where the pyridine ring carries no charge, while introduction of imidazole instead of OH⁻ eliminates the attraction effect, as is observed (Table 2)

Zinc is also implicated as the centre of the ternary complexes[18], one bond which, in *ER*, accommodates a water molecule and in *EO* is joined to the pyridine ring, now is occupied by the substrate. Caprate was found to compete with DPNH and alcohol, *iso*butyramide with aldehyde and DPN, and imidazole, with the exception of DPNH, with all four partners This suggests that alcohol and caprate have a different site of attachment from aldehyde and *iso*butyramide with imidazole able to go on either site Thus, while the *A*-bond of the DPN complex would be the bond at which alcohol is attached, it would not seem to be the bond for aldehyde However, two possibilities arise (*a*) *Two bonds* here a type of three-centre problem arises with one of the adenine bonds switching or flicking over Thus, alcohol would be on bond *A* and DPN across *B* and *C* During the rapid interconversion *EO*,alc. ⇌ *ER*,ald the adenine-N(7) would remain on bond *C* and the adenine-NH₂ would switch to the *A*-bond, DPNH thus being across *AC* and aldehyde on the *B*-bond (or the N(7) could flick to *A*, *C* becoming the aldehyde bond) Thus with DPN on two of the zinc bonds, *B* and *C*, the *A*-bond will only

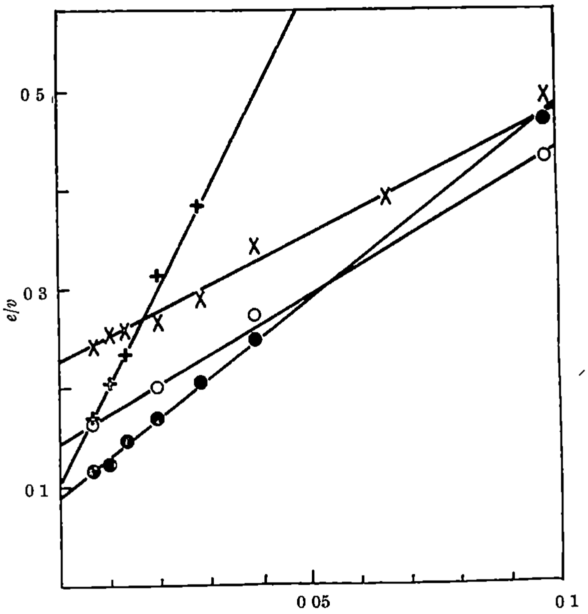


Fig 2 Stimulation and inhibition by imidazole (0 1μ phosphate, 23 5° C and pH 9) ×, No imidazole, ○, 2 mM, ●, 10 mM, +, 50 mM, [alc] = 6 1 mM

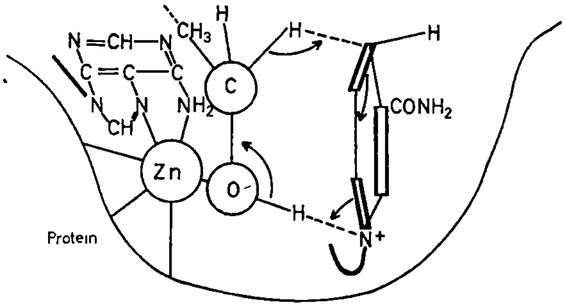


Fig 3 LADH ternary complexes (schematic)

accept alcohol, caprate or imidazole. DPN changing to DPNH implies a bond flick over, so that *B* (or *C*) becomes free, which only accepts aldehyde, *iso*butyramide or imidazole Consequently, as a result of a presumably small intermolecular rearrangement, the coenzyme determines which of two bonds is available, each bond determining what can become attached This concept gains some support from the different structures of the substrates *S* and *S'* and the stereospecific requirement that they change their position somewhat, relative to the enzyme surface[23] (*b*) *One bond* bond switching is unnecessary if the coenzymes stay on the same two bonds, but affect the third differently, so that it is a particular type of bond for each This is indeed the case with the binary complexes and presumably the positively charged pyridinium ring (DPN) allows or directs alcohol, caprate and imidazole on to the zinc bond, while the neutral pyridine ring (DPNH) only allows on aldehyde, *iso*butyramide and imidazole This mechanism, depending on the different charges of the pyridine (DPNH) or pyridinium (DPN) rings, with one bond and no molecular shifts, is of course attractive, but meets with some difficulties Perhaps the alcohol hydroxy group takes the place of the water molecule or OH^- involved in the Zn—OH-—N+ interaction (Fig 3) As has been remarked above, the bonds involved in the stability of the complexes are, apart from the zinc–protein bonds, weak to varying extents[24], but this seems necessary as strong bonds result in inhibition rather than catalysis

Thus in our opinion octahedral zinc is the centre of the ternary complex, although there have been suggestions previously that, like the protein, it is not joined to the substrates[25,26], but to the coenzyme pyrophosphate groups[26] The tertiary structure of LADH seems to depend on SH-groups[1], and, while the coenzymes bind to the enzyme through adenine, SH-bonds in addition seem necessary with DPNH, but not DPN, where there is the zinc–N+ interaction The amide group is not considered joined to the zinc, nor necessarily to the protein at another site, as the protein SH-addition to the pyridine ring in DPNH and the pyridinium–zinc bond in DPN can give the ring its necessary stereospecific rigidity[27]

This mode of bonding gives an explanation of how enzymes cause coenzymes and substrates to select, with great preference, their active reaction partners, while substances like imidazole, which can stimulate reaction at high substrate concentrations and inhibit at low substrate concentrations, could be physiologically significant in having a homeostatic function to maintain substrates at a suitable concentration An excess would be removed rapidly, but the process would be inhibited strongly when lower concentrations were reached

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ANTIGEN-ANTIBODY PRECIPITATION CONTRASTED IN GELS OF HIGH AND PHYSIOLOGICAL SALT CONCENTRATIONS

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RABBITS are the usual source of supply of precipitins for serological analyses. The chicken, however, is becoming increasingly popular for this purpose as an animal both readily housed in the laboratory and able to provide volumes of antiserum matching those produced by rabbits[1] The use of chicken antiserum in precipitin tests performed in liquid medium has led to the discovery that it differs from rabbit antiserum in usually, although not always, precipitating antigen optimally in electrolyte solutions of 10–12 times higher ionic strength than that of physiological saline[2] At these high electrolyte concentrations, specific precipitation of antigens by mammalian antisera is grossly inhibited[3]. The recent rise in popularity of chicken antiserum also has extended to its utilization in immunodiffusion tests, that is, in antigen-antibody precipitin tests carried out in gels. We wish to show here that it is false to deduce that if high salt concentrations are optimal for chicken precipitin reactions in liquid media they also must be in semi-solid media. Indeed, not only do physiological electrolyte concentrations seem to offer considerably better conditions than high concentrations for immunodiffusion tests with chicken antisera, but also at high salt concentration in these tests the chicken precipitin reaction is as much inhibited as that of mammalian antisera.

Bovine serum albumin (Armour 'Fraction V' from bovine plasma) was used as antigen for the experiments reported here. Although in this, albumin is the predominating antigen, immunodiffusion experiments with strongly hyper-immune antiserum show that it contains at least 6 other independent antigens (Fig. 1). Since the reaction of each of these with its particular antibody can be resolved and identified in these tests, our results apply at least to these 6 independent antigen-antibody systems examined simultaneously under identical conditions. By chance it happens that at least 2 other antigens also are involved, as constituents of chicken serum (Fig. 1, arrow), bringing the total of antigen-antibody systems at least to 8.

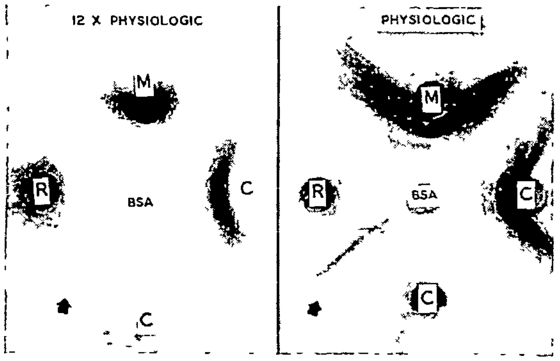

Fig. 1. Comparison in micro-immunodiffusion tests of specific precipitation produced against bovine serum albumin (*BSA*) by chicken (*C*), mouse (*M*) and rat (*R*) antisera at twelve times physiological and at physiological electrolyte concentrations. Arrow points to precipitin bands formed between rat serum and a chicken serum and presumably due to antigens found in the latter, for the rat had been immunized, in addition to *BSA*, with chicken ovalbumin, a preparation known to contain antigens in common with adult chicken serum

Mammalian antiserum is represented by mouse and rat sera; in respect to the purposes of the present investigation we have found them equivalent to comparable antisera obtained from rabbits and guinea pigs. Serum from these two species was used partly to emphasize the not widely known fact that both are useful precipitin producers. Animals from which antiserum was obtained had been vaccinated subcutaneously with water-in-oil emulsions of bovine serum albumin containing living avirulent (*H37Ra* strain) tubercle bacilli as adjuvant. For example, an animal was injected with 0.1 ml of emulsion containing 1 mgm of antigen and 1 mgm of bacilli. The injection was repeated a week later, and bleedings for antiserum were performed two weeks after the second injection. Our basic requirement was that an antiserum produce readily detectable specific precipitate with its antigen, and it has been satisfied most regularly by this particular immunizing procedure. However, several variations in vaccination protocol have been made, including deletion of water-in-oil emulsion during vaccination, without altering the basic facts pointed out here regarding mammalian antiserum and salt concentration in precipitin tests.

Chicken antisera were obtained by essentially the same procedures, although antigens were used in quantities five times larger. Again, however, vaccination procedures purposely were varied, including whether emulsions were used, to learn whether they conditioned qualitatively the results which were obtained. As among mammalian antisera, they did not. For experiments reported here, two chicken antisera were used, one of high precipitin content and the other with a content barely detectable in micro-immunodiffusion tests.

The crux of our experiments was to discover whether chicken antiserum which precipitates antigen better in aqueous medium at high electrolyte concentrations than at low also would in semi-solid medium, and how this would compare with findings for typical mammalian antisera. Therefore, it was necessary to show that chicken antisera which were to be used did indeed form more precipitate with antigen in high electrolyte concentrations and that mouse antiserum precipitated antigen best at physiological concentration. Optimal proportions for test antisera with antigen were determined at physiological and twelve times physiological salt concentration by timing the appearance of precipitates at the interfaces, in the classic ring test in aqueous medium, in series of tubes in which constant quantities of antisera were overlaid with varying dilutions of antigen. In both this and most immunodiffusion tests, the *p*H of diluent was held at 7.4 with sodium barbital buffering[4], electrolyte strength was varied solely by changes in sodium chloride concentration. Then, 1 ml volumes of antigen and antibody, used at their predetermined optimal precipitating ratios, were mixed in centrifuge tubes and allowed to stand for 1 hr at room temperature and then for 12 hr at 4° C. Specific precipitate was centrifuged out at 4° C and 41,000*g* applied for 30 min, washed twice with the same kind of buffer as used for specific precipitation, and quantitated with Boyd's technique for the Folin–Ciocalteu phenol reagent[3]. These tests established that mouse antiserum yielded 20 per cent as much specific precipitate at high salt concentration as at physiological, while chicken antiserum yielded 5 times as much at high as at low. With the classic responses of these respective antisera clearly established, their respective activities in immunodiffusion tests were next investigated.

Numerous immunodiffusion tests, most of them of micro type[5], were set up using these and other antisera and varying for them not only electrolyte concentrations but also the time of reaction development, temperature (4° or 24° C), type of buffering agent, purity of agar, and type of gel. The results from these experiments were consistent: like mammalian antiserum chicken antiserum produces heavier precipitin bands in larger numbers and more rapidly at salt concentrations equal to or close to physiological than it does at concentrations 10 or 12 times this, in which by contrast they react so well in aqueous media. Typical findings are illustrated in Fig. 1.

Our findings agree with those recently shown photographically for chicken antisera by Makinodan *et al.*[6], who also used bovine serum albumin antigen, but they are at variance with the assertions of Goodman[7] regarding the detection of certain human γ-globulin antigens with such antisera. Although possibly chicken antisera precipitate certain antigens in semi-solid media better at high salt concentrations than at low, just as they obviously do in liquid media, the present experiments suggest that this is the exception rather than the rule. Thus, none of the several antigen-antibody systems shown in Fig. 1 precipitated better (usually not at all) at high salt concentration. Precipitin band curvature for these suggests[1] that the molecular weights of antigens involved ranged from that of bovine serum albumin through that equivalent to mouse, chicken, and rat antibodies (that is, 65,000–180,000). However, in more extensive experiments at present being carried out, we are examining the possibility that human or other γ-globulin antigens reacting with chicken antiserum may indeed, because of some peculiarity of their own, precipitate better in immunodiffusion tests, as in aqueous precipitin tests, at high rather than at low electrolyte concentrations.

At present, we attribute the difference which we have found between liquid and gelled media in connexion with salt concentrations and chicken antiserum precipitin effectiveness to at least two significant differences between the physics of specific precipitation in the two media. One is that in the former, antigen and whole antiserum mix all at once mechanically, by convection, and by diffusion, while in the latter antigen and antibody, separated to some extent from other serum constituents, mix only by diffusion. The other is that in a gel antigen-antibody complexes probably are immobilized shortly after they form, so that if they have tendencies either to dissociate again or to cease growing larger, whence they might fail to precipitate in aqueous medium, these are counterbalanced by surrounding gel[8].

A few additional remarks concerning our experiments are appropriate. Inhibition of specific precipitation by mammalian and chicken antisera at high salt concentrations in semi-solid media occurs in both macro and micro double diffusion tests, although it is more obvious in the latter test because of its ability to resolve more individual precipitin bands. This inhibition occurs to similar extents in highly purified[4] as well as unpurified Difco 'Bacto' agar and in gelatin, and it is present in either barbital- or phosphate-buffered saline solutions. Superficially it seems to be greater in barbital-buffered solutions because these tend to provide resolution of precipitin bands superior to that realized with phosphate-buffered solutions.

This work was supported by U.S. Public Health Service grant *E*-3697.

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ACTION OF DEOXYRIBONUCLEASE AND RIBONUCLEASE ON THE GROWTH OF EHRLICH ASCITES CARCINOMA IN MICE

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THERE are few reports in the literature on the action of enzymes on cell growth. In tissue cultures, addition of deoxyribonuclease (DNAase) and ribonuclease (RNAase) affected the mitotic rate and the metabolism of the nucleic acids[1–6]. Haddow *et al.* have shown that *in vivo* xanthine oxidase had a definite action on the growth of spontaneous mammary tumours in mice[7,8] and Ledoux and Bergel have demonstrated that tumour growth was depressed by ribonuclease[9–11]. In relation with the above-mentioned facts, it is of interest that DNAase and RNAase activities could not be demonstrated in a variety of experimental and human tumours[12]. An *in vivo* study of the effect of DNAase, RNAase and xanthine oxidase on regenerating rat liver has shown that DNAase and xanthine oxidase affected significantly the proportions of the various mitotic phases whereas ribonuclease did not[13]. This situation prompted me to investigate the effect of DNAase on growth of tumours and to re-investigate further that of RNAase.

Swiss mice of an average body-weight of 20 gm were used. Ehrlich carcinoma in ascites form was transplanted intraperitoneally using 0·5 ml. of ascitic fluid containing an average number of 65 million cells. The total number of cells was determined as follows: the mice were killed by breaking their necks, their abdomens were skinned and a needle was introduced at the top of the abdominal cavity. The ascitic fluid was drawn out with a syringe, and the cavity was washed four times, once with 2-ml. and then with 1-ml. portions of 0·85 per cent sodium chloride solution. The ascitic fluid plus the washing liquid was adjusted to an exact volume and the cells were counted in an hæmatocymeter after staining with orcein[14].

Smears of ascitic fluid were prepared for metaphase counts and cytological examinations. They were stained by the Feulgen method[15] and, on some occasions, counterstained with methyl green[16]. At least 3,000 cells were counted at random to determine the incidence of metaphase.

In all the assays reported here the control mice were injected intraperitoneally daily with 0·5 ml. 0·85 per cent sodium chloride in a similar manner. Each morning the deaths were recorded in order to establish the survival-curves of both the control and the treated mice.

Deoxyribonuclease. Fig. 1 shows the percentage number of surviving mice in relation with time. The control and treated mice were injected daily from

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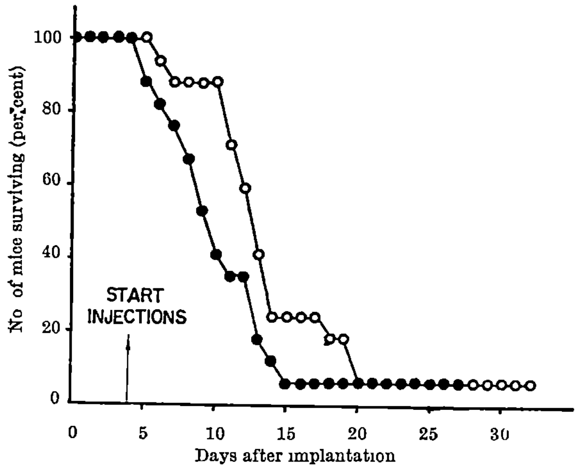

Fig 1 Effect of injections of DNAase on Ehrlich ascites carcinoma Mean life controls, 9 8 ± 3 2, treated, 13 1 ± 3 5 Increase, 34 per cent

the fourth day after implantation of the tumour except on the tenth and seventeenth day There were 17 mice in each group The curves show that when 50 per cent of the controls were dead, 88 per cent of the treated mice were still alive The mean life of the controls was 9 8 ± 3 2 days whereas the treated mice lived on the average 13 1 ± 3 5 days This increase of 34 per cent in the survival-time was statistically significant at a level better than 98 per cent

In each group there was a mouse still living 33 days after the transplantation of the tumour These mice were killed and examined The mouse in the control group did not show any apparent sign of tumour whereas the mouse of the treated group had a tumour mass of 1 5 cm in diameter

Fig 2 shows the survival-curve of the control and treated mice for an experiment in which the treatment was started on the day following the transplantation of the tumour The groups contained 10 and 9 mice respectively and the animals were injected daily When 50 per cent of the controls were dead, all the treated mice were alive The mean life of the controls was 7 2 ± 2 9 days whereas the treated mice lived 12 0 ± 3 6 days This increase of 67 per cent in the survival-time is statistically significant at a level better than 99 per cent

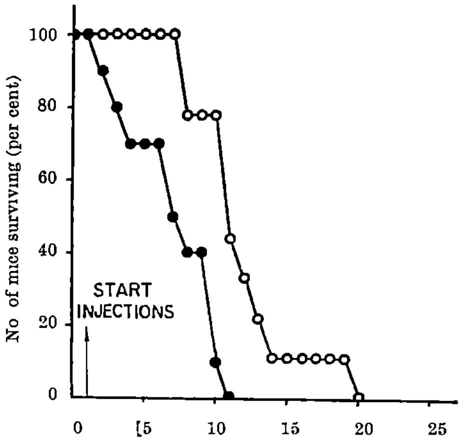

Fig 2 Effect of injections of DNAase on Ehrlich ascites carcinoma Mean life controls, 7 2 ± 2 9, treated, 12 0 ± 3 4 Increase, 67 per cent

Thus the injection of DNAase one day after the transplantation of the tumour instead of the fourth day doubles the increase in survival-time

From these results it would seem that DNAase exerted an action on the growth of Ehrlich ascitic carcinoma, and that the magnitude of this action depended on the time the injections were started following the transplantation of the tumours

The action of DNAase on the growth of Ehrlich ascites tumour cells was also ascertained by counting the total number of cells in control and treated mice The incidence of metaphase was also determined The mice were injected daily for four days starting the day following the transplantation of the tumour Table 1 shows that the total number of cells in the treated mice was one-third that of the control mice The number of cells in the controls from the day of transplantation to the fifth day, increased nearly six times whereas in the treated mice it increased only four-fold The incidence of metaphase in the treated mice was only one-half that in the controls, indicating that cell division was impaired in the presence of the injected DNAase

Table 1 EFFECT OF DNAASE ON CELL NUMBER AND INCIDENCE OF METAPHASE IN EHRLICH ASCITES CARCINOMA

Animals	Days	Total No of cells ($\times 10^{-6}$)	Incidence of metaphases
Controls	0	56	1 5 ± 0 3
	5	320	
Treated	0	56	
	5	230	0 7 ± 0 3

Daily injections of enzyme started the day following the implantation of the tumour

The action of the injected DNAase on the deoxyribonucleic acid of the cells was followed by Feulgen staining Examination of Feulgen-stained smears of tumour cells from control and treated mice indicated that the nuclei of the tumour cells from the treated mice were not stained at all or were much less stained than those from the controls This shows that some deoxyribonucleic acid of these cells was most probably destroyed by the enzyme A large number of cells also contained Feulgen-positive material in their cytoplasm

These facts seem to indicate that the injected DNAase acts on the growth of the tumour, impairing cell division by attacking deoxyribonucleic acid Similar results were obtained by Chevremont *et al* [2-5] on the action of DNAase in tissue culture These authors reported that DNAase impaired the growth of fibroblasts and myoblasts of chicken embryos in tissue culture and stopped cell division A lack of DNA was observed in the nucleus and some found in the cytoplasm

The activities of five enzymes of the purine-catabolizing enzyme system have been determined as previously described[17] in control and treated mice to gain information on the possible effect of DNAase injections since the enzyme seemed to depolymerize the deoxyribonucleic acid of the cell Table 2 shows that the activities per average cell of 5-adenylic acid nucleotidase, inosine phosphorylase, guanase, xanthine oxidase and uricase were very low in Ehrlich ascites tumour cells, even lower than in primary liver tumours or Novikoff hepatoma[17,18] Injections of DNAase for four consecutive days did not seem to affect the activities of these enzymes except for 5-nucleotidase, which was increased about

Table 2 EFFECT OF DNAASE ON PURINE CATABOLIZING ENZYMES IN EHRLICH ASCITES CARCINOMA

Enzymes	Activity in μM/min /cell (× 10^9) Controls	Treated
Uricase	0	0
Xanthine oxidase	0 021 ± 0 013	0 024 ± 0 009
Guanase	0 24 ± 0 10	0 21 ± 0 11
Inosine phosphorylase	1 43 ± 0 37	1 14 ± 0 42
5-nucleotidase	0 38 ± 0 21	2 13 ± 0 70

six-fold However, this activity was still so low that the significance of this increase was questionable It would thus seem that, under the present conditions, the catabolism of purine or purine derivatives was not affected

Ribonuclease Fig 3 shows the percentage number of surviving mice in relation with time The injections were started the day following the transplantation of the tumour Both groups contained thirty mice, and the animals were injected daily It can be seen from the curves that the period of 50 per cent death was the same for the two groups The mean life of the controls was 8 3 ± 3 9 days whereas the treated mice lived on for 10 1 ± 4 5 days This increase of 22 per cent in the survival-time was not statistically significant

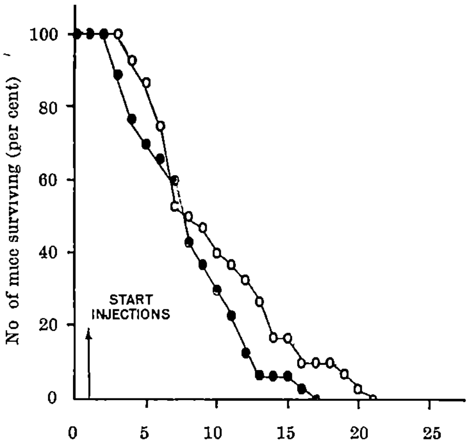

Fig 3 Effect of injections of RNAase on Ehrlich ascites carcinoma Mean life controls, 8 3 ± 3 9 , treated, 10 1 ± 4 6 Increase, 22 per cent

To ascertain that the difference between the action of RNAase and DNAase was not due to a lack of penetration of the RNAase in the cells, ribonuclease activity of the ascites cells and of the ascitic fluid from control and treated mice has been determined Immediately after withdrawal of the fluid with a syringe, the cells were centrifuged and re-suspended in sodium chloride, and RNAase activity was determined on both cell suspension and supernatant as previously described[19] Table 3 shows that the RNAase activity, either at acid or alkaline pH, was the same for both the supernatant and the cell re-suspension for the controls For the treated mice, however, the enzyme activity of the cell re-suspension was more than twice that of the supernatant Furthermore the ribonuclease activity, measured at acid pH, of the treated cells was nearly five times that of the control cells, and the activity, measured at alkaline pH, nearly seven times This indicated that the injected enzyme penetrated inside the cells as shown by Ledoux and Pileri[20] and that the lack of effect on tumour growth was not due to a lack of penetration of the enzyme

Table 3 EFFECT OF RNAASE INJECTION ON THE ENZYME-LEVEL OF THE CELLS

	Ribonuclease activity* Acid Controls	Treated	Alkaline Controls	Treated
Supernatant	0 070	0 127	0 110	0 426
Cells	0 058	0 262	0 134	0 902

* Expressed in O D

The lack of action of RNAase on the growth of Ehrlich ascites carcinoma does not confirm previous results of Ledoux, who reported significant effect of the enzyme on the growth of this tumour[9] However, this might be due to a different source of RNAase since Ledoux has shown that the biological activity of different RNAases from various sources varied tremendously Furthermore, the tumour inoculum, in the present work was much larger than that employed by Ledoux and the injected dose of RNAase was much smaller

Conclusions Injections of DNAase increased the survival-time of mice bearing the Ehrlich ascites carcinoma by 34 and 67 per cent, depending on the time the injections were started following transplantation This increased survival-time was accompanied by a decreased metaphase index and by the disappearance of deoxyribonucleic acid from a number of cell nuclei The injections of DNAase had no effect on the activities of five enzymes involved in the catabolism of purine and purine derivatives

Injections of RNAase had no significant action on the survival-time of mice bearing Ehrlich ascites tumour This lack of action was not due to a lack of penetration of the enzyme inside the tumour cells

The advice of Dr R Daoust for the cytological examinations is gratefully acknowledged The work has been supported by grants to Dr A Cantero, director of the Research Laboratories, from the National Cancer Institute of Canada

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VACUOLATION AND LEAF REGENERATION IN *SPLACHNUM AMPULLACEUM* (L.) HEDW.

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Formation of Secondary Vacuoles in De-differentiating Cells

IT was shown in ref 1 that cellular de-differentiation in *Splachnum* involves profound changes in the intracellular configuration, one of the first signs of this is the formation of intravacuolar cytoplasmic lamellæ leading to systrophe This communication is concerned with the formation of these intravacuolar cytoplasmic layers and some considerations arising from these observations

Plant material and methods are the same as described in ref 2 Leaves isolated from three-week-old plants were, however, transferred into liquid Beijerinck solution adjusted to *p*H 6 8 Regeneration takes place readily from isolated leaves at this *p*H

Fig 1 represents a composite drawing of a leaf cell in cross-sectional view parallel to the leaf surface showing various stages in the development of the primary cytoplasmic lamellæ It is evident that these lamellæ originate in the form of secondary vacuoles These vacuoles are formed in various parts of the peripheral cytoplasm, predominantly in the vicinity of the nucleus One secondary vacuole expands initially into the central vacuole and moves about considerably until it reaches ultimately the peripheral cytoplasm across the vacuole Fusion of separate intravacuolar cytoplasmic layers appears to take place not infrequently Lamellæ may originate also in the form of a secondary vacuole from the peripheral cytoplasm opposite the nucleus expanding toward the nucleus Secondary vacuolar formation continues over considerable periods of time from other sites of the peripheral cytoplasm during and after the formation of the primary intravacuolar cytoplasmic lamellæ

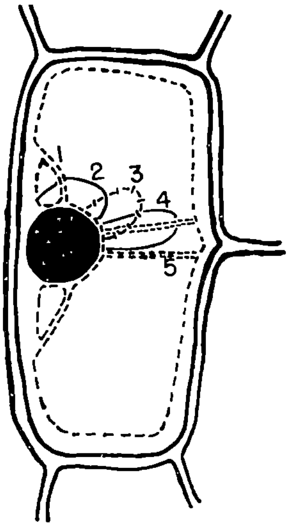

Fig 1 Composite drawing of leaf cell (marginal position) showing formation of primary intravacuolar cytoplasmic lamellæ The numbers represent successive stages in the expansion of a secondary vacuole

The pattern described is characteristic of cells near the leaf margin In other portions of the leaf, only one primary intravacuolar lamella is formed initially, originating in the form of a secondary vacuole from the peripheral cytoplasm, frequently not in the vicinity of the nucleus

The similarity between secondary vacuole formation in de-differentiating cells and that in plasmolysed cells should be noted Haberlandt[3-5] observed intravacuolar lamellar structures in plasmolysed cells and his observations were fully confirmed by those of Pfeiffer[6] Both Haberlandt and Pfeiffer found cellulose incorporation into the intravacuolar lamellæ Kuster[7] has been unable to confirm this incorporation, and doubts, therefore, that intravacuolar formation of lamella has anything to do with cross-wall formation or cell-division Although the present work has not been concerned with this question, cellulose incorporation is unlikely to occur up to the time of phragmosome formation and would appear to be restricted to it Haberlandt[5] suggested, on the basis of his interpretation of the nature and fate of the lamellæ, that divisional substances are normally present in cells which increase in concentration following the decrease in water concentration resulting from the plasmolytic treatment of the cell Division, according to Haberlandt, may set in, once a critical concentration in divisional substances has been reached The present results make this explanation unlikely since plasmolysis and, therefore, loss of water do not appear to take place and since primary intravacuolar cytoplasmic lamella formation does not represent division Pfeiffer[6] concluded on different grounds that Haberlandt's proposed mechanism can at least not be the primary one

Kuster[8] has discussed the ontogeny of intravacuolar lamellæ in plasmolysed cells and concluded that they are the result of a process of furrowing of the primary vacuole and do not arise as a result of secondary vacuole formation from the peripheral cytoplasm This conclusion cannot be maintained considering the present results and those of Wartenberg[9] Wartenberg has recently given strong evidence that plasmolysis represents an active swelling process, that is a process of active vacuole formation in the outer peripheral cytoplasm The present observations on early stages of secondary vacuole formation resemble Wartenberg's on secondary vacuoles arising in plasmolysed cells Similarities between changes in the intra-cellular configuration of de-differentiating cells and that of plasmolysed cells have been noted earlier[2] The resemblance between the formation of a secondary vacuole in plasmolysed cells and in non-plasmolysed but de-differentiating cells stressed in the present discussion appears to be significant It may be suggested that plasmolytic treatment results in the initiation of cellular de-differentiation This is supported by observations, for example, Isaburo, Nagai[10], that plasmolysed cells may regenerate, that is de-differentiate and re-differentiate, when the plasmolytic treatment is followed by deplasmolysis Secondary vacuole formation would then generally represent early stages in the process of cellular de-differentiation It may not be sufficient to explain the colloid chemical behaviour of the cell in terms of an interaction between the cell and the environment (for example, plasmolyticum), it may rather have to be considered also in terms of the interaction between different cells or the lack of it, that is, in terms of the correlative influences between different parts of the organism

Effect of Kinetin on Attached Leaf Regeneration

It has been shown recently[11] that the realization of regenerative potentialities in cells of a leaf attached to a stem segment isolated from a gametophore is inhibited by this stem segment, while the presence of the attached leaf increases regeneration from cells of the stem segment In the course of an investigation into the nature of this correlation system, the effects of locally applied kinetin have been investigated

Stem segments with one attached leaf from which the distal third was removed were isolated from the middle region of the gametophore, one segment for each gametophore They were planted on Beijerinck's medium solidified with 2 per cent agar with various concentrations of kinetin added, only the region near the wound surface of the attached two-thirds leaf being in contact with the medium They were grown under continuous white light provided by daylight fluorescent lamps. Gametophores used were always 20–21 days old

The number of protonematal regenerates for the attached two-thirds leaf was determined three days following planting and the results are shown in Fig 1 Regeneration is almost absent in attached leaves planted on the medium without kinetin In the presence of certain kinetin concentrations, however, attached leaf regeneration is promoted It should be noted that the effective concentration range shown in Fig 2 was obtained only with freshly dissolved kinetin Kinetin dissolved and kept in the refrigerator showed effectiveness only at higher apparent concentrations Kinetin locally applied to the distal end of the attached leaf may therefore overcome, to some extent, inhibition of attached leaf regeneration The stem segment represents apparently a region of attraction for substances essential for regeneration and produced predominantly by the leaf The drainage of the leaf by the stem prevents attached leaf regeneration while it promotes regeneration from the stem segment Basipetal transport of these substances is locally counteracted in the leaf by kinetin application, transport taking place also toward the site of kinetin application leading to regeneration in cells near this site

This suggestion is supported by the following experimental observations When intact gametophores are grown on Beijerinck's medium containing twice the normal ammonium nitrate concentration, increased regeneration takes place from a two-thirds leaf attached to a stem segment in the absence of kinetin It appears that in this case nitrogenous compounds are produced at higher concentrations and that basipetal transport of these substances from the leaf toward the stem segment does not lead to concentrations in the leaf limiting regeneration

The present concept of the nature of the correlation system and kinetin action described here is supported by the original observations by Richmond and Lang[12] and the extensive investigations by Mothes *et al* [13] on the effects of kinetin on the directional transport of soluble nitrogenous compounds Localized application of kinetin in isolated leaves of *Nicotiana rustica* led to a localized accumulation and retention of soluble nitrogen compounds against concentration gradients, these substances being available for renewed protein synthesis Since regeneration involves increased synthetic activities, particularly in terms of proteins (see ref 14 for present material), such accumulation would appear to be significant also for regeneration In Heitz's view[15], accumulation of sugars is the most important factor for the initiation of regeneration in isolated leaves Although regeneration did not result, he obtained increases in nuclear size by applying sucrose to leaves Stange[16] observed a dependence of increases in size of nucleus in regenerating cells of *Riella* on length of periods of photosynthesis

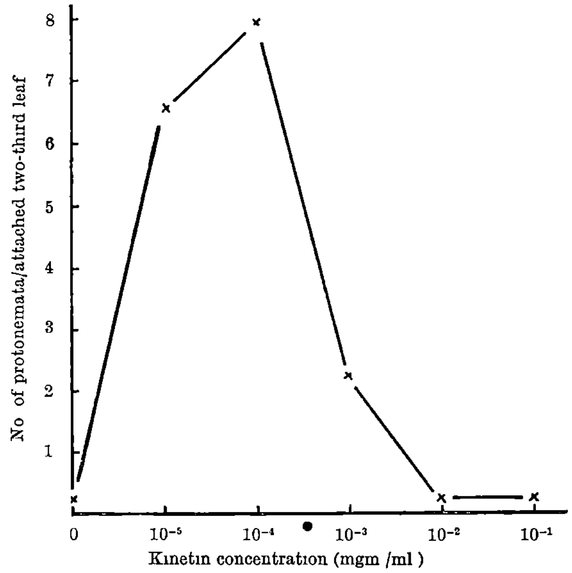

Fig 2 Protonematal regeneration from two-thirds leaves attached to a stem segment in the presence of various concentrations of kinetin

The present interpretation of the system described here may have wider applications for correlations inhibiting the realization of regenerative potentialities and the distribution of processes of dedifferentiation taking place in normal plant development[14]

This work was supported by a grant from the National Research Council of Canada

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LETTERS TO THE EDITORS

COSMOLOGY

Dirac's Cosmology

DIRAC's cosmology was based on the principle "that any two of the very large dimensionless numbers (of the order 10^{39} and 10^{78}), occurring in Nature, are connected by a simple mathematical relation in which the coefficients are of the order of magnitude unity"[1] From this principle Dirac deduced that the gravitational 'constant' $G \propto (\text{epoch})^{-1}$ In order to describe a cosmological model according to the principles of the general theory of relativity he then changed the units of distance and time so as to make G constant With the new units the metric can be written

$$ds^2 = c^2 dt^2 - R^2(t)\,(dx^2 + dy^2 + dz^2) \qquad (1)$$

with $R(t) = t^{2/3}$ Afterwards I considered this model, and by making certain assumptions, which appeared reasonable, obtained $G \propto \frac{1}{t}$, $t \approx 4 \times 10^9$ years[2]. This gives the values

$$H \approx 160 \text{ km /sec /}10^6 \text{ parsec}, \; q = -0\;5 \qquad (2)$$

for Hubble's constant $H\left(= \frac{R}{R}\right)$ and the acceleration parameter $q\left(= \frac{RR}{R^2}\right)$

Recently, L Egyed[3] has proposed a theory of the origin of the solar system which uses these results $\left(G \propto \frac{1}{t}, t \approx 4 \times 10^9 \text{ years}\right)$, and P Hédervári[4] has used the same results in a theory of the internal structure of the Moon My main object in this communication is to point out that the assumptions made in these interesting theories are not necessarily associated with the values of H and q given above, but may also give the values

$$H \approx 80 \text{ km /sec /}10^6 \text{ parsec}, \; q = -2 \qquad (3)$$

from a different description of the Dirac model in Riemannian space-time It seems advisable to point this out, since the empirical values of H and q may soon be sufficiently accurate to be able to distinguish between (2) and (3)

Rindler[5] shows how cosmological models, including those which are not described according to the principles of the general theory of relativity, may be described in Riemannian space-time, and he gives a metric for the Dirac model of the form (1) with $R(t) = t^{1/3}$ This method of discussing the model is based on the results derived from Dirac's principle, and does not provide a theory of gravitation for the model Recently, however, I have shown that a theory of gravitation with $G \propto \frac{1}{t}$ may be based on field equations derived from a principle of stationary action[6] In that theory the characteristic metric was (1) with $R(t) = t^{1/3}$, and the gravitational theory may therefore be associated with the Dirac model If one assumes $t \approx 4 \times 10^9$ years, this description of the Dirac model gives (3) for H and q

When one considers the empirical values of H and q it is of interest to observe that McVittie[7] discussed the observational results available up to July 1955 and found values for H of 143–227 km /sec /10^6 parsec Later, Sandage[8], in studying galaxies in the Virgo cluster, found that Hubble mistook glowing H II regions for brightest stars and, by applying corrections to Hubble's data, found that H can range between 50 and 100 km /sec /10^6 parsec with a very large uncertainty De Vaucouleurs[9] suggested that the local group is part of a super cluster of galaxies, and, by making allowance for the velocity of our own system which would occur on account of the differential rotation and expansion of the cluster, he obtained the value $H \approx 140$ km /sec /10^6 parsec The empirical value of q is also very uncertain at present Humason, Mayall and Sandage[10] give $q = -3\;0 \pm 0\;8$ and Baum[11] finds from photoelectric determinations of red shifts that q has a value in the range $-0\;5$ to $-1\;5$

Thus it would appear that no definite conclusions can be reached from consideration of the empirical results, and that the values of H and q given by either (2) or (3) are within the ranges of these results

Finally, consider the assumption that $t \approx 4 \times 10^9$ years, in relation to some recent astrophysical calculations Hoyle[12] has estimated the time required for the evolution of certain stars as $> 10^{10}$ years, but in his calculations he has taken $G =$ constant, whereas in the Dirac model $G \propto \frac{1}{t}$ Hoyle's figures are not, therefore, strictly comparable with the assumption that the age of the universe $\approx 4 \times 10^9$ years This age is, however, too small to allow time for the synthesis of the heavy isotopes of uranium in a star ($\approx 6\;6 \times 10^9$ years), by the process described by Burbidge, Burbidge, Fowler and Hoyle[13]

I am indebted to Dr G J Whitrow for information concerning the empirical results

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RADIOPHYSICS

Spectral Characteristics of Type II Solar Radio Bursts

IT has been recognized for some time that Type II solar radio bursts are due to the excitation of plasma oscillations in the solar corona by some exciting agency which moves through the corona. Roberts[1], in reviewing observations and theories of these phenomena, leaves unexplained two salient characteristics: (1) the appearance of radiation at the fundamental and second harmonic at comparable powers and the lack of radiation at all higher harmonics; (2) the frequent splitting of each of these two 'lines' into a pair of lines, the fractional splitting of each of these two pairs being the same, typically a few per cent.

Plasma oscillations are inherently non-radiative, so that we must ascribe our observation of these oscillations to their coupling to electromagnetic waves. In a homogeneous plasma, such coupling occurs only through non-linearity of the field and dynamical equations. It has been pointed out elsewhere[2] that the dominant coupling process is between two plasma-oscillation modes and one electromagnetic wave, which leads to radiation at $2\omega_p$, that is, twice the plasma frequency ω_p, where $\omega_p{}^2 = 4\pi ne^2/m$, and that this is the key to understanding of point (1). The next most important non-linear process couples three plasma-oscillation modes and one electromagnetic wave, and leads to radiation at ω_p and at $3\omega_p$ with comparable intensities. Since the third harmonic is not observed, we may conclude that the plasma-oscillation amplitude (measured as a fractional change in electron density) is small, and that emission at the fundamental is not due to non-linearity. Radiation at the fundamental may be ascribed to inhomogeneity of the coronal plasma, due to thermal fluctuations, to turbulence, or to a shock wave associated with the exciting agency.

In order to understand point (2), we should now consider the plasma to be permeated by a magnetic field. In the 'electrostatic' approximation, $\omega/k \ll c$, the dispersion relation has the form

$$\omega^2 = \tfrac{1}{2}\left[\omega_p{}^2 + \omega_g{}^2 \pm (\omega_p{}^4 + \omega_g{}^4 - 2\omega_p{}^2\omega_g{}^2\cos 2\theta)^{1/2}\right]$$

where θ is the angle between the wave vector k and the magnetic field vector B, and ω_g is the electron gyro-frequency eB/mc. It is noteworthy that ω does not depend on $|k|$. For $\omega_g < \omega_p$, this separates into two bands, one extending from 0 to ω_g and the other from ω_p to $(\omega_p{}^2 + \omega_g{}^2)^{1/2}$, the former of which one would not expect to radiate through the corona. Since one observes two closely spaced frequencies rather than a wide band, we must inquire whether some modes of the upper band are more strongly excited than others.

The significance of group velocity in the problem of the excitation of waves in a plasma by a moving source of finite dimensions has been recently discussed[3]. The problem may be regarded as that of the excitation of a resonator by an aperiodic force, and we know that the excitation varies proportionately with the Q of the resonator. Modes of highest Q will be those which lose their energy most slowly. If the wave-number spectrum and the dimensions of the source are subject to certain limitations, then loss of energy radiation, by electron-ion collisions and by Landau damping, will be subordinate to loss of energy by convection, a process which is characterized by the group velocity. We then expect that the most highly excited modes will be those of lowest group velocity.

By setting $\partial\omega/\partial k_x = \partial\omega/\partial k_y = \partial\omega/\partial k_z = 0$, we may find the modes of zero group velocity. Since ω here depends only on θ, the condition reduces to $d\omega/d\theta = 0$, which is satisfied at $\theta = 0$ and at $\theta = \pi/2$. Hence, considering only the upper band, we should expect the frequencies ω_p and $(\omega_p{}^2 + \omega_g{}^2)^{1/2}$ to be preferentially excited. It is proposed that these are the two frequencies to be identified with the pair of frequencies of the observed spectrum when it is split. The separation is seen to be approximately $\tfrac{1}{2}\omega_g{}^2/\omega_p$. This leads typically to estimates of 20 gauss for the strength of the magnetic field in the region associated with the beginning of a Type II burst.

The above interpretation of the spectral characteristics of Type II bursts would make it possible to obtain, from observations, information about the magnetic field in the solar corona.

The observed frequency ratio of 'first harmonic' to 'fundamental' is slightly less than 2:1. This effect has been attributed[4] to the propagation characteristics of the corona. It may be due in part to differences between the coupling mechanisms for certain forms of the plasma oscillation and inhomogeneity spectra, one finds that radiation at the fundamental is due to a band of wave numbers, and hence of frequencies, higher than that due to radiation at the second harmonic, thermal velocities of the plasma electrons now being taken into account.

An amplified version of the above arguments and a discussion of the nature of the exciting agencies of Type II and Type III bursts will be given in a forthcoming article.

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Decimetre Radio Bursts Concurrent with Solar Type IV Radiation

IT is known that when there is an important H_α flare on the Sun's disk there is often considerable radio emission over a wide band of frequencies. While the spectrum at metre wave-lengths is relatively well understood, the decimetre and centimetre radio bursts have not been related very clearly to the overall spectrum.

Especial interest is attached to the broad-banded centimetre and decimetre radiation that occurs concurrently with the metre wave-length continuum emission, known as Type IV radiation, and thought to be 'synchrotron' emission. Takakura[1] suggested that the microwave emission might be an extension of the metre wave-length Type IV spectrum to shorter wave-lengths.

From many observations of 1,420-Mc./s. solar radio bursts with the Christiansen crossed-grating interferometer used to provide a fan-beam (2′ of arc to half-power in the east–west direction) we have chosen for study eight occasions characterized by intense Type IV activity at metre wave-lengths

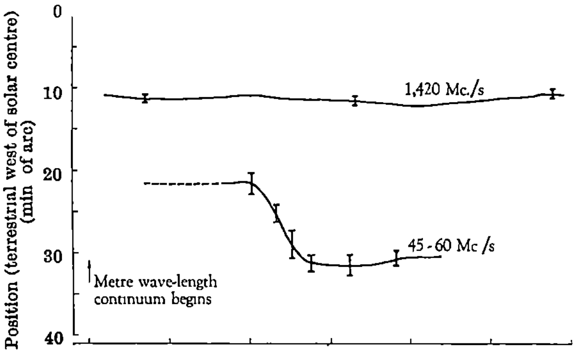

Fig 1 Position of decimetre and metre wave-length sources, July 29, 1958

Accurate estimates of position were obtained at 4-min time intervals, and were compared with the position of the Type IV source in the frequency range 45–60 Mc /s While the metre wave-length sources were located in the high corona, the 1,420-Mc /s sources remained at rest in the chromosphere or lower corona, probably at the same height as the H_α flares and certainly not more than about 50,000 km. above them The positions of the microwave burst and the flare were always closely coincident The low-frequency sources showed large-scale movements (presumably outward) on two occasions (one of which is shown, Fig. 1), while the decimetre source was stationary

Typical sizes of the 1,420-Mc /s sources were 2′–5′ of arc, and brightness temperatures of 10^8–10^9 ° K, were observed concurrently with the Type IV continuum at lower frequencies, indicating a non-thermal mechanism at decimetre wave-lengths.

Since the decimetre burst and the metre wave-length Type IV source are located at widely different parts of the solar atmosphere they must be different This conclusion supports a recent view of Takakura and Kai[2], who suggest, from spectral considerations at 4,000-Mc /s, that the microwave bursts "are not the high-frequency component of Type IV bursts on metre-waves but another distinctive type of burst emitted from radio sources fixed in the coronal condensations"

These results were first presented at an Institute of Physics Symposium (Brisbane, May, 1961)

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PHYSICS

Gravitational and Electrostatic Forces

In a recent communication[1], it was pointed out that dimensional difficulties met with in the usual gravitational equation $F = GM^2L^{-2}$, where F is the attractive force and G the gravitational constant, are avoided if the mass of a body is replaced by N_1e, where N_1 is the number of positive (or negative) charges contained in the body and e is the elementary charge It then follows that for two bodies placed a distance r apart and containing N_1 and N_2 charges, respectively, $F \propto N_1N_2e^2r^{-2}$

For two electrons, the ratio between their electrostatic repulsion and their gravitational attraction is close to 4 2 × 10^{42} Attempts have been made, notably by Eddington, to explain this enormous value, but without much success It is now possible to make a fresh approach along the lines suggested here

Consider two very small bodies r cm apart and each containing 1 gm of matter, and suppose that each body contains N protons (or electrons), a neutron for our present purpose being regarded as equivalent to one proton and one electron Then the electrostatic force in dynes between the protons (or electrons) in the two bodies is N^2e^2/r^2 and the gravitational force between the protons is very close numerically to G/r^2 If the ratio of proton mass to electron mass is α, the gravitational force between the groups of electrons in the two bodies is G/α^2r^2, and the ratio of electrostatic force to gravitational force is $N^2e^2\alpha^2/G = R$, where R is the numerical value of this ratio Thus R/N^2 is a pure numeric, equal to 4 23 × 10^{42} divided by (6 023 × $10^{23})^2$ The result, 1 166 × 10^{-5}, is equal to $e^2\alpha^2/G$ and it is reasonable to inquire if this value can be derived in terms of the fundamental constants e, h and c, where h is Planck's constant and c is the velocity of light *in vacuo* Only one dimensionless combination of these constants is known, namely, $2\pi e^2/hc$, equal very nearly to 1/137, and it is natural to see whether this quantity can be utilized. A possible relationship is $\frac{e^2\alpha^2}{G} = \frac{2}{9}\left(\frac{2\pi e^2}{hc}\right)^2$, since $\frac{e^2\alpha^2}{G}\left(\frac{hc}{2\pi e^2}\right)^2 =$ 0 219 when the experimentally determined values are substituted. Assuming that $\frac{\alpha^2}{G} = \frac{2}{9}\left(\frac{2\pi e}{hc}\right)^2$, it is tempting to split this equation into separate expressions for α^2 and G One possibility is

$$\alpha^2 = \frac{4\pi e(q_0 = 1)}{9hc} \text{ or } \alpha = \frac{2}{3}\sqrt{\left(\frac{\pi e}{hc}\right)}, \text{ numerically, and}$$

G (referred to masses of 1 gm)

$$= \frac{hc\,(q_0 = 1)}{2\pi e} = \frac{hc}{2\pi e}, \text{ numerically}$$

It is necessary to introduce q_0, with the dimensions of charge, into these equations to make them dimensionally correct, and $q_0 = 1$ simply means that q_0 is the charge which when placed 1 cm away from a similar charge causes an electrostatic force of 1 dyne to act between them, the unit of mass being 1 gm If the unit of mass is changed, q_0 and G also change, but α is unaltered Using the best available data for e,

$$h \text{ and } c,\ hc/2\pi e = 6\ 58 \times 10^{-8} \text{ and } \frac{2}{3}\sqrt{\left(\frac{\pi e}{hc}\right)} = 1{,}837\ 4.$$

These results compare with the experimentally determined values 6 67 × 10^{-8} and 1,836 1 The slight disagreement between the calculated and experimentally determined values of G is not surprising, since the assumption that mass is proportional to the number of positive (or negative) charges is not strictly true For hydrogen there are 1 008 mass units for one pair of positive and negative charges, whereas for most of the Periodic Table the ratio is close to 1 mass unit per pair of charges In every atom but hydrogen, mass is used in stabilizing the nucleus, and since the electrical value of the gravitational constant, $hc/2\pi e$, takes no account of this loss of mass, the value 6 58 × 10^{-8} must refer to hydrogen atoms Now the gravitational force between two ordinary bodies r cm apart may be expressed as $F = GN_1N_2/N^2r^2$, where N in this case is the number of protons in 1 gm. of average matter. For hydrogen N is replaced by $N/1.008$, so that $F = (1.008)^2N_1N_2hc/2\pi N^2r^2e$. The normal value of G is therefore equal to $1.008^2 \times 6.58 \times 10^{-8}$, that is, 6.68×10^{-8}, in close agreement with the experimentally determined value.

For two 1-gm. masses of hydrogen r cm. apart, the gravitational force between the electrons is given by $G/(1 + \alpha)^2r^2$ rather than G/α^2r^2, so that the calculated value of α, 1,837.4, should really be equated to $\alpha + 1$. Thus the ratio of proton mass to electron mass becomes 1,836.4, in excellent agreement with the experimental value.

Summarizing, it is possible, apart from minor corrections, to represent the electrostatic force between two charged bodies and also the gravitational force between protons and between electrons by the following equation:

$$F = \frac{n^2N_1N_2e^2}{8kr^2}\left(\frac{hc}{\pi e^2}\right)^{n-1}$$

where n has, respectively, the values 1, 2 and 3 for the three cases in the order given, and k is a dimensionless constant with numerical values as follows:

n	1	2	3
k	1/8	N^2e	N^2

For electrons and protons contained in each case in 1 gm. of matter and placed 1 cm. apart, the gravitational force in dynes is given numerically by

$$F_{e,p} = \frac{3}{4}\left(\frac{hc}{\pi e}\right)^{3/2}$$

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Radio-Luminescence in Sodium Chloride and Potassium Chloride

Work on radio-luminescence in rock salt and fluorite and other naturally occurring minerals excited by X-rays and nuclear radiations has been reported by some workers and summarized by Przibram[1]. The present communication deals qualitatively with the production and quenching of luminescence in pure and additively coloured crystals of sodium chloride and potassium chloride, excited by X-rays from a copper target.

A narrow beam of X-rays was allowed to enter a lead box impervious to light through a small circular aperture. The crystal was mounted on a holder with 'Apiezon *Q*', and was centred in such a way that the X-ray beam fell squarely on to the crystal. 'Apiezon' was found to scatter a minimum of X-rays. An E.M.I. 9502 photomultiplier was mounted at right angles to the direction of the X-ray beam, and was made to look on to the crystal through a lead glass window and a shutter. The recording of continuous readings was made possible by such an arrangement, without any interference from the X-ray beam. The micro-ammeter readings at the output of the photomultiplier gave a measure of the luminescence. The photomultiplier was worked with a cathode to first dynode voltage of 160 V. and subsequent interdynode voltage of 80 V.

The crystals were continuously irradiated with 30-keV. X-rays at 10 m.amp., and at various time intervals the luminescence output for different intensities was noted. In Figs. 1 and 2 the luminescence output is plotted against time of exposure for sodium chloride and potassium chloride, at all the intensities used. In both cases the luminescence output is found to rise steeply in the first few minutes and to fall slowly to the saturation value as more and more luminescent centres are destroyed.

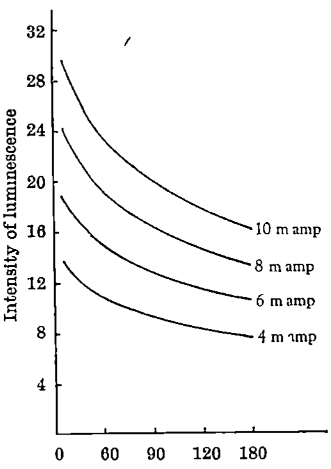

Fig. 1. Results for sodium chloride

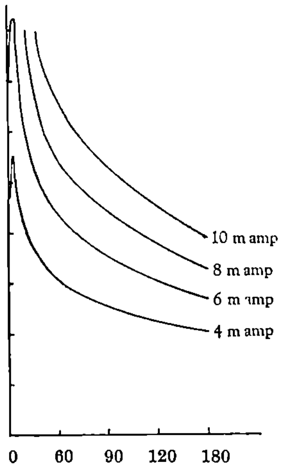

Fig. 2. Results for potassium chloride

The luminescence output is plotted against X-ray intensity at different times of exposure, in Fig. 3 for pure sodium chloride and in Fig. 4 for pure potassium chloride crystal and for the same crystal additively coloured with about 10^{17} F-centres/c.c. These plots bring out the interesting feature of the variation of the slopes of these linear relations with the time of exposure. As the time of exposure increases, the slope decreases, and the curves tend to that due to the additively coloured sample, showing that after high times of exposure a crystal irradiated with X-rays reaches a saturation value, where it behaves like an additively coloured crystal; although it can still lose its trapped electrons by exposure to white light. The intercepts of the y-axis gives the level of phosphorescence introduced into the crystal due to irradiation.

Preliminary results with a number of alkali halides, pure and additively coloured, using the photon-counting technique, showed similar behaviour with sodium chloride and potassium chloride. Potassium

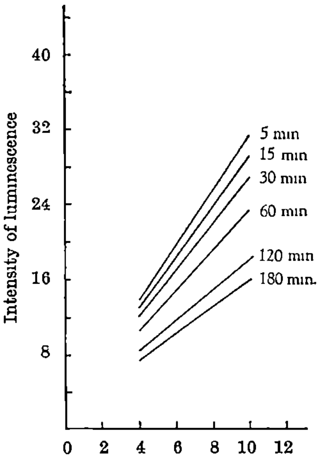

Fig. 3. Results for sodium chloride

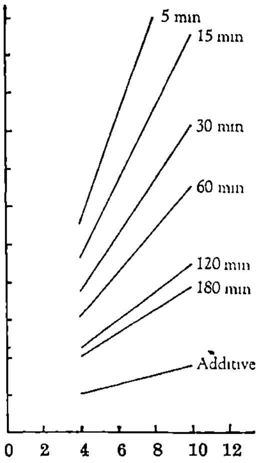

Fig. 4. Results for potassium chloride

iodide showed very fast quenching with virtually no phosphorescence, whereas potassium bromide gave excellent response but long-lasting phosphorescence. The deterioration of luminescence efficiency was almost exponential in potassium chloride and rather slow in sodium chloride, showing that quenching centres are produced in potassium chloride faster than in sodium chloride.

Thanks are due to Dr. K. S. Iyengar for his keen interest and constant encouragement during the course of this investigation and to Mr. R. Thyagarajan for the crystals.

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GEOLOGY

Occurrence of Graywacke in the Lower Siwaliks, Simla Hills

We would like to direct the attention of scientists regarding the occurrence of graywacke in the Nahan Series near Kalka, Simla Hills, Panjab, which has remained unnoticed in Indian geology.

During the course of a traverse near Kalka, while studying the terraces of Kaushallia and Jhajjar Rivers, we came across a grey to greenish micaceous 'sandstone' type of rock which in hand specimens appeared to be graywacke. This was confirmed in thin sections of the rocks, which revealed the presence of angular grains of quartz (approximately 50 per cent) embedded in a matrix composed of fragments of slate, schist, quartzite, chert, felspar, chlorite and authigenic sericite. The rock grades imperceptibly into the underlying red clays.

This graywacke is considered to be a part of the lower Siwaliks known as Nahan Series of the area. All the existing text-books on the geology of India state that these deposits are of fresh-water origin. They are considered to be made up of sandstones, grits, conglomerates, clays, and silts having the characters of fluviatile deposits of torrential streams and floods in shallow water basins.

It is interesting to note that as early as 1937, Krynine had referred to the presence of graywacke from the Siwalik formations of north-west India. However, it has failed to direct the attention of the Indian geologists and hence remained out of the present-day Indian geological literature. We would like to state that the present findings were made independently of Krynine's observation.

Although very diverse types of graywacke having variable mineral composition have been described from different parts of the world, it is generally accepted that the graywackes are characteristic of the geotectonic belts. The presence of this rock in the Himalayan foothills is therefore noteworthy as it will throw light on the sedimentation history of the Siwaliks and other adjoining formations at least of this area. It is therefore probable that the notion that the Siwaliks are entirely of fresh-water origin may no longer be true at least in this part of the sub-Himalayan region.

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RADIATION CHEMISTRY

Behaviour of Neutron-irradiated Ammonium Sulphate on Thermal Annealing

The behaviour of a neutron-irradiated substance in thermal annealing is known to depend on the temperature and on the period of heating. Normally, the retention reaches a saturation value after a few hours of heating. Very recently, experimental evidence was obtained which indicates that the ammonium group in a compound can influence the course of thermal and radiation annealing[1]. Potassium and ammonium dichromate were irradiated with thermal neutrons under the same conditions and their behaviour in thermal annealing was compared. Retention of potassium salt for various temperatures was as expected, while the retention of ammonium salt passed through a maximum above 170° C. This suggests that a reduction process is taking place at the same time as thermal annealing. Similar results have been obtained for the radiation annealing process.

Experiments with ammonium sulphate were carried out in order to see if this anomaly is also common to other ammonium compounds. The sample of 'Analar' grade ammonium sulphates was irradiated in a polythene tube in the thermal column of the *Bepo* reactor at Harwell for four weeks at a neutron flux of 10^{10} n./cm.^{2}/sec. (pile factor 0.1). The temperature during the irradiation was below 50° C. Together with sulphur-35 formed by the nuclear reaction ^{34}S(n,γ)^{35}S, ^{32}P[^{32}S(n,p)^{32}P] and ^{33}P[^{33}S(n,p)^{33}P] were also produced. Phosphorus-32 and -33 were subjected to separate investigations.

Under the conditions of irradiation used in these experiments sulphur-35 is distributed into several fragments because of recoil. It was found that about 60 per cent of the total sulphur activity was in the form of sulphate, the rest being present as sulphite. It is possible that other fragments of sulphur could be formed as intermediates which, however, are readily transformed into sulphate and sulphite under the conditions of analysis. The samples were dissolved in oxygen-free, boiling, diluted hydrochloric acid together with sulphate and sulphite carrier and phosphate hold-back carrier. Thereby sulphite and other volatile sulphur compounds were immediately separated as gases. These were removed by a stream of nitrogen and absorbed under oxidation in strongly alkaline hydrogen peroxide solution. This part of the sulphur activity as well as the remaining sulphate activity were precipitated as barium sulphate and measured with a suitable end-window Geiger counter. All the necessary corrections have been made.

The thermal annealing of the irradiated ammonium sulphate was carried out in an oven at various temperatures and various heating periods in the presence of air. The heated samples were analysed as stated here. The retention at 100° ± 0.5° C. was plotted against heating time and found to follow a typical annealing process curve. However, at temperatures above 150° C., the curve passed through a maximum. This anomaly was most conspicuous at 180° ± 0.5° C., as shown in Fig. 1. A certain radiation annealing is assumed to occur also during the pile radiation.

It is supposed that free radicals are formed in the target under the influence of the γ-radiation in the pile, probably in a relatively large 'steady-state'

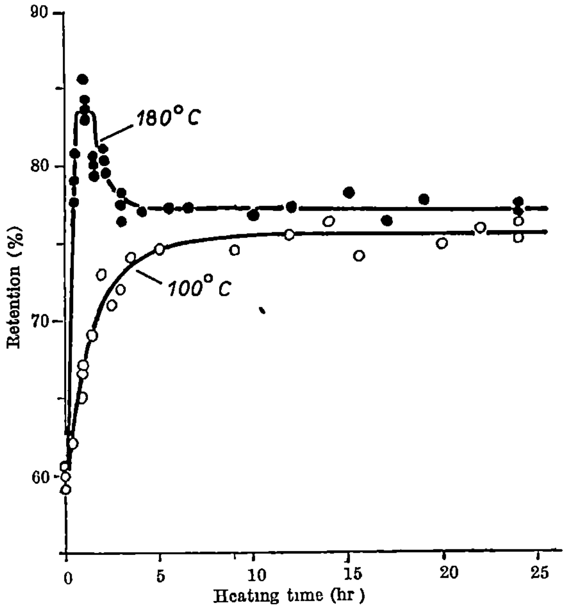

Fig 1 Thermal annealing at 100° and 180° C of ammonium sulphate irradiated in *BEPO*, Harwell, for 4 weeks

concentration, which are available for reaction with the recoil-atoms The ammonium group can be transformed under these conditions into certain fragments, which could give rise to formation of reducing compounds, for example, hydrazine This could either occur during the irradiation in the pile or later in the thermal annealing process All these products formed from the ammonium group can act as reducing agents during the annealing process, although the reducing effect becomes clear only at higher temperatures

The reformed sulphate arises chiefly during the process of thermal annealing directly from the reduced sulphate fragments Another contribution could also be the oxidation of some recoil fragments which form sulphate on dissolving Consequently, two counteracting processes are taking place in the irradiated ammonium sulphate during the period of heating at temperatures greater than 150° C , namely, thermal annealing and reduction

A full report on the role of the ammonium group in thermal annealing will be published in the *Mitteilungen des Instituts fur Radiumforschung in Vienna* (Proceedings of the Austrian Academy of Sciences)

We are indebted to Prof B Karlik for her interest in this work One of us (M N) wishes to thank the Bundesministerium fur Unterricht in Austria for a fellowship

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[1] Getoff, N , and Maddock, A G (in the press) See Getoff, N , *I A E A Internal Report*, No 6, *SC/202/A/7* (Oct 31, 1960)

Radiolysis of Liquid Acetone : Effect of Dose-rate and Linear Energy Transfer

The effect of the linear energy transfer parameter of the radiation and of dose-rate on the radiolysis of aqueous solutions can be understood in terms of interactions between spurs along a track and between spurs on adjacent tracks[1] In contrast the radiolysis of organic liquids shows (1) no effect of linear energy transfer on pure alkane liquids[2], although a slight effect has been found with solutions containing iodine[3], and a pronounced effect with aromatic liquids[4] (see also ref 5), (2) effects at much lower values of dose-rate than in the case of aqueous solutions[6].

In the course of a more general study of the radiolysis of liquid acetone I have found that the yields of products are affected both by the linear energy transfer of the radiation and also the dose-rate The *G* value for carbon monoxide production was found to decrease with increasing dose-rate this is somewhat surprising, but may be due to scavenging of radicals by carbon monoxide, for which there is some evidence[7] Since this trend would imply that less methyl radicals are available for reaction at high dose-rates than at low ones, I have normalized the yields of methane, ethane and methyl ethyl ketone (these are thought to be the only products arising from reactions of methyl radicals) to the yield of carbon monoxide and plotted them against dose-rate in Fig 1 The radiation used was γ-rays from a cobalt-60 source and 1-MV electrons from a Van de Graaff machine All runs were carried out at 20° C. and in a dose-rango where it had previously been shown that *G* values were independent of absorbed dose

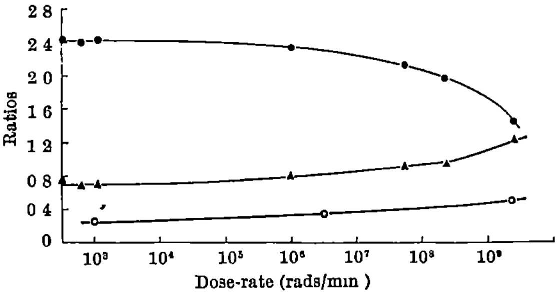

Fig 1 Effect of dose-rate on product ratios ●, CH_4/CO, ▲, $(C_2H_6)^{1/2}/CO$, ○, $CH_3CH_2CO\ CH_3/CO$

It is known that more than three-quarters of the yield of methane in the radiolysis of pure liquid acetone at low dose-rates is derived from the reaction of a thermal methyl radical[8]

$$CH_3 + CH_3\ CO\ CH_3 \longrightarrow CH_4 + CH_2\ COCH_3 \quad (1)$$

and it is probable that ethane and methyl ethyl ketone arise from the reactions

$$CH_3 + CH_3 \longrightarrow C_2H_6 \quad (2)$$

$$CH_3 + CH_2\ CO\ CH_3 \longrightarrow CH_3\ CH_2\ CO\ CH_3 \quad (3)$$

The variation of the product ratios with dose-rate shown in Fig 1 can then be explained in terms of the competing reactions (1), (2) and (3) and the overlapping of 'diffused' spurs at higher dose-rates The dose-rate at which such an effect starts to occur is mainly determined by the efficiency of reaction (1) in removing methyl radicals Since reaction (1) is very slow compared with 'normal' scavenging reactions, the effect on the yields of products occurs at much lower dose-rates than in aqueous solutions[9] Knowing the rate of reaction (1) one can calculate the dose-rate above which combination of methyl (and other) radicals diffusing from spurs on adjacent tracks might be expected to occur This gives the same value (within the limits of the calculation) as that shown in Fig 1 The decrement in CH_4/CO on going from low to high dose-rate (= 0 97) is greater than the increment in $(2C_2H_6 + CH_3\ CO\ CH_2\ CH_3)/CO = (0\ 71)$ this one might anticipate, since we have no experimental measure of the reaction

$$CH_3 + CO\ CH_3 \longrightarrow CH_3\ CO\ CH_3 \qquad (4)$$

which should be more important at higher dose-rates·

Table 1 contains G values of some products as a function of dose-rate (cobalt-60γ and electron irradiations) and of linear energy transfer of the radiation at the same dose-rate (electron and proton irradiations) Comparison of the results of γ- and proton-irradiation illustrates more markedly the effect of dose-rate and linear energy transfer on the products of the radiolysis of acetone, which can be explained as an enhancement of reactions (2) and (3) at the expense of reaction (1), occurring between radicals from spurs on adjacent tracks and between radicals from spurs on the same track It will be noted that G_{CO} appears to be the same for the γ- and proton-irradiations and that G_{CH_3} (total) for γ-irradiation is equal to 2 47 ± 0 18, compared with G_{CH_3} (total) equal to 2 24 ± 0 25 for proton irradiations. the difference, if significant, may again be a measure of reaction (4)

Table 1 PRODUCT YIELDS AS A FUNCTION OF DOSE-RATE AND LINEAR ENERGY TRANSFER

Radiation dose-rate in rads/min	Cobalt-60γ 3 3 × 10^2	1-MV electrons 5 4 × 10^7	2-MV protons 5 4 × 10^7
G_{H_2}	0 44 ± 0 09	0 47 ± 0 10	0 92 ± 0 06
G_{CO}	0 76 ± 0 01	0 47	0 73 ± 0 08
G_{CH_4}	1 85 ± 0 05	0 78	0 59 ± 0 14
$G_{C_2H_6}$	0 25 ± 0 04	0 25 ± 0 04	0 56 ± 0 03
G_{MEK}	0 21 ± 0 01	0 20 ± 0 01	0 53 ± 0 05*

* Dose-rate = 9 6 × 10^9 rads/min

These experiments together with scavenging experiments allow some speculation to be made about the mode of formation of some of the products It has been suggested[8] that ethane is formed in the low dose-rate irradiation entirely from radical–radical reactions in the spur, and this is supported by the present work The suggestion[7,8] that hydrogen formation arises from an abstraction reaction of hot hydrogen atoms appears in conflict with the dependence of the yield on linear energy transfer the latter indicates a hydrogen atom recombination process in the spur Scavenging experiments (with iodine) showed that at low dose-rates at least half and probably appreciably more of the yield of methyl ethyl ketone was unaffected· this product is therefore likely to be formed in radical–radical reactions in spurs also

I thank Dr W Wild for helpful criticism and the United Kingdom Atomic Energy Authority for the award of a fellowship.

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CHEMISTRY

Fundamental Relaxation of Classical Oscillators

THE relaxation of systems of gaseous oscillators and the related process of non-equilibrium chemical reaction have recently received detailed consideration However, while satisfactory formal theories have been presented both for the quantum[1–4] and classical[5,6] cases, little progress has been made in formulating the detailed transition probabilities which control the time evolution of these systems, and little can be expected in view of the extraordinarily complex nature of the collision process

The present purpose is to direct attention to an important limiting case in the classical theory Vibrational energy alone is considered and is assumed to be conserved during all collisions If now the precise details of the collisional act are ignored and it is assumed instead that the total energy is redistributed 'statistically' in every collision, then the transition probability distribution (transition kernel) governing the exchange between single harmonic oscillators and a heat bath consisting likewise of single oscillators may easily be shown to be the following.

$$k(E_1,E_2) = (Z/kT)\exp(E_1/kT)\,\mathrm{ei}[\max(E_1,E_2)/kT] \qquad (1)$$

Here Z is the collision number per molecule per unit time, $\max(E_1,E_2)$ is the maximum of a given initial and final energy, kT has the usual significance, T being the heat-bath temperature, and $\mathrm{ei}(x) = \int_x^\infty (1/s)e^{-s}ds$ is the exponential integral ($k(E_1,E_2)dE_2/Z$ is the probability that an oscillator initially with energy E_1 will undergo a transition to dE_2 at E_2 on collision with a heat-bath oscillator)

This result follows immediately on considering the probabilities that (1) an oscillator with energy E_1 will form a collision complex with specified total energy, (2) the complex will divide to leave the oscillator of interest with energy in dE_2 at E_2, all distributions assumed equally probable, then finally integrating over all possible collisions and multiplying by the collision number.

It will be seen that $k(E_1,E_2)$ has a singularity at $k(0,0)$ while from a given energy all down-transitions are equally probable

The relaxation process is described by a time-dependent distribution function $c(E,t)$ which is the solution of the following continuity equation[6]

$$\left(\frac{\partial}{\partial t} + Z\right) c(E,t) = \int_0^\infty k(\xi,E)c(\xi,t)d\xi \qquad (2)$$

Now it is known that the transition kernel (1), with a symmetrizing factor, is essentially the Green's function corresponding to the Laguerre polynomials, and the solution of the above equation with this kernel is in fact

$$c(E,t) = (1/kT)\exp(-E/kT) \left[1 + \sum_{k=1}^{\infty} c_k L_k(E/kT)\exp\left(-\left(\frac{k}{k+1}\right)Zt\right)\right] \qquad (3)$$

where c_k is determined by the initial distribution $c(E,0)$ through the orthogonality property of the polynomials.

$$c_k = \int_0^\infty c(E,0)L_k(E/kT)\,\mathrm{d}E \qquad (4)$$

In the special case where the initial distribution is itself a Boltzmann one, though at a different temperature from the heat bath, it can be shown that

$$c_k = (1-T/T_0)^k \qquad (5)$$

T, T_0 being the temperatures of heat bath and initial distribution respectively

The importance of the transition kernel derived here is that it expresses what, within the scope of the model, is the most efficient possible coupling between heat bath and system

An extended account of the above approach, together with its application to the theory of chemical reaction, is in preparation

I am indebted to the Office of Naval Research, Washington, and the Department of Scientific and Industrial Research, London, for financial assistance at different stages of this work

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Thermal Decomposition of Ammonium Perchlorate in Presence of Zinc Oxide

THE decomposition of ammonium perchlorate has been examined by Bircumshaw and Newman[1], and Galwey and Jacobs[2], respectively They have observed that the decomposition of the salt occurs according to two different mechanisms At lower temperature (between 200° and 280° C), when only about 30 per cent of the substance decomposes, the electron transfer mechanism dominates, the energy of activation is 32 kcal , and at higher temperature (between 350° and 400° C) the proton transfer mechanism plays a decisive part (E = 39 5 cal)

Recently, we have investigated the influence of several metal oxides on thermal decomposition of different solids (potassium chlorate, potassium perchlorate, etc)[3] The most effective catalyst for both reactions was nickel oxide, which not only increased the reaction-rate but also lowered the energy of activation of the process According to earlier observations the rate of decomposition of ammonium perchlorate is influenced by metal oxides[4], especially by manganese dioxide In our experiments with ammonium perchlorate from among nickel, zinc, ferric and cadmium oxides it was especially the zinc oxide which was outstanding

As a rule, the experiments were carried out both in air and *in vacuo*, and the reaction-rate was followed by determining the amount of gas evolved The decomposition of ammonium perchlorate between 200° and 300° C , in absence of a catalyst, occurred to the extent of 30 per cent Kinetical results were evaluated by the Prout–Tompkins equation, the unimolecular decay and Avrami–Erofeev's equations respectively. The activation energies calculated from the dependence of rate constants on temperature agree generally fairly well with those calculated by the authors referred to

The decomposition of ammonium perchlorate was effectively catalysed by zinc oxide and then decomposition occurred at 240° C to the extent of 75–85 per cent However, as in our earlier experiments, the rate of reaction decreased in each case with the decrease of the amount of the catalyst[3], now the rate scarcely depended on the amount of zinc oxide, and a considerable catalytic effect was observed even in the presence of about 0 1 per cent of the oxide The contracting-cube formula was found to be the best to evaluate the rate constants in the catalysed decompositions Some data on the energy of activation found at different ammonium perchlorate and zinc oxide mixtures are given in Table 1

Table 1

NH_4ClO_4	ZnO (mole ratio)	Activation energy*
50	1	29 5 kcal
16'	1	33 3 ,,
1	1	33 5 ,,

* Rate constants were calculated from the equation $1-(1-\alpha)^{1/3}=Kt$.

These values are approaching 32 kcal , which corresponds to the electron transfer mechanism, indicating that in the temperature-range investigated the electron mechanism plays a part even in the presence of zinc oxide As is well known, zinc oxide belongs to the n-type group of semiconductors Its electric properties can be effectively influenced by doping with small amounts of foreign ions of different valence Thus we have studied the effect of changes in the electric conductivity of zinc oxide on the catalysed decomposition of ammonium perchlorate According to our measurements the rate of reaction was accelerated by increasing the conductivity of zinc oxide (building-in of 1 per cent of aluminium oxide) and it was lowered by decreasing the conductivity (building-in of 1 per cent of lithium oxide), and it largely lengthened the induction period On the contrary, activation energies practically do not change on doping with zinc oxide

While the catalysed decomposition of ammonium perchlorate could at 240° C be followed easily, above this temperature the course of kinetic curves changed, and already in presence of 0 1 per cent zinc oxide, after an induction period, the substance exploded. The explosion occurred very sharply at mole fraction of 300 1–10 1 ammonium perchlorate–zinc oxide and it was accompanied by a luminous phenomenon In presence of zinc oxide applied in equal mole ratio the decomposition of ammonium perchlorate similarly resulted in an explosion, but not so violently as in the case of mixtures containing a small quantity of zinc oxide By further increasing the concentration of zinc oxide, instead of explosion, degeneration occurred From dependence on temperature of the times until explosion we obtained the energies of activation, given in Table 2, which are practically independent of the amount of zinc oxide between 1 1 and 100 · 1 mole ratio of ammonium perchlorate–zinc oxide mixtures.

Table 2

NH_4ClO_4	ZnO (mole ratio)	Activation energy
100	1	29 5 kcal
50	1	28 0 ,,
25	1	27 0 ,,
10	1	27 6 ,,

According to Galwey and Jacob's measurements[5] the explosion of ammonium perchlorate at 1 atm. pressure occurs only at temperatures above 440° C. From the dependence on temperature of the induction period these authors obtained almost identical value for the activation energy (41 5 kcal) as in the decomposition of ammonium perchlorate at high temperatures from what they stated it appears that the proton transfer mechanism plays a part in the explosion In our experiments the explosion temperature was lowered almost to 200° C by a very small amount of zinc oxide (0 2 per cent) The values of activation energy found by us are considerably lower than those obtained by Galwey and Jacobs and well approach those corresponding to the electron transfer mechanism and determined at a lower temperature

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Separation, Identification and Determination of the Fatty Acids of Montan Wax

Crude montan wax is essentially a mixture of the esters of long-chain carboxylic acids, with free acids, resin and asphalt[1]. Much of the work devoted to a study of the wax acids prior to 1930 is clearly unreliable In that year, using micro-distillation and X-ray analysis, Holde and Bleyberg[2] isolated and identified a normal C_{28} acid, and indicated the presence of a normal C_{26} acid Further progress was made by Fuch and Dieberg[3] using absorption and elution chromatography and infra-red spectroscopy They succeeded in isolating and identifying all the normal straight-chain carboxylic acids with even carbon numbers from C_{22} to C_{34}

Using gas-chromatography, we have succeeded in resolving the methyl esters of carboxylic acids from a saponified bleached montan wax A total of 11 components were separated, and identified as the esters of the normal straight-chain acids with both even and odd carbon numbers from C_{22} to C_{32}, inclusive The C_{34} acid identified by Fuch and Dieberg was not detected

This separation was achieved using a 'Shandon' gas-chromatograph and a flame-ionization detector The column originally used was 4 ft in length and contained 100–200 mesh 'Celite' with 10 per cent polyethyleneglycol adipate as stationary phase. More recently the columns have been reduced in length and the packing has included also 1 per cent of orthophosphoric acid[4]

The identifications were made using the well-known relation of the logarithms of retention times with carbon numbers for each homologous series From the work of Fuch and Dieberg, establishing the presence of the normal straight-chain carboxylic acids C_{22} to C_{34}, it should be possible to assign carbon numbers to the retention times recorded, provided that one or preferably two reference retention times are known Such reference times were obtained from stearic and palmitic acids present in a commercial sample of stearic acid From these it was possible to identify not only the C_{22} to C_{32} even-numbered acids noted by Fuch and Dieberg but also the remaining 5 components, as the intervening odd-numbered acids

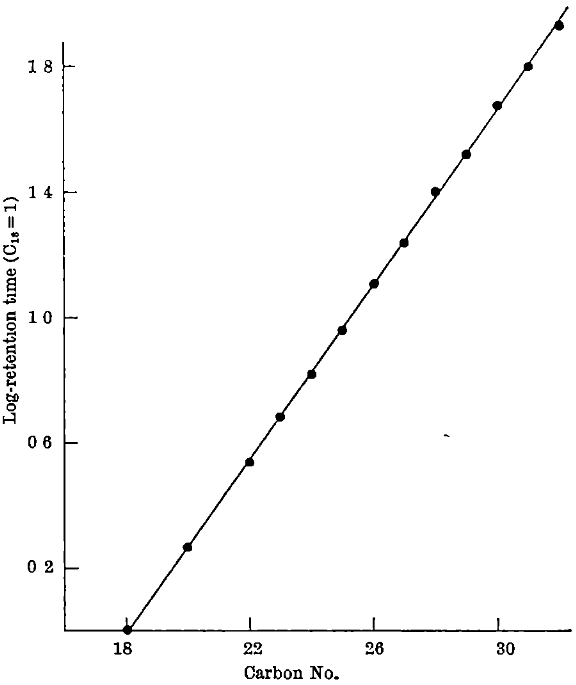

Fig 1 Linear relationship between carbon number and log-retention time

The validity of this identification was confirmed by an examination of arachidic C_{20} and behenic C_{22} methyl esters The linear relation of retention for all the methyl esters recorded is shown in Fig 1 (methyl stearate = 1).

It has been possible to derive an approximate composition of the mixture of esters by measuring the areas below the chromatogram, when the following was obtained

	$C_{16}C_{18}C_{20}$	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}
Per cent	less than 0 05 each	0 25	0 25	7 5	2 0	22 0	3 5	27 5	3 0	24 0	2 0	8 0

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BIOCHEMISTRY

Distribution of Potassium in Baker's Yeast

Baker's yeast in the resting state normally contains a relatively large amount of potassium In addition, it has been shown by a number of workers that during active metabolism (aerobic or anaerobic utilization of glucose[1] or aerobic respiration of certain other substrates[2]) considerable net absorption of potassium can occur even though the concentration of potassium in the external medium may be very much less than that inside the cells Accumulation of potassium, and of other cations, by yeast involves active transport of the ions across the cell membrane[3], the inward movement of ions being mediated by relatively specific carrier mechanisms[4] therein Many facts require this interpretation and have been stated in detail elsewhere

The results of an investigation recently carried out in this laboratory[5] showed that the presence of relatively large amounts of bound potassium within the yeast cell is very unlikely In these experiments the total intracellular concentration of free solutes in washed and centrifuged yeast, which had previously been subjected to a repeated process of freezing in liquid oxygen and thawing, was determined by a microcryoscopic method, a mean value, for resting yeast, of 0 59 *M* being obtained This value was in close agreement with that calculated from the known concentrations, determined by chemical analysis, of low-molecular weight substances, including potassium, in the same strain of yeast (0 60 *M*) In dialysis experiments in which the fluid material obtained by alternate freezing in liquid oxygen and thawing of the centrifuged cells was allowed to come to equilibrium with a relatively small volume of distilled water, it was found that the total intracellular concentration calculated from the freezing-point depression of the dialysate was in good agreement with that obtained from direct measurements on the frozen and thawed yeast. From these results it seemed possible that a determination of the proportion of the total potassium in yeast appearing in such dialysates might yield some information about the extent to which this ion is bound in the cells

A series of experiments was carried out in which fresh yeast was twice washed in de-ionized water and centrifuged, the second centrifuging being continued for 20 min at 3,000 r p m The packed cells from this second centrifuging (which were contained in stoppered plastic centrifuge tubes) were then frozen in liquid oxygen and allowed to thaw, this procedure being repeated twice 2-ml samples of the liquid material from the freezing and thawing process were dialysed overnight in the cold against an equal volume of de-ionized water and the potassium content of the dialysate determined This was compared with the total potassium estimated directly on a sample of the frozen and thawed yeast In a group of nine experiments of this kind, the total potassium ranged from 102 5 to 130 0 m equiv /kgm washed and centrifuged yeast, the mean value being 119 0 m equiv /kgm The potassium concentration in the dialysates ranged from 94 0 to 125 0 m equiv /kgm with a mean of 109 0 m equiv /kgm

These results indicated that by far the greater proportion of the total potassium content of yeast is free to diffuse out of the cells once the membrane has been broken by suitable means, and that the amount bound to high-molecular weight cytoplasmic constituents is relatively small This small amount of bound potassium, amounting in the average to about 8 per cent of the total, appears to be quite firmly held In experiments in which the solid residue from the dialysis process was washed in about 50 times its own volume of de-ionized water it was found that, after three such washings, some 50–60 per cent of it still remained bound to the solid cell debris The unlikelihood of relatively large amounts of potassium, previously bound, being released during dialysis is indicated by the close agreement observed[5] between the total intracellular concentration determined from the freezing-point depression of such dialysates and that of the frozen and thawed yeast

Further experiments were concerned with an attempt to determine the distribution between these two fractions of the extra potassium entering the cells during fermentation under a variety of conditions In these experiments the yeast, after fermentation, was twice washed in de-ionized water as before, frozen in liquid oxygen and thawed and the total and 'dialysable' potassium estimated as described above. In each case the results were compared with those obtained from a sample of the same yeast in the resting state These results are summarized in Table 1

From Table 1 it is seen that, with the exception of the long-period fermentation in potassium citrate (sample 4), virtually all the extra potassium accumulated during fermentation appears in the freely dialysable fraction In the case of samples 1 and 2, where the external potassium concentration is relatively low, the results shown in Table 1 would appear to indicate the accumulation of free potassium against a high concentration gradient

Table 1 ACCUMULATION OF POTASSIUM BY YEAST FERMENTED UNDER VARIOUS CONDITIONS

Sample	Total potassium		Potassium accumulated	Potassium in dialysate		Potassium accumulated
	Resting	Fermented		Resting	Fermented	
1	127 5	145 5	18 0	121 0	139 5	18 5
2	118 0	129 0	11 0	114 0	122 5	8 5
3	123 0	150 0	27 0	111 5	137 0	25 5
4	111 0	140 0	29 0	98 0	112 0	14 0

In all cases, 1 part by weight of washed and centrifuged yeast was suspended, with aeration, in 20 vol of fluid containing 5 per cent glucose Other conditions were as follows Sample 1 fermentation for 1 hr in the presence of 10 m moles/litre of potassium chloride plus collidine/hydrochloric acid buffer Final *p*H of suspensions, 4–4 5 Sample 2 conditions as for 1, but with 2 m moles/litre external potassium chloride Sample 3 fermentation for 45 min in the presence of 200 m moles potassium acetate Sample 4 fermentation for 4 hr in potassium citrate (200 m equiv potassium ions/litre)

All values are in m equiv /kgm washed and centrifuged yeast Each sample represents the mean of two experiments with duplicate estimations in each experiment

Table 2 DISTRIBUTION OF SODIUM AND POTASSIUM IN SODIUM-RICH YEAST

Yeast	Potassium (m equiv /kgm) Total	Potassium (m equiv /kgm) In dialysate	Sodium (m equiv /kgm) Total	Sodium (m equiv /kgm) In dialysate
Resting	112 0	97 0	—	—
Sodium-rich	55 0	34 0	80 0	80 0

Results give the mean for three experiments each with duplicate estimations

Conway and Moore[6] have shown that when yeast is fermented in 5 per cent glucose with the addition of sodium citrate, there is a marked accumulation of sodium, potassium being lost. In Table 2 is shown the results of an investigation by the technique outlined here of the distribution of sodium and potassium in a sodium yeast prepared by fermenting fresh yeast for two successive 2-hr periods in 20 volumes of a sodium citrate-glucose medium containing 200 m equiv sodium ions per litre. From Table 2 it appears that virtually all the sodium accumulated is in a freely diffusible form In these experiments there appeared to be some increase in the amount of bound potassium in the cells

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Comparison of the Mitochondria of the Small Intestine of Vertebrates

MITOCHONDRIA of the intestinal mucosa of the following vertebrates have been examined by electron microscopy: the rat (*Rattus norvegicus*), guinea pig (*Cavia percellus*), chicken (*Gallus domesticus*), lizard (*Tiliqua rugosa*), toad (*Bufo marinus*) and carp (*Carassius auratus*) (Fig. 1). The mitochondria in the intestinal cells of these species showed no major differences in structure from the other mitochondria that have already been described[1,2]. The intestinal mitochondria from all these species swelled when they were isolated by differential centrifugation in 0·25 *M* sucrose (Fig. 2), but all except those from the rat exhibited considerable succinoxidase activity as measured manometrically (Table 1). The oxygen uptake of homogenates of the intestinal mucosa of rats was also low and variable compared with that of homogenates of intestinal mucosa prepared from guinea pigs. In contrast, the oxygen uptake (per mgm. dry weight) of whole intestinal cells of both the rat and the guinea pig was similar.

Homogenates of the intestinal mucosa of rats were found to inhibit the oxygen uptake of homogenates of the intestinal mucosa of guinea pigs. The inhibitor present in the rat intestinal homogenate was not destroyed by heating for 3 min. in a boiling water bath, or by adding hydrochloric acid to a final concentration of 2 *N*. The inhibitor was not dialysable, but it could be extracted with iso-octane. The iso-octane extract contained a substance that absorbed light strongly at wave-lengths below 250 mμ. This suggested the presence of long-chain unsaturated fatty acids. Chromatographic examination of the extract showed the presence of oleic acid and a smaller amount of linoleic acid. Small quantities of oleic acid (≏ 0·005 ml./mgm. mitochondrial N), or of the material recovered from the iso-octane extract, inhibited by 95 per cent the succinoxidase activity of mitochondria isolated from guinea pigs. The inhibitory effect of oleic acid on a succinoxidase preparation from ox-heart has previously been reported[3]. Nakamura *et al.*[4] also ascribed the low succinoxidase activity of rat intestinal mucosa to the presence of fatty acids. That mitochondria from the intestine of the rat do contain succinic dehydrogenase can be shown cytochemically. When these mitochondria are incubated with 2 : 3 : 5 triphenyl tetrazolium chloride in the presence of succinate, deposits of formazan can be seen. No reduction of the dye occurs in the absence of succinate. As cytochrome oxidase has been shown[5] to donate electrons to triphenyl tetrazolium chloride, it is suggested that the inhibitor of succinoxidase released on the rupture of rat intestinal cells acts between cytochrome oxidase and oxygen. Some evidence to confirm this has been obtained. The cytochrome oxidase activity (measured manometrically) of mitochondria isolated from the intestinal mucosa of the rat is less than one-tenth of that of intestinal mitochondria isolated from the guinea pig.

Table 1. SUCCINOXIDASE ACTIVITY EXPRESSED AS THE $Q_{O_2}N$ OF MITOCHONDRIA ISOLATED FROM THE INTESTINAL MUCOSA OF VARIOUS VERTEBRATES

Animal	$Q_{O_2}N$	Standard error
Rat	< 15	—
Guinea pig	420	13
Chicken	526	13
Lizard	467	13
Toad	212	25
Carp	356	13

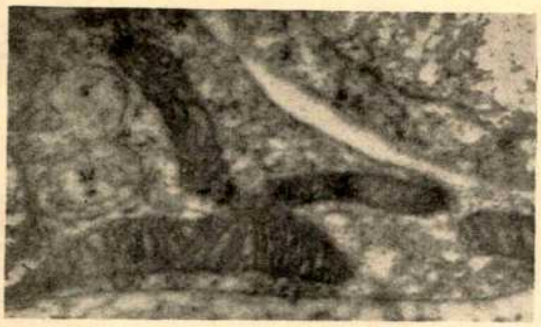

Fig. 1. Electron micrograph of a section of a cell of the intestinal mucosa of *Carassius auratus* showing the mitochondrial structure. × 35,000

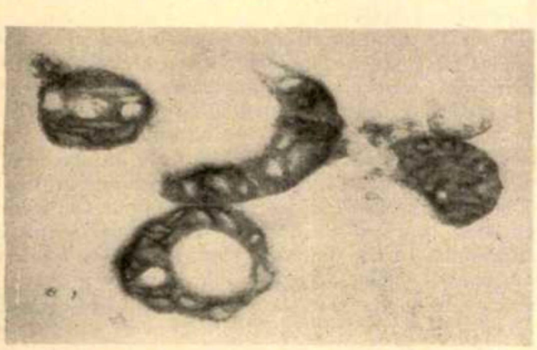

Fig. 2. Electron micrograph of a section of mitochondria isolated from the intestinal mucosa of *Carassius auratus*. × 37,500

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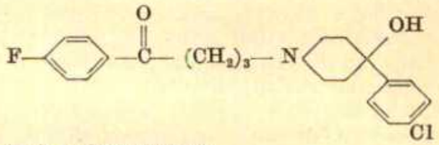
Combustion Analysis of Paper Chromatographic Spots

THE quantitative determination of paper chromatographic spots is usually carried out by using a variety of photometric techniques. In many instances, however, the known methods lack specificity, reliability and sensitivity.



Haloperidol (*R*1625)

For haloperidol[1-3], a new potent neuroleptic agent recently developed in this Laboratory, a satisfactory method of photometric analysis, suitable for the analysis of minute amounts in chromatographic spots, has not yet been found. I have therefore developed an alternative method, based on the quantitative micro-analysis of the organic fluorine content of the spots.

Fluorine (μgm.)			
Actual	Estimated		
0·40	0·25	0·45	0·50
0·80	0·77	0·72	0·60
1·20	1·28	1·14	1·08
1·60	1·68	1·44	1·60
2·00	2·05	2·20	1·70

After one-dimensional chromatographic separation (using methanol, amyl alcohol, benzene, water, 40/20/40/5) on buffered[4] Whatman No. 1 paper, of a solution containing 8–40 μgm. of haloperidol, the haloperidol spots are located by inspection under an ultra-violet light source. The fluorescent area is then cut out and burned in a modified Schöniger oxygen flask[5-8], 150 ml. in volume and with a small valve constructed at the bottom of the flask. The combustion products are absorbed in 3 ml. of water and the inorganic fluoride determined by the colorimetric method of Singer and Armstrong[9]. In a series of 15 experiments, the estimated haloperidol content of the spots varied between 85 and 110 per cent of the actual amounts (8–40 μgm.) which were put on the paper chromatogram.

This general method should be applicable to the quantitative analysis of paper chromatographic and paper electrophoretic spots of compounds containing other halogens, sulphur, phosphorus, arsenic, boron, cadmium, mercury and zinc[10].

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Inactivation of *A*-Methopterin by the Acetylating Enzyme System from Pigeon Liver

A-METHOPTERIN (4-amino-N^{10}-methyl pteroyl glutamic acid) inhibits the growth of certain tumours[1] as well as of many folic acid dependent micro-organisms[2]. It is a powerful inhibitor of enzyme systems reducing folic acid to tetrahydrofolic acid[3]. Recent investigations[4,5] have shown that *A*-methopterin inhibits acetylation of aromatic amines. Small quantities of *A*-methopterin have been found to decrease considerably the urinary excretion in mice of the acetylated aromatic amine formed from injected folic acid (unpublished results). Evidence presented here shows that *A*-methopterin is inactivated by the acetylating enzyme system of pigeon liver.

Crude enzyme preparations employed in the present work consisted of 5 per cent extracts of pigeon liver acetone powder in 0·2 *M* sodium fluoride. *A*-methopterin was assayed by the inhibition analysis using *S. faecalis* as the test organism[6]. Initial experiments showed that the assay system was most sensitive in the presence of 0·8 mμgm. of folic acid per ml. The dilutions of the test material were made so that the final concentration of *A*-methopterin in the assay system was 0·5–0·8 mμgm. per ml. Each determination was done in duplicate at 3 different levels of dilution.

Table 1. EFFECT OF OMISSION OF COMPONENTS OF THE ACETYLATING SYSTEM ON THE INACTIVATION OF *A*-METHOPTERIN

Exp. No.	System	Aliquot (ml.)	Time of incubation (min.)	Growth of *S. faecalis*: Klett units 660 mμ	Growth of *S. faecalis*: Expressed as percentage of control in absence of *A*-methopterin
1	Complete system				
	(Crude enzyme extract)	0·6	0	76	42·0
	(Crude enzyme extract)	0·6	180	118	65·6
	Autoclaved system*	0·6	180	87	48·0
2	Complete system				
	(Fractions*A*-40 + *A*-60)	0·6	0	90	45·5
	(Fractions *A*-40 + *A*-60)	0·6	180	132	65·7
3	Fraction *A*-40 omitted	0·6	0	78	36·4
	Fraction *A*-40 omitted	0·6	180	76	35·0
4	Fraction *A*-60 omitted	0·6	0	70	35·0
	Fraction *A*-60 omitted	0·6	180	66	33·0
5	Acetate omitted	0·6	0	91	45·5
	Acetate omitted	0·6	180	91	45·5
6	CoA omitted	0·6	0	35	17·8
	CoA omitted	0·6	180	33	17·0
7	ATP omitted	0·6	0	92	48·0
	ATP omitted	0·6	180	84	44·5

Incubation system: Na_2HPO_4, 0·02 *M*; $NaHCO_3$, 0·028 *M*; acetate, 0·02 *M*; ATP, 0·004 *M*; *A*-methopterin, 13·2 μgm.; CoA, 12·5 μgm.; Mg^{++}, 0·0055 *M*; cysteine, 0·005 *M*; crude enzyme extract (0·5 ml.) or fraction *A*-40 and *A*-60, 0·4 ml. each. Volume, 3·0 ml. *p*H 7·0; temperature, 37° C.

* Autoclaved at 10 lb. pressure for 10 min.

Table 2. EFFECT OF *p*-AMINOBENZOIC ACID ON THE INACTIVATION OF *A*-METHOPTERIN

Exp. No.	System	Aliquot (ml.)	Time of incubation (min.)	Growth of *S. faecalis*: Klett units 660 mμ	Growth of *S. faecalis*: Expressed as percentage of control in absence of *A*-methopterin
1	Crude enzyme system	0·4	0	75·0	43·0
	Crude enzyme system	0·4	180	125·0	71·8
2	Crude enzyme system				
	+ PABA (13·2 μgm.)	0·4	0	86·0	49·7
	+ PABA (13·2 μgm.)	0·4	180	107·0	61·5

Incubation system same as in Table 1.

The inactivation of *A*-methopterin in the test system occurred rapidly. Values more consistent with the quantity of added *A*-methopterin were, however, obtained by autoclaving the zero-time samples at 10 lb. for 10 min. prior to assay. The sterilization of the test samples was carried out by filtration through sintered-glass bacteriological filters.

Results given in Table 1 show that incubation of *A*-methopterin for 180 min. with the complete acetylating system from pigeon liver leads to more than 40 per cent decrease in the activity of *A*-methopterin as a growth inhibitor of *S. faecalis*. The fact that the original inhibitory activity of *A*-methopterin is recovered by autoclaving the incubated mixture indicates the formation of a less active or an inactive derivative of *A*-methopterin which is unstable to high temperatures.

The effect of omission of various components of the acetylating system on the inactivation of *A*-methopterin was investigated using pigeon liver extracts prepared according to Chou and Lipmann[7]. In these preparations, fraction *A*-40 activated acetate to form acetyl-coenzyme A and fraction *A*-60 catalysed the transfer of the acetyl group to the acceptor amine.

The results (Table 1) demonstrate that the inhibitory activity of *A*-methopterin is decreased only in the presence of the enzyme system containing all the co-factors (adenosine triphosphate, coenzyme A and acetate) required for the acetylation of aromatic amines. By extensive dialysis, it was possible to demonstrate the requirement for acetate. The inactivation of *A*-methopterin did not occur in absence of any one of the active protein fractions. This excludes the possibility of inactivation due to non-specific adsorption on proteins *p*-Aminobenzoic acid (PABA), which can be acetylated by the pigeon liver enzyme system and so may be expected to compete with the acetylation of other amines, was consistently found to diminish the inactivation of *A*-methopterin (Table 2) The quantity of extra PABA added in these experiments did not affect the growth in the control system These observations, and the fact that the *A*-methopterin molecule contains the necessary structural features, suggest that the inactivation of *A*-methopterin is brought about through the formation of acetyl derivatives which are less active or completely inactive as inhibitors for the growth of *S faecalis* Aminopterin is also inactivated by a similar enzyme system, but to a lesser extent than *A*-methopterin Earlier work of Jacobson[8] on the effect of aminopterin on cell division have indicated the formation of an inactive derivative from aminopterin on incubation with various animal tissues, including leukæmic cells Fountain *et al* [9] have reported that the concentration of *A*-methopterin detected in liver and kidney of mice injected with *A*-methopterin increased several fold when the samples were boiled before assay Since both these organs contain the acetylating enzymes, the increased activity produced on boiling the tissue could have resulted from breakdown of acetyl derivatives of the type which the present work has shown to be unstable to high temperatures

A-methopterin was found to inhibit the acetylation of sulphanilamide in pigeon liver preparations but had no effect on acetyl-CoA formation Similar results have been obtained also by Johnson *et al* [4]. Apparently *A*-methopterin inhibits the acetylation of aromatic amines by interference with the transferase component of the overall system The removal of acetyl groups for the acetylation of *A*-methopterin as inferred from the present findings could explain the type of inhibition produced by *A*-methopterin on biological acetylations

Acknowledgement is made to the International Division of the Lady Tata Memorial Trust for a research assistantship (to V K) and a grant in aid

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Improved Benzidine Sugar Reagent for Paper Chromatograms

BENZIDINE has been found to be a useful reagent for sugar detection on paper chromatograms[1,3] and for quantitative determination in test-tubes[4] Horrocks[3] first found that benzidine–acetic acid detected reducing aldoses on paper chromatograms, but ketoses were detected only at high concentration Bacon and Edelman[1] added trichloracetic acid to the benzidine–acetic acid reagent to increase its acid strength and claimed that it detected less than one microgram of glucose in free or combined form Its sensitivity to fructose was considerably less An acid is required for the formation of furfuraldehyde from sugars Furfuraldehyde, or a degradation product of it, then reacts with an aromatic amine or phenol (benzidine in this case) to form a coloured product It is important that the acid should be strong enough to cause the reaction to proceed, but, in the case of paper chromatography, it should not cause partial destruction of the paper or produce a dark background colour For this purpose, organic acids (acetic, phthalic, or trichloracetic acid) have been used However, when mineral acids at low concentrations are used, an improved reagent is obtained without paper destruction or serious background interference

In an attempt to improve this reagent, it was discovered that addition of mineral acids to the benzidine–trichloracetic–acetic acid reagent caused precipitation, but this difficulty was overcome by the application of the components separately to the paper The use of mineral acids with the benzidine reagent permits the detection of aldoses and ketoses in free or combined form in 1-mgm quantities Through the manipulation of the procedures to be described below, classes of simple sugars and monomers of oligosaccharides can be distinguished on one chromatogram Procedure *a* is relatively specific for aldoses, and procedure *b* for ketoses If both aldoses and ketoses are to be revealed in one chromatogram, procedure *c* or *d* should be used

The benzidine reagent is prepared by dilution of 10 ml. of stock 1 (5 0 gm. benzidine in 100 ml glacial acetic acid) plus 10 ml of stock 2 (40 0 gm trichloracetic acid in 100 ml distilled water) to 100 ml with 95 per cent ethanol[1] The stock solutions keep indefinitely in brown bottles The mineral acids are 0 3 *N* hydrochloric acid in 95 per cent ethanol and 1 *N* phosphoric acid in 95 per cent ethanol.

Procedure *a* Spray a chromatogram with the benzidine reagent and heat at 70° C for 10–15 min Aldoses of 1 mgm quantity in free or combined form are revealed as brown spots against a white background Ketoses are detected only at high concentration

Procedure *b* Spray a chromatogram with the benzidine reagent Follow this with a light spraying of 0 3 *N* hydrochloric acid in 95 per cent ethanol, and then heat at 70° C for 10–15 min Ketoses of 1 mgm. quantity in free or combined form are revealed as yellow spots against a pale background Aldoses at high concentration give faint brown spots The yellow colour intensity is enhanced under ultra-violet light

Procedure *c* It is essentially the same as procedure *b*, except that hydrochloric acid is replaced by 1 *N* phosphoric acid in 95 per cent ethanol Both aldoses and ketoses in free or combined form are revealed in their characteristic colours on a chromatogram which appears moist and has a light background

Procedure *d* Spray a chromatogram with the benzidine reagent and heat at 70° C for 10–15 min for the detection of aldoses, spray lightly with 0 3 *N* hydrochloric acid in 95 per cent ethanol and heat again at 70° C for 10–15 min to detect ketoses This procedure is better than procedure *c* because of its high sensitivity and relative specificity for classes of sugars By this procedure both aldoses and ketoses in either free or combined form are revealed as brown and yellow spots respectively against a light background Stachyose, raffinose, cellobiose, melibiose, sucrose, maltose, lactose, rhamnose galactose, glucose, fructose, mannose, arabinose, xylose and ribose give their characteristic colours, brown for aldoses, yellow for ketoses, and the colour of the oligosaccharides containing aldoses and ketoses depends on the sugar concentration and heating temperature

By the addition of mineral acids at low concentration the benzidine reagent[1,3] is improved to detect simple sugars and oligosaccharides with high sensitivity and relative specificity

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Crystallization of the Complex of D-Amino-Acid Oxidase and 'Substrate-Substitute'

In an earlier communication[1], it was reported that a crystal was obtained from the mixture of D-amino-acid oxidase apo-protein, flavin-adenine-dinucleotide and benzoate, a 'substrate-substitute'. It was also found that the crystal is composed of equimoles of the apo-protein, coenzyme and benzoate By adding D-alanine to the solution of the crystal, the complete decolorization of the coenzyme accompanied by the liberation of the benzoate was observed This result indicated that the benzoate can be replaced by the substrate, and that the enzyme is not denatured when crystallized by combining with benzoate In the present experiment, we sought to find another 'substrate-substitute' to crystallize this enzyme

Among the benzoic acid derivatives, *p*-nitrobenzoic acid was also considered to be a 'substrate-substitute' from our former experiment[2] *p*-Nitrobenzoic acid was expected to be detected more easily by its absorption peak at 275 mμ than benzoate Using *p*-nitrobenzoic acid as 'substrate-substitute', a crystal was obtained from the mixture of the apo-protein, flavin-adenine-dinucleotide and 'substrate-substitute' in the same way as reported in the previous report[1]. The solution of the crystal retains its full activity after heating at 60° C for 3 min , indicating that the apo-protein is considerably stabilized in the crystal The shape of the crystal is nearly the same as that of the crystal obtained from the mixture of the apo-protein, coenzyme and benzoate. The crystals were collected by filtration, and the filter cake was washed twice by distilled water The crystals thus obtained were dissolved in pyrophosphate buffer (*M*/60, *p*H 8 3)

The absorption spectrum of the crystal in the visual wave-length region is shown by curve I in Fig 1 It exhibits the same characteristic feature as in the case of the crystal of the apo-protein-coenzyme-benzoate complex the peaks appeared at 380 mμ and 465 mμ, a marked shoulder being recorded at 485 mμ

To liberate the combined coenzyme and the 'substrate-substitute' from the apo-protein, the *p*H of the solution of the crystal was adjusted to *p*H 5 0 and heated at 90° C for 3 min The completely denatured protein was removed by centrifugation After the *p*H of the supernatant was corrected to 8·3, its absorption spectrum was measured as shown by curve II in Fig 1 The spectrum was considered to be the sum of the spectra of two compounds, the coenzyme and *p*-nitrobenzoic acid The spectrum, however, is identical with that of flavin-adenine-dinucleotide in the range from 370 mμ to the longer wave-length, and *p*-nitrobenzoic acid has no absorption in this region. So the theoretical spectrum of the coenzyme (curve III) was calculated from the optical density at 450 mμ of curve II Consequently, the absorption spectrum of *p*-nitrobenzoic acid in the supernatant can be calculated to be curve IV, which has the same shape with that of the standard *p*-nitrobenzoic acid (curve V) Comparing the optical density at 275 mμ of curve IV with that of curve V, the molar concentration of *p*-nitrobenzoic acid as compared with those of the apo-protein and coenzyme in the crystal was calculated as follows 1 2 moles of *p*-nitrobenzoic acid, 1 1 moles of the apo-protein, and 1 0 mole of flavin-adenine-dinucleotide.

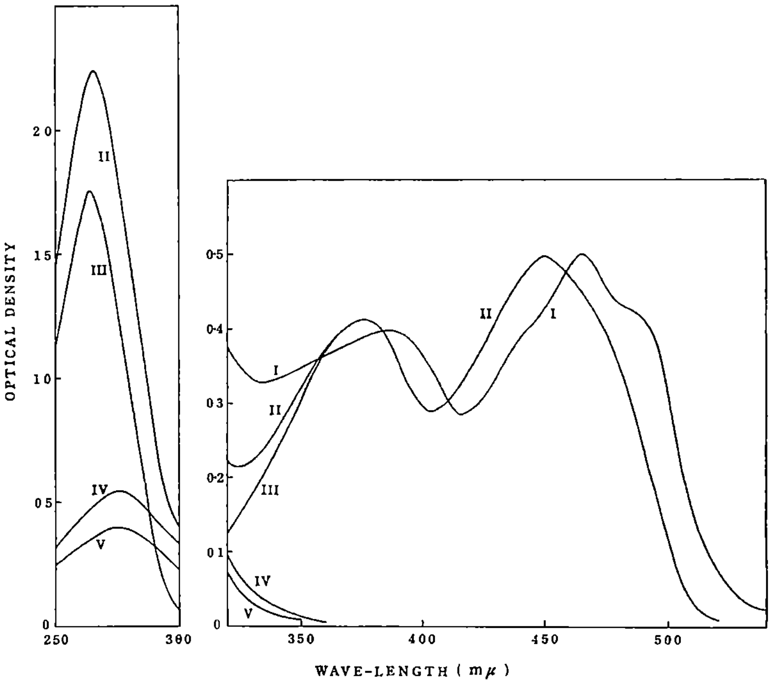

Fig 1 Absorption spectra of the crystal and *p*-nitrobenzoic acid in pyrophosphate buffer (*M*/60, *p*H 8 3). I, The crystal, II, the supernatant obtained after heating I to denature the apo-protein (see text), III, flavin-adenine-dinucleotide calculated from the optical density of II at 450 mμ, IV, theoretical spectrum of II–III, V, *p*-nitrobenzoic acid (4 0 × 10^{-5} *M*)

From these results, it was concluded that D-amino acid oxidase apo-protein can also be crystallized by combining with equimoles of *p*-nitrobenzoic acid and coenzyme in the same way as in the case of benzoate. This fact may be attributed to the low specificity of this enzyme

Furthermore, it seems clear that the enzyme can be easily crystallized by combining with a suitable compound as 'substrate-substitute' which can combine with the protein at the same binding sites as the substrate, but not be oxidized by the enzyme The crystal of the complex of the enzyme and such a 'substrate-substitute' may provide a valuable clue in elucidating the actual features of enzyme-substrate compounds

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PHYSIOLOGY

Persistence of Sterols other than Cholesterol in Chicken Tissues

Recent work by Davison and his collaborators[1-3] has shown that cholesterol may be incorporated in the nervous tissues of myelinating chickens and rabbits and that this cholesterol will persist in these tissues for considerable lengths of time Our interests in the metabolism of cholesterol and other sterols prompted us to compare the distributions in chicken tissues of injected sitosterol and lanosterol with that of cholesterol Emulsions of cholesterol-7-^{3}H (purchased from New England Nuclear Co , Boston, Mass), sitosterol^{3}-H (randomly labelled, gift of Dr Leon Swell), and lanosterol-^{3}H (randomly labelled, gift of Dr George J Alexander) in saline were prepared by the method used for preparation of cholesterol-^{14}C emulsions for experiments involving mitochondrial oxidation of this sterol[4] Newly hatched White Leghorn chicks were used and the appropriate sterol emulsion (0 50 ml) was injected directly into the yolk sac After four days, radioactivity could be detected in the brain tissue of chickens from each group After 9 weeks the one surviving chicken in each group was killed by exsanguination. The blood was collected in centrifuge tubes and the erythrocytes separated. All the brain, cord, liver, spleen and aorta was excised, weighed and dissolved in strong potassium hydroxide The non-saponifiable fraction was assayed for tritium radioactivity in a liquid scintillation counter Small pieces of sciatic nerve were taken from each chicken and all showed slight but consistently detectable radioactivity Other findings are shown in Table 1

It is of interest that all three sterols were incorporated into brain tissue The rather large incorporation into erythrocytes is also noteworthy as is the small but persistent incorporation of radioactivity into the aortic tissue. That a small percentage of orally ingested cholesterol will be deposited in the aorta of adult rabbit[5] or man[6] has been shown The relative persistence of lanosterol-^{3}H in the liver could indicate greatly reduced turnover as compared with the other two sterols In view of reports that some insects and mammals may convert sitosterol and ergosterol to cholesterol or cholesterol-like compounds[7-10], it was necessary to prove the presence of the injected material Gas chromatography of the non-saponifiable fractions from the brains and livers of all three chickens showed the presence of only the injected sterol and cholesterol (We are greatly indebted to Dr. W M Holmes of Smith, Kline and French Laboratories, Philadelphia, for these analyses)

Table 1 Recoveries of Tritium Radioactivity from Non-saponifiable Fractions of Chick Tissues Nine Weeks after Injection of Certain Sterols

	Recovery (per cent)		
Sterol	Cholesterol-^{3}H	Sitosterol-^{3}H	Lanosterol-^{3}H
Input (c p m)	4 3 × 10^{5}	8 2 × 10^{4}	8 7 × 10^{5}
Brain	0 60	0 76	0 28
Cord	Lost	N D *	0 02
Erythrocytes	0 33	0 24	0 49
Liver	0 40	0 25	1 07
Spleen	0 14	N D *	0 35
Aorta	0 02	0 18	0 13

* N D , not detectable

Work with larger groups of chickens is in progress Preliminary results are consistent with those presented here Experiments involving more accurate characterization of the labelled sterols as well as autoradiography of specific tissues are under way

This work was supported, in part, by grants from the National Institutes of Health (*H*–3299) and the American Cancer Society (*E*88 and *E*89)

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Effect of Sheep Pituitary Extract on Hypercholesterolæmia in Intact and Hypophysectomized Rats

The prolonged administration of cortisone or adrenocorticotrophic hormone (ACTH) has sometimes been found to cause a change in the concentration of cholesterol in serum in human beings and in experimental animals While some workers[1,4] report that they have found such an elevation, others have noted no effect[7]; still others have reported the opposite[8]

A study of endocrine factors influencing the cholesterol[8] content of serum has shown that, although a mild increase in the concentration of serum cholesterol occurred in rats from daily subcutaneous injection of 2·5 mgm. of cortisone acetate for two weeks, there was no significant change from corticotropin[5] in a dose of 0 3 mgm (about 20 i u.) suspended in peanut oil and given subcutaneously over the same period of time However, in hypophysectomized rats there was an increase of 80 per cent or more in the cholesterol content of serum on treatment with 0 5 mgm of cortisone acetate daily for a period of 4 days or with daily injection of 0 3 mgm corticotropin in oil for two weeks The effect of corticotropin was mediated through the adrenals as there was no such elevation in adrenalectomized hypophysectomized animals.

The increased sensitivity of the hypophysectomized rat, over the normal animal, to the development of hypercholesterolæmia from cortisone or corticotropin was found to be much greater in the case of hypercholesterolæmia induced by feeding 5 per cent cholesterol and 0 1 per cent sodium cholate in 'Purina' fox chow diet for periods of from four days to three weeks A crude pituitary extract was found to lower the concentration of cholesterol under these circumstances

Acetone-dried sheep pituitary powder was extracted by stirring with distilled water and adjusting the pH to 10 with 2 5 N sodium hydroxide, after centrifugation the extract was lyophilized The dry material easily dissolved in saline at a concentration of 100 mgm per ml The animals were injected subcutaneously with a daily dose of 40 mgm over the period of cortisone treatment, or over the last four days of feeding a high-cholesterol diet Forty mgm of this extract was given to hypophysectomized rats twice daily for four days On the fifth day body weight and the weight of thyroid, adrenals, testes, seminal vesicles and ventral prostate were noted It was found that the extract was rich in gonadotropins and, in addition, contained small amounts of growth hormone, thyrotropin and corticotropin

None of the purified or semi-purified fractions of pituitary gland tested inhibited cortisone-induced or dietary hypercholesterolæmia completely The fractions tested were growth hormone of Raben *et al* [5,6] in a dose of 0 3–3 0 mgm daily, an extract prepared by the method of Koenig and King[2] in a daily dose of 2–6 mgm , Armour thyrotropin ('Thytropar'), 0 2 unit daily, Armour lactogenic hormone ('Panlitar'), 2–4 mgm daily, Schering pregnant mare serum gonadotropin ('Anteron'), 20 I U daily, desiccated thyroid, 0 006–0 01 per cent in diet, and testosterone propionate, 2 5 mgm twice weekly Œstrogen increased somewhat the serum cholesterol in the rat Animals given posterior pituitary powder in a dose of 5 mgm daily all died by the third day The extract of Koenig and King and desiccated thyroid had only a moderate effect alone or in combination However, the effect of these compounds was more pronounced when given in higher doses There was also a moderate effect noticed with testosterone and when bovine liver extract, prepared in a similar way and administered in the same dosage as the pituitary extract, was given (Table 1)

Table 1 EFFECT OF DIFFERENT HORMONES ON THE SERUM CHOLESTEROL CONTENT OF INTACT AND HYPOPHYSECTOMIZED RATS
For details of dosage and period of treatment, see text

	Normal (mgm per cent)	Hypophysectomized (mgm per cent)
Control	85 (117)*	73 (193)
ACTH	89 (8)	134 (10)
Growth hormone	84 (7)	73 (7)
ACTH + growth hormone	79 (7)	118 (6)
Cortisone alone	91 (5)	120 (18)
Cortisone + pituitary extract	—	71 (15)
Cortisone + growth hormone	—	181 (5)†
Cortisone + thyroxine (20 μgm daily)	109 (6)†	150 (6)†
Cortisone + thyrotropin	—	127 (5)
Cortisone + Koenig's extract	—	113 (5)
Cortisone + chorionic gonadotropin	—	126 (5)
Cholesterol diet‡	285 (4)	373 (4)
Cholesterol diet + pituitary extract	121 (6)	124 (5)
Cholesterol diet + Koenig's extract	224 (7)	241 (5)
Cholesterol diet + testosterone	—	205 (6)
Cholesterol diet + desiccated thyroid (0 006 per cent)	—	306 (10)
Cholesterol diet + desiccated thyroid (0 01 per cent)	164 (10)	150 (7)
Cholesterol diet + liver extract	245 (5)	210 (5)

* Number of rats used
† 2 5 mgm of cortisone given daily
‡ 5 per cent cholesterol diet for three weeks

In the doses used these fractions contained greater activity, as judged by the weight of the respective end organs in hypophysectomized rats, than was obtained from alkaline extract of sheep pituitary powder

This work was supported in part by research grants from the Massachusetts Heart Association and the United States Public Health Service (*A*–612) I am indebted to Drs E B Astwood and M. S Raben for their many valuable suggestions and helpful advice

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Antagonism of Reserpine by Intraventricular Bretylium

BRETYLIUM, as shown by Boura and Green[1], causes an inhibition of sympathetic nervous transmission by a blocking action at adrenergic postganglionic neurons Reserpine also inhibits peripheral sympathetic nervous transmission but does so by depleting adrenergic nerve endings of the transmitter substance norepinephrine[2] It also causes a depression of central nervous activity (tranquillization or sedation) which is associated with depletion of brain stores of norepinephrine[3] and 5-hydroxytryptamine[4]. It seemed of interest to see if bretylium could block the actions of reserpine on the central nervous system In order to study interactions of the two drugs on the central nervous system both had to be given so that effective concentrations of each could be attained at this site. Investigations by Boura *et al* [5] in which labelled bretylium, which is a quaternary amine, was administered to cats, demonstrated that only small quantities reached the central nervous system In order to attain a higher concentration the bretylium in the present work was administered intraventricularly

Male albino mice of *CF*–1 strain were used Bretylium tosylate, 4 mgm /kgm dissolved in normal saline, was given subcutaneously or in a single intraventricular injection of 0 03–0 05 ml following the method described by Haley and McCormick[6] Control intraventricular injections of equal volumes of normal saline were also made Reserpine phosphate, 2 5 mgm /kgm , was injected subcutaneously 0 5 hr after the intraventricular injection The mice were kept in individual jars The following tests were begun on the mice 0 5 hr after the reserpine injection (1) *spontaneous activity* as each jar cover was removed the mouse was scored 100 (moving body), 50 (moving head), or 0 (no movement), (2) *evoked*

Table 1 EFFECT OF INTRAVENTRICULAR BRETYLIUM ON RESERPINE IN MICE

Treatment No 1 (intraventricular)	Treatment No 2 (subcutaneous)	No of mice	Time tested after treatment No 2 ½ hr	1 hr	2 hr	3 hr	4 hr
			Blepharospasm (complete closure = 100)				
Bretylium	—	5	0	20	0	0	0
Saline	—	17	0	0	0	0	0
—	Reserpine	15	0	6	30	52	67
Bretylium	Reserpine	15	0	7	12	13	23
Saline	Reserpine	20	0	6	30	44	56
—	Reserpine plus bretylium	5	0	0	0	53	80
			Plasticity (complete immobility = 100)				
Bretylium	—	5	100	90	90	20	0
Saline	—	17	0	47	29	24	8
—	Reserpine	15	0	30	30	73	90
Bretylium	Reserpine	15	75	90	60	47	60
Saline	Reserpine	20	95	63	70	68	80
—	Reserpine plus bretylium	5	40	20	30	80	100
			Evoked activity (normal mouse = 100)				
Bretylium	—	5	60	80	100	100	100
Saline	—	17	86	88	91	94	92
—	Reserpine	15	90	90	63	37	17
Bretylium	Reserpine	15	40	40	47	57	53
Saline	Reserpine	20	87	83	40	33	25
—	Reserpine plus bretylium	5	90	100	90	50	0
			Spontaneous activity (normal mouse during first ½ hr = 100)				
Bretylium	—	5	50	0	20	60	80
Saline	—	17	93	41	29	32	38
—	Reserpine	15	95	57	50	30	10
Bretylium	Reserpine	15	0	0	23	33	30
Saline	Reserpine	20	17	10	5	10	0
—	Reserpine plus bretylium	5	100	80	60	70	40

activity the mouse was picked up by the tail and scored 100 (marked sideways movement or arching of body), 50 (slight movements of body), or 0 (no movement), (3) *blepharospasm* while held by the tail the degree of opening of the palpebral fissure was scored 100 (complete closure), 67 (almost closed), 33 (slightly narrowed), or 0 (wide open), (4) *plasticity* the mouse was replaced in the jar in a semi-standing position with its forefeet resting on the vertical sides of the jar and the degree of plasticity or immobility in this unusual position was scored 100 (retains unusual position for more than 15 sec), 50 (retains unusual position for 5–15 sec), or 0 (gets back on all four feet in less than 5 sec) The scores resulting from each series of mice are given in Table 1

The most marked effect observed was the block by intraventricular bretylium of the reserpine induced blepharospasm. Four hours after reserpine all mice without intraventricular bretylium had marked or complete blepharospasm (scores 67 or 100) while those with bretylium ranged from no closure to marked blepharospasm Subcutaneous bretylium was not effective in counteracting this action of reserpine

The intraventricular injections of saline or bretylium produced some plasticity, as defined above, in the responses of the mice This was more marked with bretylium, but disappeared over the 4-hr test period Reserpine had a slow onset, gradually producing plasticity, and this appeared to be inhibited by intraventricular bretylium

At 4-hr , evoked activity of reserpine-treated mice was depressed Subcutaneous bretylium and reserpine also blocked evoked activity Intraventricular bretylium did not depress evoked activity of the mice and tended to counteract the depression caused by reserpine

Spontaneous activity was the most variable parameter tested Mice receiving reserpine without bretylium showed somewhat less activity at 4 hr than those receiving intraventricular bretylium in addition to reserpine

The interpretation which we would give to the above results is that bretylium is capable of antagonizing the central effects of reserpine if it is injected so that it reaches the same sites of action as reserpine This correlates with the interaction of bretylium and reserpine peripherally reported recently by Costa[7], who has shown that when the two drugs are administered together the ability of reserpine to deplete cardiac stores of norepinephrine is markedly reduced.

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Effect of Aldosterone on the Living Cornea *in vitro*

EARLIER work has shown that the cornea of the rabbit, excised and mounted between two chambers, can be kept alive and functioning for 8 hr [1] during which time it can be demonstrated that the epithelium generates an electrical potential of 20–50 mV [1], that sodium is actively transported across the epithelium into the corneal stroma[2], and that the net flux of sodium is equivalent to virtually all the neutralizing current[2,3] This system appeared to afford a satisfactory model for the *in vitro* study of the effect on sodium transport of aldosterone, the principal sodium-retaining steroid of the adrenal cortex of most mammals studied[4] Aldosterone is assumed to affect sodium exchange in the renal tubule[5], and has been demonstrated *in vitro* to stimulate transport of sodium across the isolated bladder of a toad[6] and skin of a frog[7] In the present investigation the effect of aldosterone on the excised cornea of the rabbit was assessed *in vitro*

The cornea was excised and mounted in a lucite clamp between two chambers, each containing a facsimile of rabbit aqueous humour[1] (The composition of this fluid was the same as that used in the earlier work[1,2] except that 2 0 instead of 1 0 ml of glucose 12 per cent were added Ascorbic acid (20 mgm per cent) was added in several experiments, also[8]) The pressure within the chamber was adjusted to approximate normal intraocular pressure, 20 cm of water Thirty experiments were carried out with measurements of potential difference, electrical resistance, and neutralizing current being made at 20-min intervals The preparation was allowed to stabilize over a 2-hr period. Measurements were then made over an additional hour to establish a base-line for readings during the experimental period At the end of the hour, *d*-aldosterone (kindly provided by Dr Robert Gaunt of CIBA, Inc) was added to the bathing medium, in 15 instances to the epithelial side, and in 15 to the endothelial side The *d*-aldosterone was dissolved in distilled water The steroid was added to the preparation in such quantities as to arrive at a final concentration in the ambient fluid of 1–1,000 μgm /100 ml, that is, in concentrations ranging from 10^2 to 10^5 times those encountered under physiological conditions Such high concentrations are effective in enhancing sodium balance *in vivo*[9] and sodium transport *in vitro*[7] In two experiments, cortisol, dissolved in small quantities (0 1–1 0 per cent, final concentration) of ethanol, was added in addition to aldosterone, in order to assay a possible 'permissive' effect of cortisol on aldosterone action, as shown in the whole organism[9]

In order to provide for optimal diffusion and tissue binding of the steroid, 2–3 hr. were allowed to pass In two instances, tritiated aldosterone was added to the bathing fluid in order to determine the permeability of the cornea to steroid At the end of a 5-hr period, fluid was removed from both sides of the cornea, carrier aldosterone was added, the fluid specimens were extracted with ethyl acetate, and the extracts were washed, dried, and chromatographed in the Bush *B*5 system (benzene methanol water)[10], the steroids being localized by reaction with blue tetrazolium and eluates counted in a liquid scintillation counter

Table 1 PERCENTAGE CHANGE IN ELECTRICAL INDICES OF RABBIT CORNEA *in vitro* DURING THE ADDITION OF *d*-ALDOSTERONE*

Exp No	Experimental conditions	Electrical indices		
	Compound added to fluid bathing epithelium	% Δ *I*	% Δ *PD*	% Δ *R*
	(per cent)			
1	20 μgm aldosterone	0	+ 4	+ 9
2	1 μgm aldosterone	− 2	+10	+14
3	10 μgm aldosterone	− 4	− 6	− 9
4	20 μgm aldosterone	+28	+58	+18
5	10 μgm aldosterone	+ 3	+ 8	+ 8
6	10 μgm aldosterone	+ 8	+10	+ 1
	Compounds added to fluid bathing endothelium			
	(per cent)			
7	10 μgm aldosterone 20 mgm sodium ascorbate	+ 2	+ 9	+ 6
8	10 μgm aldosterone	− 5	+ 6	+ 5
9	10 μgm aldosterone 20 mgm sodium ascorbate	−14	+ 7	+26
10	10 μgm aldosterone 1 mgm cortisol	− 4	+ 7	+10
11	10 μgm aldosterone 1 mgm cortisol	+ 1	− 4	− 5
12	100 μgm aldosterone 10 mgm cortisol	+11	+14	+20
13	Control—nothing added	−14	+ 6	+21

* These figures represent percentage changes in current (*I*), potential difference (*PD*) and resistance (*R*) from a time one half-hour before the addition of aldosterone to a time 2 hr after addition of the hormone There is no large or consistent change in neutralizing current (sodium flux) Experience has shown that the small increases in *PD* and *R* cannot be interpreted as indicating anything more than the viability of the preparations

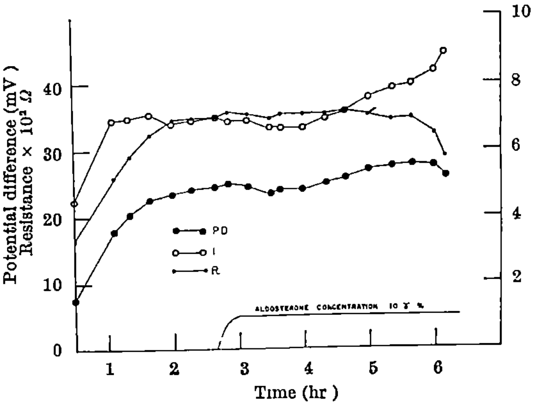

Fig 1 Results of experiment 6 No significant changes in electrical indices can be found in response to the addition of aldosterone to the bathing media The changes at the far right of the graph indicate the end of the useful life of the preparations

The results of 13 representative experiments are summarized in Table 1, and one of these experiments (No 6) is illustrated in Fig 1 It will be noted that the addition of *d*-aldosterone over a wide range of concentrations elicited no significant change in any parameter of electrical activity, that is, addition of the steroid did not enhance the active transport of sodium Further, addition of cortisol was without effect Aldosterone was shown to pass through the cornea, the concentration on the opposite side being about 1/100 that on the side to which the steroid was originally added

The results do not agree with those of Crabbé[6] or of Penney *et al* [7] They are, however, in harmony with those of Maetz *et al* [11], who failed to demonstrate an enhancement by aldosterone of active sodium transport in the skin of a frog *in vitro*, but showed such an enhancement in the isolated skin of frogs which had been pre-treated with the steroid A possible explanation for the lack of *in vitro* effect of aldosterone in this preparation is that more than 6 hr may be required for such an effect to become manifest However, it is not possible to keep the rabbit cornea alive and functioning effectively much in excess of 6 hr An alternative hypothesis is that sodium transport in this multi-layered, avascular membrane occurs in large part independently of hormonal control There is no *a priori* reason for supposing that aldosterone acts ubiquitously on cellular membranes

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HÆMATOLOGY

Use of Phenol in the Isolation of Erythropoietic Glycoprotein

THE erythropoietic activity in the plasma of rabbits made anæmic with phenylhydrazine can be isolated as an acid glycoprotein. A dialysed and lyophilized ethanol fraction from heat-coagulated plasma consistently gives us a potent stable highly water-soluble preparation. Six laboratories have found good correlation in a comparative assay of a batch prepared by us[1]. Further di-ethyl-amino-ethyl cellulose column fractionation removes 40–50 per cent of inert protein and results in the active glycoprotein previously described[2].

While the active peak could be re-chromatographed on di-ethyl-amino-ethyl cellulose without apparent change in electrophoretic and elution properties, there was usually a drop of 25–75 per cent in specific activity. Even more striking losses occurred in the fractionation of erythropoietin from human urines. Urine from patients with congenital hypoplastic anæmia was precipitated with 4 volumes of ethanol. The non-dialysable portion of the precipitate, a mixture consisting in the main of glycoproteins, contained essentially all the erythropoietic activity of the original urine. It could be further resolved on di-ethyl-amino-ethyl cellulose but with variable, sometimes complete, loss of activity.

One ethanol precipitate from human urine, after removal of the dialysable part, had this activity in a fasted rat assay[1]: 1 mgm. total dose per rat gave 26.8 per cent ^{59}Fe-uptake in the blood. When 100 mgm. portions of the precipitate were fractionated on di-ethyl-amino-ethyl cellulose we repeatedly lost 90–100 per cent of the activity although all the protein was recovered in successive eluates with 0.01 to 1.0 *M* sodium chloride.

We had observed before that plasma erythropoietin is not damaged by phenol and is extractable into a phenol upper layer like some other glycoproteins in the procedures of Morgan and Partridge[3], Westphal *et al.*[4] and Kirby[5]. By using as little as 0.1 per cent phenol in all equilibration and elution buffers in di-ethyl-amino-ethyl cellulose chromatography, as well as on both sides of the membrane during dialysis, we are now consistently recovering more than 50 per cent of the activity contained in the original urine.

The dialysed and lyophilized ethanol fraction from plasma, fractionated as previously described on di-ethyl-amino-ethyl cellulose[2], but in the presence of phenol, yields a peak with 2–3 times the specific activity of the original fraction, corresponding with the removal of inert material.

Evidence to date indicates that erythropoietic activity requires integrity of a glycoprotein since proteolytic enzymes as well as neuraminidase inactivate. Erythropoietin could be a glycoprotein associated with inert glycoprotein or even some other compound which becomes labile on disintegration of carrier glycoprotein. Enzymes like those mentioned, or other as yet undefined carbohydrases, either secreted with the urine or coming from other sources, could be responsible for large losses of urinary activity. The presence of phenol during isolation may prevent subtle changes through bacteriostasis, enzyme inactivation, anti-oxidant action, etc.

If it were not for measurement of the biological activity the protective effect of phenol would not have been noted. We hope that this preliminary report might be useful to those working with glycoproteins with or without biological activities.

This work was supported by a grant of the American Cancer Society.

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Thalassæmia in Melanesia

THALASSÆMIA has previously been regarded as non-existent in Melanesians[1]. In August 1960, a Papuan female from the Milne Bay area of New Guinea, aged 18–20 yr., was admitted to the General Hospital, Port Moresby, for re-investigation of a refractory anæmia first noticed during pregnancy in 1957. On that occasion she had been delivered of a full-term, apparently normal, infant, her hæmoglobin-level having been maintained by transfusions. Since discharge she had received no treatment and had been able to carry on normal village life, in spite of a hæmoglobin-level remaining fairly constant at 5–6 gm. per cent. Investigation in August 1960 showed typical bony changes, a hypochromic microcytic anæmia with target cells and normoblasts present in the peripheral blood, and the alkali denaturation test[2] showed 67 per cent undenatured hæmoglobin. The presence of hæmoglobin *F* as a major component was confirmed by hæmoglobin electrophoresis on cellulose acetate strips. Since then three other cases of thalassæmia major in Papuan children have been diagnosed, two of the children aged 5 yr. and 1.5 yr. came from the Kerema district, and one aged 3 yr. from the Oro Bay area in Papua.

All showed typical bony changes on radiological examination, a hypochromic microcytic anæmia with normoblasts and target cells in the peripheral blood and excess hæmoglobin *F*—32 per cent, 39 per cent and 46 per cent—by alkali denaturation, confirmed by hæmoglobin electrophoresis. All had been treated by transfusion on many occasions. In two of them diagnosis was delayed, presumably in one because of suppression of endogenous erythropoiesis by transfusion[3], and in the other by the presence of an aplastic crisis[4]. One of the children from Kerema has undergone splenectomy and his transfusion requirements have been greatly diminished since operation. Glucose-6-phosphate dehydrogenase estimation in this child on two occasions showed 46 and 56 units/100 ml. red blood cells respectively, suggesting that he has the negroid type of glucose-6-phosphate dehydrogenase deficiency in addition to thalassæmia.

In view of the fact that these cases have come from widely separated districts, it is considered that thalassæmia may be widespread in New Guinea.

Thanks are due to Dr. P. Brain of the Red Cross Transfusion Service, Perth, who carried out the hæmoglobin electrophoreses, Dr. D. Curnow and

Dr W Pitney, of the Royal Perth Hospital, Perth, who performed the alkali denaturation tests and examined the peripheral blood and marrow smears, and Prof J B Chatterjea of the School of Tropical Medicine, Calcutta, who confirmed the presence of hæmoglobin *F* in two of the cases, being the first to demonstrate it in one case Mr Ian Parsons, of the Peter MacCallum Institute, Melbourne, carried out the estimations of glucose-6-phosphate dehydrogenase

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HISTOLOGY

Antidiuretic Hormone and Renal Collecting Tubules

GINETZINSKY[1] has reported that, in the kidneys of rats treated with posterior pituitary extract, the epithelium of the collecting tubules becomes flattened and its basement membrane less distinct We have attempted to repeat Ginetzinsky's observations

Fifty-two young male white rats, 150–200 gm in weight, were fasted overnight with free access to water and then treated in one of four ways

(1) Eighteen rats were given water by stomach tube (5 per cent of body-weight followed by 3 per cent after 1 hr , or 7 per cent of body-weight with replacement of lost water every $\frac{1}{2}$ hr) and were killed during a diuresis of 2 0–3 5 ml (11 rats) or 1 0–2 0 ml (7 rats) per $\frac{1}{4}$ hr

(2) Sixteen rats were treated similarly, but the water diuresis was inhibited by a subcutaneous injection of 'Pitressin' (Parke Davis), 25–30 m units per 100 gm body-weight These rats were killed when urine flow, which was rapidly inhibited, had fallen to 0 3 ml /15 min or less, in 4 rats the flow had ceased completely for a half-hour or more

(3) Nine rats were given the hormone but no water, and (4) nine were given neither water nor hormone but killed after being handled similarly to the other groups In groups (3) and (4) the output of urine did not exceed 0 3 ml in any single 15-min period

The following methods were used for staining sections of kidneys of these rats (*a*) Bharadwaj and Love's method for mitochondria[2], (*b*) LaCour, Chayen and Gahan's method for lipids[3], fixation in Zenker's fluid, followed by (*c*) hæmatoxylin and eosin, (*d*) Heidenhain's azan, (*e*) toluidine blue or (*f*) the periodic acid–Schiff reaction (PAS), (*g*) fixation in formol saline, followed by Heidenhain's iron hæmatoxylin In each group, Bharadwaj and Love's method, the PAS reaction and either azan or hæmatoxylin and eosin were each used for at least half the rats and the other methods for two animals of the group

The staining of mitochondria and lipids showed differences between rats and between tubules in individual kidneys These variations are reported elsewhere[9]

From the examination of sections stained by the PAS reaction, by toluidine blue, by azan, and by Heidenhain's iron hæmatoxylin, the following generalizations can be made

(1) In all rats the collecting ducts were lined by cuboidal epithelium, which became low columnar in form near the tip of the pyramid The height of the epithelium of these ducts did not differ perceptibly from one rat to another or between groups of rats, if similar distances from the tips of the pyramids were taken in choosing fields for comparison

(2) The collecting ducts converge early in their course, so that sections parallel with the plane of the pelvis and ureter must be central if the ducts are to be seen in the pelvic half of the pyramid Besides these ducts, the only tubular organs in the innermost medulla are blood vessels and thin segments of Henle's loops Thus it is possible to obtain longitudinal sections of the pelvic half of the medulla which show only tubules lined by low cells, such as those in Ginetzinsky's Fig 4, but it does not follow that some of these must be collecting ducts, which in this region are to be found only in the centre of the pyramid

(3) The basement membranes varied in appearance between sections of the same block when toluidine blue was used, but, after the PAS reaction, these membranes were clearly seen, constant in appearance for each part of the nephron, and did not vary appreciably between the experimental groups

(4) The PAS reaction showed the collecting ducts to differ between individual kidneys in the proportion of intercellular boundaries which showed stained cement In the ducts of some kidneys PAS-positive material was present in the lumina in masses up to 50μ in diameter These two features were not obviously correlated with each other, nor did either provide a point of distinction between experimental groups

These findings do not support the suggestion that hyaluronidase, which Ginetzinsky[1,6] reported to be secreted in increased concentration in the urine under the influence of antidiuretic hormone, acts by altering the visible form of basement membranes and intercellular cement in the collecting ducts Since such variability in tubular mucopolysaccharides as was found in our experiments could not be correlated with the treatment of the rats, our results may be compared with those of Berlyne[4,5], who, estimating hyaluronidase in the presence of a constant concentration of electrolytes[7], reported that the rate of excretion of hyaluronidase in the urine was unrelated to the rate of urine flow We did not obtain any evidence for the apocrine activity which, according to Ginetzinsky[1], is indicated by a loss in height of the epithelium of the collecting ducts

The findings reported here agree with those of Heller and Lojda[8], who examined renal histology under the influence of water and antidiuretic hormone but did not record rates of urine flow

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PATHOLOGY

Acetylcholine in Sweat in Fibrocystic Disease of the Pancreas

THE varied secretory abnormalities in fibrocystic disease of the pancreas in which elevated sweat electrolytes and deficient pancreatic enzymes are linked to viscid mucus glycoproteins and increased parotid secretory rate suggest an underlying mechanism of overstimulation of exocrine glands with cholinergic innervation[1-4]. Roberts[5] further hypothesizes that excessive exocrine gland stimulation might result should acetylcholine (ACH), released at secretory nerve endings, persist owing to defective enzymic breakdown by cholinesterase, or, alternatively, to excessive quantities released. It seemed to us that a stimulating neurohumor not completely inactivated at its site of release might be found in the secretion of the gland it stimulates. Thus we examined sweat, a secretion abnormal in fibrocystic disease, for evidence of any characteristic neurohumoral activity. We have found in thermal sweat well-defined levels of a free choline-ester indistinguishable from acetylcholine. The activity was detected in sweat samples from normal individuals as well, but appeared to be of a significantly higher order in the fibrocystic.

Patient subjects were twenty-nine children in hospital at the National Institutes of Health, sixteen with and thirteen without fibrocystic disease of the pancreas. Their average age was eight years. Each was sweated thermally for 1 hr. in a plastic bag under blankets by a modification of Schwachman's method[6], in which collections were made from a restricted area

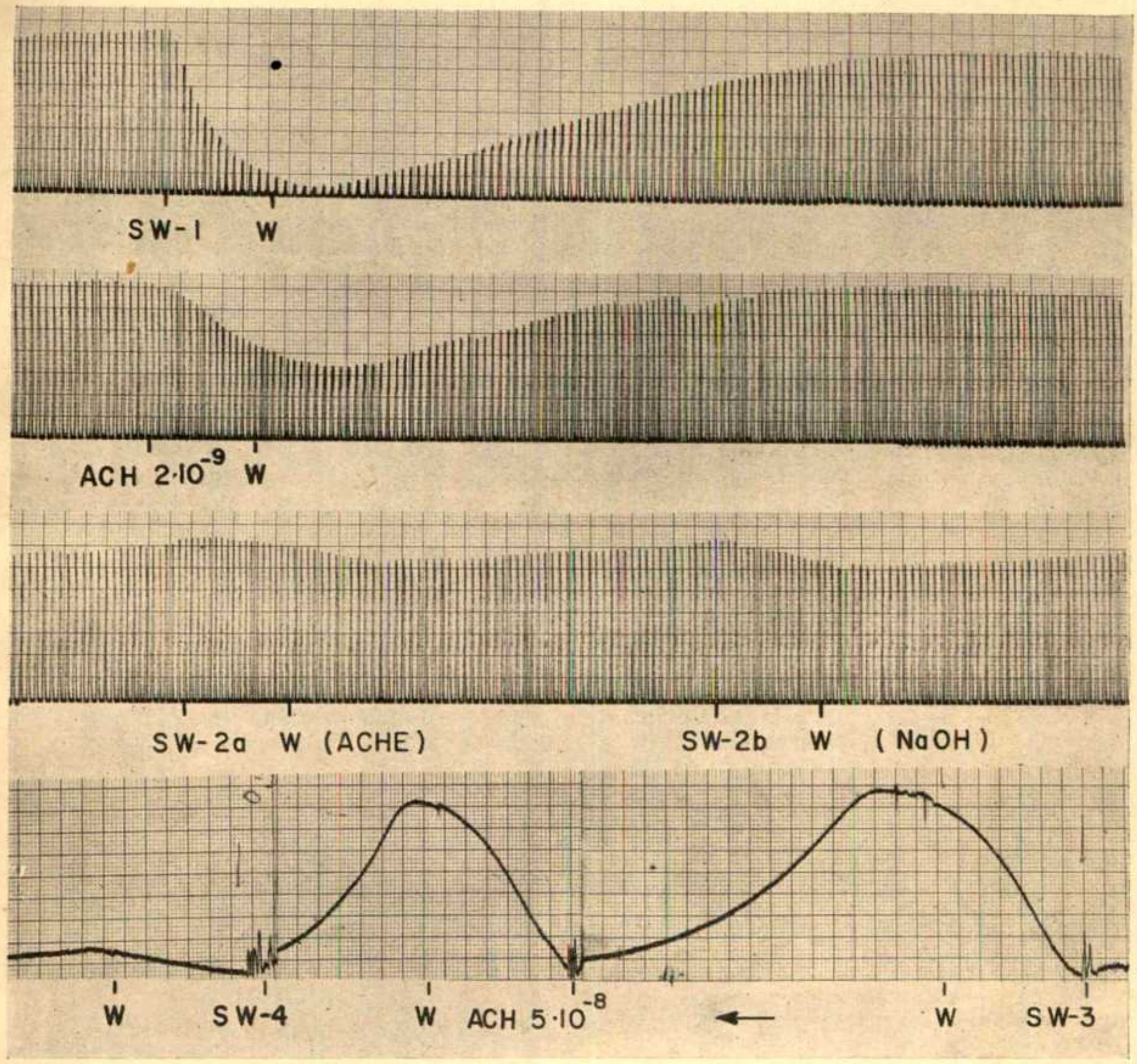

Fig. 1. Bioassay (*Venus* heart and frog rectus) of sweat from fibrocystic patient (lowest tracing reads right to left). *SW*-1, 0·4 ml. of sweat inhibiting *Venus* heart 95 per cent, giving estimated ACH content of 0·1 μgm./ml. of sample; *W*, wash, at 1·5 min. with *Venus* heart, 2 min. with frog rectus assay; ACH 2×10^{-9}, acetylcholine chloride, final concentration in bath of solution containing 0·02 μgm./ml. giving 50 per cent inhibition of heart; *SW*-2*a*–*SW*-2*b*, 0·4 ml. vol. of sweat inactivated by acetylcholinesterase (*SW*-2*a*) and sodium hydroxide (*SW*-2*b*); *SW*-3, 1·0 ml. of sweat giving 43 mm. contraction of frog rectus muscle, equivalent, after correction for interfering activity to ACH content of 0·15 μgm./ml. (several constituents of sweat affect the frog rectus contraction—we have found the highly specific *Venus*-heart assay gives a more accurate ACH level)—0·5 ml. of sweat gave a 17-mm. contraction, not shown); ACH 5×10^{-8}, acetylcholine chloride, final concentration in bath of solution containing 0·25 μgm./ml., giving 38 mm. contraction of muscle; *SW*-4, 0·55 ml. of sweat inactivated for ACH with acetylcholinesterase treatment; 4 mm. contraction from non-inactivated substances remains

of the back and simultaneously from the rest of the body surface. Sweat obtained by another method[7] and for shorter time-periods gave similar results. Measurement was made of sweat volume, pH, sodium and potassium. Samples were acidified to pH 4·0 with 1 N hydrochloric acid and assayed immediately or frozen to preserve activity. Sweat was tested in volumes of 1 ml. or less for choline-ester activity by bioassay on the heart of *Venus mercenaria* according to Welsh's method[8] and occasionally, when feasible, on the eserinized frog rectus muscle[9].

Two inactivation procedures used on each sample distinguished choline-ester activity from that of choline and inorganic ions. Activity was abolished by boiling for 15 min., at pH 12·0, after addition of sodium hydroxide but preserved after boiling at acid pH 4·0 (ref. 9). In the second procedure the activity was destroyed on incubation for 1 hr. with 0·5 mgm. of commercially purified bovine erythrocyte cholinesterase (ACHE) in buffer at pH 6·8. pH measurements were made with a glass electrode.

Fig. 1 shows an example of the activity found in sweat from a representative fibrocystic patient. The average choline-ester activity for the sixteen fibrocystic patients expressed as acetylcholine was 0·075 μgm./ml. of sweat (range 0·01–0·25 μgm./ml.). The corresponding figure for the thirteen control patients was 0·0065 μgm./ml. (range 0·002–0·02 μgm./ml.). Five fibrocystic patients gave very high values averaging 0·19 μgm./ml. Some scatter appears in the data from the disease group. Also overlap occurs between groups as one fibrocystic failed to give levels higher than 0·01 μgm./ml. while two non-fibrocystics presented levels of 0·015 and 0·020 μgm./ml. Levels tended to be constant on retesting the same individual and did not appear to be related to sweat volume or pH.

Our work has not been extended to adults or to infants, nor is it controlled for variable degrees of acclimatization or for the finer physiological parameters of sweat collection. The relationship of the findings to function of sweat glands therefore remains to be clarified. Two previous reports describe acetylcholine in sweat from normal individuals in levels estimated at 0·001–0·002 μgm./ml. (ref. 10). The presence of this neurohumor in remarkably higher amounts in the sweat of fibrocystic patients is being investigated.

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Types of Cell in Ascites Tumours

THE mouse tumour *MCIM* (Klein) exists in two sub-lines, the M_{SS}, which only grows in the solid form, and the M_{AA}, which grows as an ascites tumour and also in solid form, designated M_{AS}. The difference between the behaviour of the sub-lines is as yet unexplained in terms of cell structure. Bergstrand and Ringertz have recently found no constant difference on examination with the electron microscope[1]. These tumours have been investigated recently by means of the aniline-blue staining technique[2].

It has been found that solid tissue cells, including those of solid tumours, show a blue rim- or surface-staining which is believed to be associated with staining of the common part of the cell walls of adjacent cells which lie between the electron-dense cell membranes. In the M_{SS} tumour such staining is seen in fresh preparations and in sections. The fresh ascites cells of the M_{AA} tumour, like all ascites tumour cells examined, fail to stain at all with the aniline-blue reagent, and stand out as clear, refractile spheres in the blue solution. However, there are also present in the fluid, and sometimes loosely adherent to the peritoneum, small compact masses of cells which, when washed to remove loosely attached free cells, are firm in consistency and show a blue staining surface which may be thick and irregular.

When fresh preparations of the M_{AS} tumour are so treated, it is found that both types of cell are present but in different proportions. There is, in young tumours, a preponderance of solid tumour masses which show blue-staining surfaces, with isolated pools of 'free' cells which can readily be washed out from the tissue and which fail to stain. It appears, therefore, that tumours of either type derived from the $_{AA}$ sub-line contain both elements, the predominating form depending on the environment. This accounts for the appearance of apparently typical 'ascites' cells in the M_{AS} tumours examined by Bergstrand and Ringertz. The softening found in the centre of older M_{SS} tumours is probably due to the cell degeneration so commonly found; in the M_{AS} tumours there may, in addition, be a predominance of 'free' cells in such areas.

Whether the two forms of cell breed true or whether the surface changes are reversible is not yet clear. Further investigations into this and into the reason for the failure of the free ascites cells to stain with aniline-blue are in progress.

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BIOLOGY

Feeding Habit of Some Central African Freshwater Fishes

FISHES of the family Citharinidae, belonging to the genera *Phago* Steind., *Eugnathichthys* Blgr. and *Belonophago* Gilt. (Figs. 1–2), together forming part of the sub-family Ichthyoborinae, have an extremely specialized and curious feeding-habit, being, namely, fin-eaters.

This most interesting fact was discovered during an ichthyological survey of Lake Tumba and the Central Congo Basin by our Institute in 1959 Amongst other biological observations, the examination of all gut contents was particularly stressed, and a large number of specimens belonging to these genera were found to have fin fragments in their stomachs All species belonging to these genera apparently are exclusively fin-eating, at least in the adult stage (Only *Phago loricatus* Gunth , from the Niger, has not been examined) Other genera, like *Gavialocharax* Pell and *Paraphago* Blgr , judging from their morphology (dentition), probably also belong to this interesting little group, while *Phagoborus* Myers , though ichthyophagous, also has been found to eat fins occasionally

Several authors, amongst others G Marlier[3], had already noticed that most fishes in the Central Congo rivers had mutilated fins, often even severely torn and cut up This condition was thought to be caused by some carnivorous species of leech, insect or fish, but was mostly ascribed to bacteria or some type of fin rot, and at first we put forward the hypothesis that it was done by a large predaceous Dytiscid beetle (*Cybister* sp), which often attacks (small) fish Mr J P Gosse, of the I N E A C , had also recently noticed this strange fin-eating habit in some of these species of fish, thus further confirming our observations

Morphologically, these fish are very specialized and possess a powerful dentition, well adapted to their method of feeding They are very elongate species, *Belonophago* even needle-like in form *Phago* and *Belonophago* are very slender, possessing large, extremely hard scales, often strongly ridged, their jaws are long, narrow and beak-like *Eugnathichthys* has numerous small soft ctenoid scales and shorter, broad and strong jaws The first two genera are small, respectively reaching a size of 16 and 12 cm , whereas *Eugnathichtys* can grow to 30 cm

The mouth is large, the jaws elongate, closing tightly on one another, the premaxillary is mobile

The teeth are cuspidate (bicuspid, usually), in two parallel rows, those in the outer one much larger, strong, sharp and firmly inserted on the external border of the jaw, forming a saw-like ridge This last character is particularly apparent in *Eugnathichthys*, the dentition of which reminds one of that of South American Piranhas (*Serrasalmus*)

The intestine is very short, nearly straight, being coiled back only once on itself, with numerous pyloric cæca, the stomach is large and muscular In spite of the bony nature of the material ingested, digestion seems to be fairly rapid, to judge from the fact that many specimens had empty stomachs The stomach contents are composed exclusively of diverse fin fragments and some scales, the intestine containing only hard concretions, largely of a calcareous nature The young (of less than 30–40 mm standard length), feed mostly on aquatic insect larvæ (*Ephemerida*, *Trichoptera*)

Observations effected in a large aquarium containing a specimen of *Belonophago hutsebauti* Gilt and several other fish species (*Distichodus*, *Chrysichthys* spp) revealed some interesting aspects of its mode of feeding The fish 'hovered' in horizontal position just below some floating grass, the fins (especially the pectorals) vibrating As soon as some other fish swam within reach, it darted out, grabbed hold of a fin between its long jaws and snipped off part with a quick twist, the startled victim unconsciously favouring this action by also twisting and pulling away minus a piece of fin

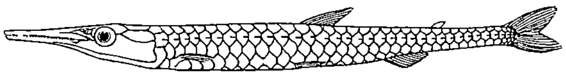
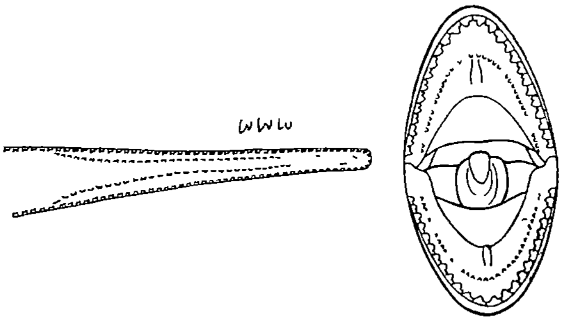


Fig 1 *Belonophago tinanti* Poll (× 2/9) (ref 10)



(a) (b)

Fig 2 (a) *B tinanti*, upper jaw, showing dentition (ref 10) (b) *E eetveldi*, mouth, showing dentition (ref 10)

The species belonging to the genera mentioned often go about in small groups in lakes and large rivers, close along the shores, and preferably in relatively calm zones where vegetation (bushes, grass) trails in the water, or near the expanses of floating grass (*Echinochloa* and *Panicum*) which clog up the river arms and coves The species attacked usually are slow-moving herbivores like *Distichodus*, *Tylochromis*, or bottom-feeders like *Chrysichthys*, though more vigorous swimmers like *Alestes*, *Lates* and even *Hydrocyon* often also show lacerated fins The dorsal, anal and caudal fins are those commonly bitten off, paired fins rarely showing mutilations Moreover, according to the native fishermen, they appear to attack man on some occasions

A similar feeding habit has been suspected for the Nyassa cichlid fish *Docimodus johnstoni* Blgr , the strong jaws and cutting teeth of which are figured by Boulenger[2] Of this it is written[1] "This fish is well named Chiluma—'the thing that bites' Stomach contents suggest that the cutting teeth may be used to bite pieces from the fins of other fishes" Trewavas states (personal communication) that of eleven stomachs examined six were empty, four contained fragments of fins and one a little plant and insect débris Among marine fishes the Blenniidae *Runula*[4] and *Aspidontus*[5] are described as parasites, biting bits off fins and skin of other fishes, *Aspidontus* approaching its victim by means of a mimetic resemblance to a 'cleaner-fish' An allied habit is that of scale-eating, practised not only by certain Cichlidae of lakes Tanganyika[8,9] and Nyasa[2,6,11] but also by a Panamanian characid, *Roeboides occidentalis*[3]

Considered economically, these fishes are a nuisance in the sense that the wounds inflicted not only pave the way for various diseases (especially bacterial and fungal), like fin rot, but often contribute to make the victim an easy prey for predators, some having their fins nearly entirely amputated, thus considerably hampering their locomotion and balance

Fin-eating fish are very common in some places, for example, in Lake Tumba, where many species show serious fin mutilations Unfortunately, there exists no effective method actually of reducing their numbers, as they are hard to catch with conventional fishing gear Small-meshed gill-nets, however, placed perpendicularly to the shore in appropriate zones, often catch quite a number, but have the disadvantage of also catching the young of many economic species, at least in certain seasons

This curious mode of feeding almost certainly evolved from the voracious habits of their ancestors, this being supported by the fact that related genera, like *Mesoborus* Pell and *Phagoborus*, for example, are distinctly ichthyophagous, the latter even partly fin-eating occasionally, whereas all members of the sub-family Ichthyoborinae are predominantly carnivorous

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Mediterranean Fishes of the Family Istiophoridae

Scombroid fishes belonging to the family Istiophoridae are rather uncommon in the Mediterranean However, a species has been known for a long time and was actually discovered there It is *Tetrapturus belone*, described by Rafinesque[1] from Sicily, where it is captured chiefly during the summer months, later, it was found in other localities, for example, Spain, Genoa, Naples, Malta and the Adriatic

A second Istiophorid was described by Canestrini[2] after a specimen was caught in the bay of Genoa This was given the name *Tetrapturus lessonae*, which was synonymized by subsequent authors with *T herschelii* Gray or with *T georgii* Lowe The type of *T lessonae* is now preserved in the Museum of Natural History in Genoa; another similar specimen was also caught long ago in the Bay of Genoa and still exists in the Zoological Museum of Turin[3] Both these fishes are adult and are easily recognizable as *Makaira albida* (Poey) Such identity was suggested by La Monte[4] and is fully confirmed by careful study of all their characters, during a recent visit to Florida I became familiar with *M albida* This species is well known in the Atlantic, including its eastern part, where it has been recently observed[5] It certainly exists in the Mediterranean too, but it seems to be of very rare occurrence in this Sea, maybe it does not reproduce there It is probable, however, that some records of *T belone* really belong to *M albida* We do not know at present whether the *Tetrapturus* living in the Atlantic (for example, Florida) are the same species as those of the Mediterranean It would appear unlikely that such widespread pelagic fishes as Istiophorids should include a species restricted to the Mediterranean As new investigations on these huge fishes are carried out, it becomes more and more evident how far we still are from adequate knowledge of them *T belone* and *M albida* are very similar, both morphologically and biologically, and I do not feel certain that they deserve to be generically separated (rather than included in *Makaira* Lac 1803, which has priority over *Tetrapturus* Raf, 1810) I would entirely reject their placing in different families, Istiophoridae (*Tetrapturus, Istiophorus*) and Makairidae (*Makaira*), which is the procedure adopted by Bertin and Arambourg[6]

Ben Tuvia[7] reported a young *Istiophorus gladius* (Brouss) from Haifa (Israel) and supposed it to be an immigrant from the Red Sea through the Suez Canal I know no other Mediterranean records of this easily identifiable genus It is to be looked for chiefly in the southern parts of this Sea, not only eastward, but also in the western section, where the occasional occurrence of the Atlantic form (*I americanus* Cuv Val), that exists along the coasts of Africa and Europe, is quite possible *Makaira albida* is to be expected in the whole western basin of the Mediterranean An expert student of Istiophorids, Col J K Howard (Marine Laboratory, University of Miami), recently told me that this fish is sometimes caught off Ceuta and La Linea

In conclusion, the Mediterranean fauna certainly includes at least three species of Istiophoridae *Tetrapturus* (or perhaps *Makaira* ?) *belone* Raf (the commonest, known for a long time in the Mediterranean only), *Makaira albida* (Poey) (Atlantic, recorded from the Bay of Genoa), *Istiophorus gladius* (Brouss) (Indo-West Pacific, recorded from Haifa)

As stated above, further researches are needed With the co-operation of some ichthyologists from the United States, we hope to improve the knowledge of the Mediterranean Istiophorids and to reach a better understanding of their relationships with the species living in the Atlantic

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Development of the Polychaete *Fabricia sabella* (Ehr)

A striking feature of the rock shores of the southern coast of Northumberland is the presence of extensive silt patches which lie on top of the rock platforms in the intertidal area These patches are occupied characteristically by dense populations of the sabellid worm, *Fabricia sabella* (Ehr)

While engaged on an ecological study of this annelid the opportunity was taken to investigate its breeding Rasmussen has been quoted by Thorson[1] to have expressed the opinion that the reproduction of this species is non-pelagic A study of the Northumberland population has shown that not only is the development non-pelagic but also that the entire larval development takes place within the tube of the adult female

When the tubes of adult females collected in May were dissected open the presence was observed of small batches of eggs and larvæ in various stages of development situated in small dilations of the tube, each batch being covered with a mucus envelope Up to ten batches could be found in a single tube with 1–7 individuals in each batch The batches were distributed along the length of the tube from the

Table 1

Tube No	Top of tube 1	←— Batch No —→ 2	3	Bottom of tube 4
1	4 Stage I	6 Stage IV	Larvæ hatched, mucus envelope left	4 Stage V
2	4 Stage I	5 Stage III–IV	2 Disintegrating	
3	3 Stage I	4 Stage III–IV	2 Stage I	
4	7 Stage I			
5	4 Stage III–IV	3 Stage IV	5 Stage V	

bottom to the top and all stages of development from fertilized egg to active pre-adult worm could be observed

At no stage was any swimming larva observed, and in fact the early larvæ appear to be atrochous Five arbitrary stages of external characters have been recognized in the development (1) large yolky uncleaved egg 150–200μ, (2), cleavage stages to gastrula, (3), early hatched larvæ—pyriform in shape and without obvious external features (250–300μ), (4), a more elongated form (350μ) with 2 velum-like branchial lobes and incipient ventral lip, 8 chætigers and both anterior and posterior eyes, (5), the final stage has nine of the adult eleven chætigerous segments, the branchiæ are well developed and the worm is crawling actively At this stage the young worms apparently leave the tube of the adult Table 1 gives the data derived from the tubes of five adult females and shows the number of batches in a tube, the number of individuals per batch, and the arbitrary stages of the individual's development

The information regarding reproduction in the Sabellidae has been summarized by Wilson[2] The most similar development to *Fabricia* appears to be *Dasychone lucullana* Delle Chiaje, the eggs of which are enclosed in a mucus sac shaped like a thick ring around the mouth of the tube of the parent Although in this case the early larva has a strong prototroch, it is not clear from the account of Claparède and Mecznikow[3] whether a swimming larva occurs

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A Spartinetum at Baldoyle in Ireland

IN 1870, a species of *Spartina*, known later as *S townsendii* (H and J Groves), was collected from the mud-flats near Southampton It was growing in a mixed community of *S alterniflora* and *S maritima* (then *S stricta*) It resembled the latter species so strongly that it was labelled '*S stricta*', and was overlooked until 1878, when Townsend recorded the species officially in his *Flora of Hampshire*[1] It was assumed generally that the intermediate *S townsendii* was a hybrid between *S alterniflora* and *S stricta*, for example, Stapf[2] stated "the evidence is circumstantial but strong, resting upon many of the characters being intermediate between those of its presumed parents, *its pronounced instability, and its varying fertility*" [The italics are ours] In 1930, Huskins[3] furnished cytological evidence favouring this assumption when he found that *S townsendii* had a mitotic chromosome count of $2n = 126$, while *S alterniflora* and *S maritima* have $2n = 70$ and $2n = 56$, respectively.

From that time until recently, it was accepted that all *S townsendii* growing at Southampton would be amphidiploid In 1956, however, male-sterile plants were found at several localities near Southampton[4] They resembled the type specimens of *S townsendii*, and had a chromosome count of $2n = c$ 63 (ref 5) Thus it was established that the primary F_1 hybrid was still in existence in the area, and that it was sterile in all cases examined It was suggested that this sterile F_1 hybrid was *S townsendii*, that the seed-bearing *Spartina* had been incorrectly identified with *S townsendii*, and that this seed-bearing species was temporarily nameless

The only species of *Spartina* known in Ireland[6] was introduced as cuttings in 1925[7] It was planted soon afterwards in mud in the Baldoyle Estuary some eight miles north-east of Dublin, but was not officially recorded from that area until 1937[8] In November, 1958, an investigation was begun of the extensive and spreading meadow of *Spartina* which now fills part of the estuary The following interesting facts have already emerged from the investigation

Spartina plants show the "pronounced instability" and "varying fertility" recorded by Stapf[2] They vary in stature from short, stunted ones closely resembling *S maritima*, to tall vigorous ones which exceed *S alterniflora* in height by as much as 1 ft This range in stature is wider than that usually ascribed to the amphidiploid *S alterniflora* × *S maritima* It is paralleled by a correspondingly wide variation in the inflorescence, where one spike is very frequent on stunted plants, and up to sixteen have been found on tall, vigorous ones Lush growth is typical of older *Spartina* meadow where the plant is growing in foul-smelling, deep, soft mud, and is clearly visible at full tide, shorter stature is characteristic of meadow where the plant is growing in more sandy mud, and is completely covered for some time by every tide, finally, extreme stunting and a red colour characterize small clones growing in sandy areas bordering the muddy reaches of the estuary, they are covered by every tide Similar stunting has been observed by Chater and Jones[9] for *Spartina* plants growing in a sandy habitat in the Dovey Estuary

Male sterility is quite common In any clone, inflorescences vary from completely male-sterile to normal The term 'male-sterile' is used here to describe inflorescences in which the anthers fail to emerge, or if they do emerge, fail to dehisce, and in which pollen is variable in size and content 'Normal' inflorescences are those in which abnormalities were not observed Seeds have been found in inflorescences

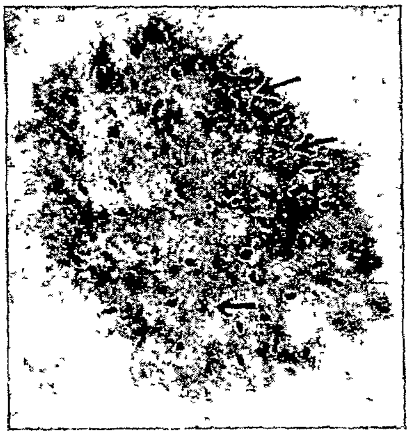

Fig 1 The three quadrivalents are marked with arrows

of all types and in all parts of the marsh, but so far, 80 per cent of the inflorescences contained 0–5 seeds only It was not possible to detect the presence of seed in spikelets except by dissection or touch The latter method was used All seeds were normal, and 100 per cent germination was obtained The chromosome number of all plants examined was $2n = 126$ (Fig 1) Three quadrivalents and 57 bivalents are present in the cell illustrated, and quadrivalents are common at meiotic metaphase Meiotic abnormalities were observed in some pollen mother cells of all anthers examined, multivalents, asynapsis and lagging chromosomes being the most usual

Male sterility has been used to distinguish the diploid hybrid *S alterniflora* × *S maritima* with $2n = 63$, from the tetraploid with $2n = 126$, but in the Baldoyle Estuary male sterility is quite frequent in clones with $2n = 126$ It is suggested, therefore, that until some clear-cut morphological distinction is observed, the name *S townsendii* (H and J Groves) is equally applicable to both known cytotypes.

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Influence of Date of Flushing on Flowering in *Pinus banksiana*

NUMEROUS attempts have been made to relate the year-to-year variability in flowering of forest trees to seasonal fluctuations in weather conditions which, in turn, are believed to influence the internal physiological processes regulating flower initiation[1] The pronounced effect of photoperiod on flowering, noted in many herbaceous species, appears to be lacking in forest trees, and observations indicate that most trees are photoperiodically day-neutral[2,3] However, until more definitive experiments have been undertaken, photoperiod cannot be ruled out as a factor influencing flower formation

Since weather factors, primarily temperature and rainfall, appear to exert an influence on flower-formation, it may be that favourable weather conditions and/or photoperiod must be synchronized with a particular developmental stage of the bud for flower primordia to form It might be assumed that such synchronization would be thrown out of phase by an exceptionally early or late spring which would shift the receptive stage of development into a more favourable or unfavourable set of environmental conditions

To test this possibility, an exploratory study was conducted with jack pine (*Pinus banksiana* Lamb) Three-year-old potted trees were overwintered in outdoor cold-frames, and the following spring (1959), prior to normal flushing, the trees were transferred to a cold-storage room On the dates indicated in Table 1, lots of 30 trees each were brought into a greenhouse maintained at minimum temperatures of 60° F (night) and 72° F (day), late April and May day-temperatures usually exceeded this minimum in the greenhouse The plants were exposed to the natural photoperiod and were watered daily Shortly after the last group (May 18) was removed from cold storage, the trees of all treatments were transplanted in nursery beds and exposed to prevailing environmental conditions The controls were 3-year-old trees transplanted directly in the nursery beds 1 year prior to the treated plants and were therefore subjected to a natural environment during the spring of 1959 At the time of floral induction, the treated trees were in their fourth, and the controls in their fifth, growing season

Table 1 FLOWERING RESPONSE OF YOUNG JACK PINE TREES FOR VARYING DATES OF FLUSHING

Treatment date	Total No of trees	No of trees flowering				Total No of strobili	
		Male	Per cent	Female	Per cent	Male†	Female
4/ 6/59	28*	5	17 8	22	78 6	199	40
4/20/59	30	12	40 0	20	66 7	482	30
5/ 4/59	30	—	—	19	63 3	—	33
5/18/59	30	1	3 3	15	50 0	1	23
Control‡	123	—	—	70	56 9	—	134

* Excluding two trees that died in the nursery
† Male strobili produced in clusters of aments varying from 1 to 32 in number
‡ Control, 5-year-old trees, treated groups, 4-year-old trees

The results, shown in Table 1, are based on strobili counts made in late May and early June, 1960 The results show a marked superiority of the April 20 date for staminate, and the April 6 date for pistillate, strobili-formation This differential response is no doubt due to the location and the sequential initiation of the flower primordia of the two sexes Male strobili occur on the basal portion of the new shoot of older lateral branches whereas the female strobili, at least in young jack pine, occur primarily on the apical end of the main shoot contiguous with the lateral branch buds Jack pine is multinodal in the bud[4], and occasional female strobili may be found at the secondary whorl positions of the new shoot as well as at the apical end of the newly formed laterals These differences in position, which are characteristic of the genus *Pinus*[5], indicate that the staminate flowers may be laid down well in advance of the pistillate flowers, as shown for other members of the hard pine group[6–8]. Although phenological data are not available for jack pine, information for other species of *Pinus* indicates that the time differential between the initiation of flower primordia for the two sexes may be in excess of several weeks

Several environmental factors may contribute to the variable flowering response between treatments, the most obvious of which are photoperiod and temperature Each set of buds would be subjected to a different régime of photoperiod and temperature from the buds of the previous and subsequent treatments as they attained the stage of development at which flower primordia were initiated Photoperiod varies uniformly and can be readily evaluated Temperature and temperature-photoperiod interactions, however, cannot be readily evaluated under natural conditions as wide fluctuations occur throughout the growing season The possibility must also be considered that neither photoperiod nor temperature exerts a direct influence on flower initiation and that the differential response to treatment may be due to the length of the growing season available for bud-development Data have been presented indicating that source of seed also influences significantly the flowering response of young jack pine[9] Because of the preliminary nature of the present investigation, definite conclusions cannot be drawn, but continuation of the work under more exacting conditions may permit critical evaluation of the influencing factors

The data obtained nevertheless suggest that the seasonal fluctuations in date of flushing may be one of the factors responsible for variable flowering behaviour Such fluctuations would place the dates of male and female flower initiation in different sets of environmental conditions and these would vary from year to year Moreover, due to the vagaries of weather conditions, the same date of flushing in subsequent years may not necessarily evoke the same flowering response

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Rootstock Influence on the Flowering of Seedling Apples

THERE has long been divergence of opinion as to whether or not the flowering of seedling trees, especially pome fruits, could be hastened by cultural practices or through environmental changes At the California Institute of Technology, it was found with *Pinus* that the 'juvenile' or initial non-flowering period of the seedling's life could be shortened following artificial lengthening of the period of summer growth and prolonged winter dormancy[1] In apples, any treatment that restricts or retards growth has been held by some to delay the onset of reproductive ability, while others have held the contrary view[2,3]

Experience at East Malling has long suggested that seedling material budded on the dwarfing Malling (*M*) IX (Jaune de Metz) rootstock fruited distinctly earlier than could be expected of seedlings on their own roots[4] No evidence was available as to whether this effect was attributable to the individual characteristics of the *M* IX rootstock as such or directly to its dwarfing and precocious character

In the course of a very considerable programme of crossing and selfing to produce, directly or through later back-crossing, apples combining eating quality with disease resistance and with the character of late flowering as a means of avoidance of spring frost damage, hundreds of seedlings selected from large families on the basis of late leafing and disease resistance have been retained on their own roots, to provide material for future propagation A bud from each of these seedlings, usually taken in the seedling's second year, has also been grown on one or other of two dwarfing rootstocks, *M* IX and 3431, the latter having the parentage *M* XIII × *M* IX Normal *T* budding on 18-month old bedded layers was practised

Table 1

Family No	No of seedlings	Trees on their own roots Flowering at five years	Flowering at six years	Flowering at seven years	Trees budded on *M* IX Flowering at three years	Flowering at four years	Flowering at five years
1	138	0	13	24	11	93	107
2	126	0	3	12	29	83	95
3	13	0	0	2	1	6	10
4	7	0	0	2	1	6	6
5	164	0	1	1	5	47	41
6	15	0	1	0	0	5	6
7	139	0	2	5	1	24	14
Totals	602	0	20	46	48	264	279

Seven families were raised in 1954 and buds from 602 selected seedlings were worked on *M* IX rootstocks in the summer of 1956 The numbers that flowered are given in Table 1

A further seven families were raised in 1955 and buds from 302 selected seedlings were worked on rootstock 3431 The numbers that flowered are shown in Table 2

Table 2

Family No	No of seedlings	On their own roots Flowering at five years	Flowering at six years	Budded on 3431 Flowering at three years	Flowering at four years
8	169	11	19	37	67
9	36	0	4	5	12
10	39	0	3	7	19
11	20	2	2	1	8
12	2	0	1	1	1
13	9	0	0	0	3
14	27	0	0	0	4
Totals	302	13	29	51	114

Thus, on *M* IX, 43 per cent had flowered in the sixth year from germination as against 3 per cent on their own roots On rootstock 3431, 38 per cent had flowered in six years from the seed as against 9 per cent on their own roots

As neither the families nor the period of experiment were identical, it is difficult to compare the effects of *M* IX and rootstock 3431 on such limited data It may not, however, be out of place to note that 3431 is more dwarfing than *M* IX[5] A more comprehensive experiment is needed if this clearly demonstrated effect of dwarfing rootstocks in reducing the 'juvenile' period is to be related more clearly to the degree of 'dwarfing' characteristic of the individual rootstock Further, it must not be forgotten that, while *M* XIII is markedly more vigorous, *M* IX is one of the parents of 3431 The possibility of a specific heritable attribute is therefore not excluded

It would seem[4,6] that the effect reported here is not to be related either to the relative mass or to the physiological age of the stock, since working small seedlings on large trees of long-established commercial varieties of apples had no effect on shortening the 'juvenile' period The work reported here supports the view that its length, while clearly susceptible to influence by dwarfing rootstocks, also varies markedly from seedling to seedling and from family to family

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Replacement of an Alternating Temperature Requirement for Germination by Gibberellic Acid

IN many species of plants, seed-germination does not take place readily except under special conditions A barrier to germination may be imposed by the seed coats or other coverings, by the presence of germination inhibitors in some part of the seed, by morphological or physiological immaturity of the embryo, by the need for light or for its absence, or by the need for alternating temperatures during germination[1] Chemical substances can in some cases substitute for specific environmental factors in effecting seed germination Nitrates, for example, promote the germination of some light-requiring seeds in the dark[1], and the results of recent work indicate that the plant hormone gibberellic acid is particularly effective in overcoming several types of germination inhibition In the germination of 'Grand Rapids' lettuce seeds, the primary light-requirement[2,3] and the light-requirement induced by high temperature or dark-osmotic treatments[3] can be replaced by gibberellic acid Gibberellic acid overcomes the inhibiting effect of coumarin on lettuce seed germination[4] and of endogenous germination inhibitors present in *Avena fatua*[5] and *Betula* spp [6] seeds Kallio and Piroinen[7] found that gibberellic acid has a marked stimulating effect on the germination of seeds of 8 species, including *Gentiana nivalis* and *Geranium sylvaticum*, their experiments were conducted in the light at 24°

We have examined the effect of gibberellic acid on another type of germination inhibition, namely, that caused by a requirement for alternating temperatures A series of experiments has been conducted with *Asclepias fruticosa* seeds, which do not germinate at constant temperatures ranging from 10 to 35° C, but only at alternating temperatures[8] Repeated cycles of alternating temperatures are not essential, if the seeds are kept at 18° C for 7 days, transferred to 35° C for 4 hr and replaced at 18° C, 80–100 per cent germination is obtained within 3 days after the return to the lower temperature[8] These seeds are not light-sensitive

Table 1 EFFECT OF GIBBERELLIC ACID ON THE GERMINATION OF *Asclepias fruticosa* SEEDS

50 seeds per treatment No germination took place before treatment with gibberellic acid

Gibberellic acid (p p m)	Percentage germination after 2 days	4 days	6 days	8 days	14 days
0	2	4	4	4	4
1	2	4	4	10	20
10	16	30	46	56	72
50	12	50	74	82	96
100	12	64	92	94	96
500	6	16	26	34	38
1,000	0	0	0	6	10

The effect of gibberellic acid on the germination of *Asclepias fruticosa* seeds was studied at a constant temperature of 18° C in the light The seeds were placed on moist filter paper in Petri dishes After 3 days, the seeds were pricked by passing a fine needle through the seed coats, endosperm layers and cotyledons, and were transferred to Petri dishes containing filter papers moistened with gibberellic acid solutions at concentrations ranging from 0 to 1,000 p p m The results of a typical experiment are presented in Table 1 Rapid, almost complete, germination was obtained when the seeds were treated with 50 or 100 p p m gibberellic acid Concentrations of gibberellic acid lower than 50 p p m were less effective in promoting germination and concentrations higher than 100 p p m were inhibitory Gibberellic acid was completely without effect if the seeds were not pricked, which indicates that the seed coats and/or the endosperm layers may be impermeable to gibberellic acid In this respect, *Asclepias fruticosa* seeds are similar to those of *Geranium sylvaticum*[7], which do not respond to gibberellic acid unless they are first scarified with sulphuric acid

These results show that gibberellic acid is highly effective in replacing the need for alternating temperatures in the germination of *Asclepias fruticosa* seeds Experiments are in progress with the seeds of *Argemone mexicana*, which normally germinate only if subjected to repeated cycles of alternating temperatures in the presence of potassium nitrate, and the preliminary results indicate that gibberellic acid promotes rapid seed germination at a constant temperature in the absence of nitrate It will be of interest to determine how general the promotive effect of gibberellic acid on the germination of seeds having special requirements is The effect of gibberellic acid on dark-germinators, for example, does not seem to have been investigated

This work was supported by grants from the South African Council for Scientific and Industrial Research and the Department of Agricultural Technical Services We thank Messrs Chas Pfizer Inc, New York, for a gift of gibberellic acid

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Relationship between 3,4-Dihydroxyphenylethylamine Content and the Genome, *acuminata*

THE major polyphenoloxidase substrate responsible for the blackening of the fruit of the banana has been identified[1] already as 3,4-dihydroxyphenylethylamine Although the original work was carried out using material of the clones Gros Michel and Mysore, a range of clonal varieties was investigated afterwards to determine whether this substrate was responsible for blackening in other clones or whether other phenolic compounds were implicated in the blackening reaction

It has been found that in all clones of the edible banana investigated the major polyphenoloxidase substrate is 3,4-dihydroxyphenylethylamine and furthermore that a positive correlation could be shown between the content of 3 4-dihydroxyphenylethylamine and the extent of the contribution of the genome *acuminata* to the genotype

It is now recognized[2] that the majority of the edible bananas had their origin in only two wild species, *Musa acuminata* and *M balbisiana* Simmonds and Shepherd[3], using a scoring method, were able to establish the relative contribution of the two wild-species to any given clone The genetic composition of each of the clones investigated is shown in Table 1 All plant material for the investigation was obtained from the collection of banana plantings at the Imperial College of Tropical Agriculture, Trinidad Earlier work on the distribution of 3,4-dihydroxyphenylethylamine in the banana indicated that although

Table 1 CONTENT OF 3,4-DIHYDROXYPHENYLETHYLAMINE IN PREPARATIONS OF CLONES OF DEFINED GENOTYPE

Clone	Genotype (ref 2)	Dihydroxyphenylethylamine content*
Kelantan	*AA*	+ + + +
Ceylon	*BB*	+
Pancha Bontha Bathees	*ABB*	+ + +
Nendra Padaththi	*AAB*	+ + +
Red	*AAA*	+ + +
Calcutta	*AA*	+ + +
SH 62	*AB*	+ +
Ney Mannan	*ABB*	+
Guindy	*AB*	+
Mporo Moka	*AAB*	+ +
Bluggoe	*ABB*	+
Tiparot	*ABBB*	+
Rajah	*AAA*	+ + +
Gitigi	*AAA*	+ + + +
Nkonjwanshansha	*AAB*	+ + +
Dwarf plantain	*AAB*	+ + +

* Amounts assessed by size and intensity of the corresponding *p*-nitroaniline spots on two-dimensional chromatograms

A, Genome, *acuminata*, *B*, genome, *balbisiana*

present in small amounts in the pulp, larger amounts were present in the skin of the fruit Extracts of the skin were therefore used for the comparative investigations described here

Methanolic hydrochloric acid extracts of skin tissue obtained from a range of clones were prepared by methods described before 0 02 ml of each extract was applied to duplicate paper chromatograms (Whatman No 1) and subjected to two-dimensional development with butanol/acetic acid/water (40 10 50) followed by 2 per cent acetic acid One member of each pair of duplicate chromatograms was sprayed with *p*-nitroaniline reagent and the other with a banana polyphenoloxidase preparation After incubation of the enzyme-sprayed chromatogram in a humid chamber, brown colorations developed in the position corresponding to that of 3,4-dihydroxyphenylethylamine The size and intensity of the spots obtained with *p*-nitroaniline reagent in the position were proportional

The results which are shown in Table 1 indicate that the content of 3,4-dihydroxyphenylethylamine is determined by the extent of the contribution of the *acuminata* genome to the genotype

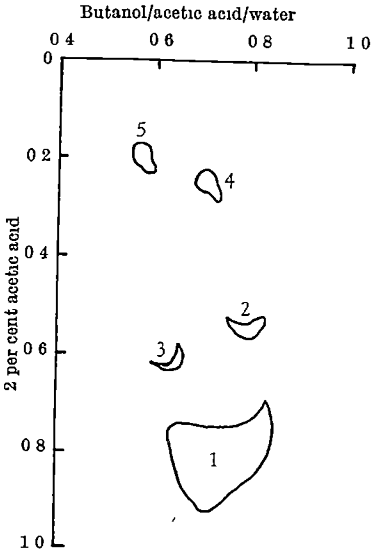

Fig 1 Composite diagram showing the position of fruit phenolics of banana cultivars 1, 3,4-dihydroxyphenylethylamine, 2-5, unknown minor phenolic constituents (Colorations with *p*-nitroaniline 1, purple, 2 and 3, red, 4 and 5, brown Ultra-violet fluorescence 2, white, 3, green, 1, 4 and 5, non-fluorescent)

Two-dimensional chromatography (Fig 1) showed that 3,4-dihydroxyphenylethylamine is the only major phenolic present in the fruit investigated Although a number of minor phenolic constituents were detected, these were present in trace quantities only and clearly did not contribute to any appreciable extent to the blackening of the fruit Clonal differences were almost wholly quantitative, the same phenolics being present in all the clones to a varying degree The major source of variation was (as noted in Table 1) the content of 3,4-dihydroxyphenylethylamine, the amounts present ranging fron trace quantities in *M balbisiana* varieties to very high levels in the *M acuminata* cultivars

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CYTOLOGY

Cytology and Origin of the Dwarf Coconut Palm

DISTINCT varieties of dwarf coconut (*Cocos nucifera* L) occur in Malaya, Philippines, Fiji Islands, Viet Nam, Ceylon and India These varieties grow to a height of 15–30 ft compared with more than 50 ft among normal tall types and need only 3–5 years to commence bearing in contrast to 8–10 years taken by the tall strains While the tall varieties are largely obligatorily cross-pollinated, the dwarfs usually undergo self-pollination owing to the overlapping of the female and male phases in the same inflorescence As a result, many dwarf strains yield homogeneous progenies and they can be used with advantage for identifying the most pre-potent males[1] The poor vigour and copra quality of the dwarf palms, however, render them unsuitable for cultivation on a plantation scale[2] In addition to the typical tall and dwarf palms, various intermediate categories occur in Nature and have also been obtained by crossing the tall and dwarf strains[3]

Several semi-tall and dwarf varieties of coconut show 15–35 per cent pollen sterility, as judged by a tendency to become stained with aceto-carmine The tall varieties, on the other hand, contain less than 5 per cent of sterile pollen[4] A detailed study of meiosis in microsporocytes was hence undertaken in two tall, one semi-tall and three dwarf palms While meiosis was always regular in the tall plants, the following abnormalities occurred in several cells in the others (*a*) one quadrivalent at diakinesis and metaphase I, suggesting heterozygosity for a reciprocal translocation, (*b*) a dicentric bridge and an acentric fragment at anaphase I indicating heterozygosity for an inversion, (*c*) nucleoli which persist at metaphase and anaphase I, (*d*) pollen mother cells with varying chromosome numbers, (*e*) lagging chromosomes at the first and second anaphase and (*f*) sporads with varying number of spores There was also a significant reduction in chiasma-frequency in Apricot (semi-tall) and Green (dwarf), as compared with the two tall varieties The percentage of cells with abnormalities at different stages of meiosis in the varieties examined is given in Table 1

Table 1 MEIOSIS IN SOME COCONUT VARIETIES

Variety	Type	Percentage of cells with abnormalities at Metaphase I	Anaphase I	Second division	Sporad
Laccadive ordinary	Tall	0	0	0	0 8
Local type	Tall	0	0	0	0
Apricot	Semi-tall	20 4	40 1	24 7	29 2
Dwarf red	Dwarf	4 0	23 6	8 6	7 9
Dwarf green	Dwarf	0	5 1	2 9	4 3
Dwarf yellow	Dwarf	0	4 6	2 2	1 3

The occurrence of various abnormalities and 'chromosome mosaic' cells at meiosis, and a poor development of the endosperm in the dwarf palms, indicate that the efficiency of the chromosome mechanism has been affected in somatic, gametic and endosperm cells It is probable that the imposition of inbreeding on a normally out-crossed species is responsible for this situation, since it is known that inbreeding upsets the chromosome mechanics in cross-pollinated species[5-7] Some authors[3,8] have suggested that the dwarf strains of coconut may have originated through a sudden discontinuous mutation from the common tall palm From the cytological results it seems equally probable that the dwarf coconuts occurring in Nature in different countries may be the products of inbreeding among different tall varieties This view is also supported by the following First, the tall and dwarf palms differ in not one but a series of characters all of which seem to lead to reduced vegetative vigour and aberrant cytological behaviour Secondly, all gradations between the two contrasting categories represented by typical talls and dwarfs occur in Nature Thirdly, the F_2 progeny of a dwarf × tall cross gave a wide range of segregates and the data did not suggest any simple genetic explanation[2] The more regular meiosis observed in the dwarf varieties in comparison with the semi-tall type suggests that with progressive inbreeding an equilibrium condition may be reached with regard to cytological behaviour The same may also be true of vegetative vigour An initial mutation, if any responsible for the origin of the dwarf coconut might have led to the overlapping of the male and female phases of the inflorescence, thus rendering self-pollination both possible and predominant However, self-pollination can occur in the tall palms during certain seasons and a mutation facilitating this process is not a prerequisite for inbreeding to occur Alternatively, a situation analogous to that described by Harland[9] in the maize strains of Trinidad may be responsible for the evolution of stable self-pollinating dwarfs

Whatever may be the mode of origin of the dwarf coconut, the fact that many geographically distinct and true-breeding dwarf strains exist indicates the possibility that they may be useful both to estimate and exploit heterosis in coconut Much of the hybridization work carried out on coconut so far relates to crosses between tall and dwarf varieties or among tall strains The only record of a cross between two geographically distinct dwarfs seems to be that made by Marechal[10] between two dwarf strains from Malaya and the Fiji Islands The F_1 plants of this cross showed outstanding performance and have been much sought after in Fiji[11] Harland[1] has suggested the induction of haploidy in coconut for obtaining homozygous genotypes and, if the view that some dwarf coconuts represent stable inbred lines is correct, they may serve the same purpose as the autodiploids derived from haploids Harland's technique for identifying pre-potent males would be ideal for estimating combining ability in different dwarf × dwarf crosses

We are indebted to Drs B P Pal and A B Joshi for their interest in this work and to Prof S C Harland for advice on the manuscript

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MICROBIOLOGY

Factor converting Mesophilic into Thermophilic Micro-organisms

IN the course of work on thermophilic, aerobic, spore-forming bacteria, we have observed that the thermophilic cells contain, in certain conditions, a principle, which, when added in small quantity to a fresh culture medium, permits an inoculum of a mesophilic organism to grow at elevated temperatures Moreover, this induced thermophily is carried from one transfer to another for an indefinite period

Concentrated preparations of this factor produced the effect in quantities of 5 μgm per ml of medium The properties, described here, suggest that the factor is in the nature of a nucleoprotein We have also observed that it is only present, or at least extractable, during vigorous growth of the thermophilic culture, but not after the exhaustion of nutrient has led to sporulation

The addition to a medium of an autolysate obtained from *Saccharomyces cerevisiae* also permitted the growth of a mesophilic inoculum at 55°, but this effect appears to differ from that obtained by the homologous factor inasmuch as thermophily cannot be indefinitely maintained without renewed addition of the yeast factor

The source of the principle is *Bacillus stearothermophilus* No 194, grown in a medium described elsewhere[1], and supplemented with 1 per cent casein hydrolysate This medium was inoculated with a stock culture of the thermophilic bacillus and incubated overnight at 55° This culture, now germinated, was inoculated into fresh basal medium and incubated at 55° for 9–11 hr, this procedure resulted in a small amount of spore-formation Incubation for 15 hr or longer periods, when sporulation had set in, did not yield the principle

After an incubation period of 9–11 hr, cells were collected in a Sharples refrigerated centrifuge at 50,000 r p m Packed cells were washed by homogenization in a Waring blendor with cold distilled water and again centrifuged The packed bacilli were lyophilized and stored at 4° C The converting factor was obtained from the lyophilized bacillus powder by suspension in 0 05 *M* sodium citrate and grinding with glass beads (type 112, Minnesota Mining and Mfg

Co , U S A) for 10 min in a Waring blendor at 4° C The debris was centrifuged off at 2,000 r p m , the remaining cell suspension was further centrifuged at 5,000 r p m for 6 hr at 4° C The supernatant was discarded The residue was extracted twice with 50 ml of 2 *M* sodium chloride, the transforming factor is not stable in aqueous solution The extract, diluted with 0 16 *M* sodium chloride, was added in varying amounts to a medium, which was then inoculated with a mesophilic strain of *B sphaericus* This mesophil was found to grow at 37° in 0 5 per cent beef extract (Difco) plus 0 5 per cent proteose peptone (Difco) but not at 55° It now grew at 55° on addition of the converting factor down to dilutions of 5 µgm /ml , growth is still obtained at 37° with or without addition to the principle

The converting principle is destroyed by deoxyribonuclease, ribonuclease, pepsin and papain (Worthington Biochemical Corp , U S A), while trypsin, chymotrypsin, and carboxypeptidase do not inactivate it The principle is non-dialysable against distilled water, and is unstable in aqueous solution as compared with 2 *M* sodium chloride This behaviour is similar to that of deoxyribonucleic acid[2]

The factor will stand 1 min of heating at 100°, but its heat stability is influenced by the rate of cooling, its efficacy will be less diminished if cooling is carried out slowly, for analogous observations on depolymerization of bacterial nucleic acid, see ref 3

These findings suggest that the factor is nucleoprotein Nucleic acid preparations from thermophilic bacteria were not effective In order to test substances rich in nucleic material, yeast autolysate (Albimi Laboratories, Brooklyn, N Y) was tested and a similar transformation of the mesophilic organism was observed[4] Growth in medium containing yeast autolysate was required to maintain thermophily, contrary to this finding, once thermophily was induced with thermophil extract, it was no longer required to maintain thermophily on repeated sub-culturing Attempts to purify and compare the converting factor from the thermophilic bacteria and from yeast autolysate are now under way We are also studying the specificity of the effect

This work was supported by grant *G*–8250 of the National Science Foundation

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Influence of adding Salts to the Medium on Counts of Phenol-treated *Escherichia coli* on Membrane Filters

IT has been shown[1] that suspensions of *Escherichia coli* which had been treated with phenol gave lower counts on membrane filters than on the surface of nutrient agar Many workers[2] have observed that bacteria which have been exposed to bactericides or heat exhibit a marked sensitivity to the nature of the subculture medium, and both Flett *et al* [2] and Jacobs and Harris[3] have shown that when ferric chloride was added to the medium it exerted a beneficial effect, demonstrated by longer extinction times than in control media, on the growth of phenol-treated organisms The latter workers found that the rate and extent of the death of phenol-treated cells of *E coli* and *Staphylococcus aureus* which occurred in nutrient broth[4] was reduced by the inclusion of $5 \times 10^{-3} M$ of magnesium chloride, and that the inclusion of ferric chloride and other metal chlorides in nutrient agar gave surface-viable counts of such cells greater than were obtained on nutrient agar without metals[5] In view of the present widespread use of membrane filters, and suggestions that these filters should be used to recover damaged cells from a variety of harmful environments, for example, in sterility tests, it was thought useful to examine the effect of adding salts to the nutrient broth on which the membranes are incubated

Chlorides of iron, chromium, calcium, magnesium and magnanese were added to the nutrient broth ('Oxoid *CM* 15', 16 gm /l) before sterilization to give concentrations of 0 0037, 0 00185, 0 000925, 0 00037, and 0 000185 *M* The method of counting has been described in ref 1, ten drops of a diluted suspension were placed on the surface of the membrane and counts on membranes using media containing added salts were compared with those obtained on a control nutrient broth, without salts In this work only one strain of *E coli* was used (strain *A* of Harris and Richards[1]), since two of the strains used in the earlier work were known to behave similarly when salts were added to nutrient agar

The counts of untreated cells did not differ significantly from those on the control whatever the nature or concentration of the cation used, and the results are not presented here Table 1 gives the mean percentage counts from phenol-treated suspensions in numerous trials at each concentration of the cations, and the significance of the results, given by the analysis of variance test, is indicated also In contrast to untreated cells, counts of phenol-treated cells were influenced markedly by the presence of salts However, only iron influenced favourably the counts from treated suspensions at all the concentrations used except at 0 00037 *M* of chromium and 0 000925 *M* of calcium and magnesium, with which counts did not differ significantly from the control, these three cations also gave favourable responses Manganese gave variable results and counts were greater and less than on the control with equal frequency The most favourable of the concentrations tested was 0 00185 *M* with all the cations, and the improvement in counts was most marked with chromium and iron

Possible mechanisms for the beneficial action of salts have been discussed by Jacobs and Harris[4].

Table 1 MEAN RELATIVE MEMBRANE FILTER COUNTS OF PHENOL-TREATED *E coli* ON MEDIA CONTAINING VARIOUS CONCENTRATIONS OF A RANGE OF CATIONS

Molarity of ion ($\times 10^6$)	Mean relative viable count* on Iron	Chromium	Calcium	Magnesium	Manganese
3,700	160	154	152	134	54§
1,850	841	1,421	364	478	208
925	221	179	100†	99†	41§
370	174	159†	167	140	136†
185	166	183	165	180	157

* Relative to nutrient broth without added salt as 100

† No significant difference from control , § significantly less than control, all others significantly greater than control, all at the 5 per cent level at least

They suggested that substances in the medium which are toxic to damaged cells could be removed by adsorption on metal precipitates or by co-precipitation with them, and that cations which are not precipitated could reduce the permeability of the damaged cell membrane and so reduce the inward diffusion of such toxic substances and the leakage from the cell of vital metabolites[6,7] The protective influence of metallic cations, especially those of high valency, against the action of phenol has been observed in ref 7

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Enzymic Inactivation of Trichothecin and Crotocin

WORK on the inhibitory action of trichothecin[1] and crotocin (substance *T*, ref 2) on the inductive amylase synthesis of *Penicillium chrysogenum*[3] suggested that these antibiotics may undergo enzymic inactivation in certain circumstances The possibility of such an enzymic inactivation was first recognized by Darpoux and Faivre-Amiot[4]

Penicillium chrysogenum was grown on a synthetic medium as described previously[5] Cultures for the experiments were prepared by inoculating 200 ml synthetic medium with 10 ml of a 72-hr -old vegetative mycelium suspension The fate of crotocin added was examined by two different methods First, the antibiotic was added in a 1 mgm /ml single dose after 48 hr incubation The level of the antibiotic in such a culture remained unchanged for a minimum of 24 hr as revealed by the Bradler[6] agar-diffusion method In the second series of experiments 10 μgm / ml crotocin was added after 40 hr and this was followed by the addition of 1 mgm /ml of the same antibiotic after 48 hr incubation Under these experimental conditions a 70 per cent decrease in the antibiotic activity of crotocin was observed in 5 hr

This phenomenon suggested the possible presence of a crotocin-inactivating enzyme in cultures pretreated with a low dose of the antibiotic The enzyme appeared to be bound to the mycelia as the supernatant was inactive, while 1 gm of washed mycelium (calculated on dry material) inactivated an average of 40 mgm crotocin per hr The preliminary examinations carried out up to now indicated that the enzyme was probably bound to the cell-wall of the mycelium

It is known that trichothecin is an ester of trichothecolon and isocrotonic acid[7] The alcoholic component of crotocin is very similar, though not identical, to that of trichothecolon It seemed plausible that the decrease of the biological activity was a result of enzymic cleavage of the ester linkage The validity of this assumption was later confirmed by isolating and chromatographing the split products

After inactivation of crotocin by washed mycelia the mixture was filtered and the fluid phase was saturated with sodium chloride and extracted with chloroform at *p*H 10 (ref 8) The extract was evaporated and the oily residue crystallized from benzene The isolated substance had a melting point of 155 5° C , identical with the melting point of the alcoholic component obtained by alkaline hydrolysis of crotocin Mixed melting points of the two materials obtained by the two different methods did not exhibit any depression The acid component was identified by paper chromatography

According to the above observations crotocin if applied in small quantities can act as an inductor The enzyme thus induced is able to hydrolyse the ester linkage in the antibiotic which destroys its biological activity On the addition of a single large dose of crotocin no inactivation of the antibiotic activity could be observed since this quantity inhibited also the synthesis of the inductive enzyme Crotocin might be replaced by trichothecin, both as an inductor and a substrate

Other fungi such as *Penicillium urticae*, *Aspergillus parasiticus* and *Aspergillus oryzae* failed to exhibit similar action *Aspergillus niger*, however, when tested under the same experimental conditions, had an effect similar to that observed for *Penicillium chrysogenum*

This work will be published in detail in *Acta Microbiol. Hung*

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Detection of Gibberellic Acid in *Azotobacter* Cultures

IT is known that *Azotobacter* cultures can affect the germination of seeds and growth of plants It is assumed that this influence is due to the presence of physiologically active substances and vitamins in the cultures Actually, indole-3-acetic acid has been detected in *Azotobacter* cultures[1,2] In a previous paper[2] the detection of another growth-active compound, in addition to indole-3-acetic acid, was described More details about this compound, which appeared to be gibberellic acid, are presented here

Azotobacter chroococcum was grown in Burk's solution containing traces of molybdenum and boron, the same procedure as that used for growth of cultures when isolating indole derivatives[2] The medium did not contain calcium carbonate, however After ten days of incubation the culture was centrifuged, the supernatant evaporated *in vacuo* to a tenth of its original volume, made acid with 1 *N* hydrochloric acid (to *p*H 2 8–3 0) and extracted with *n*-butanol[3] or

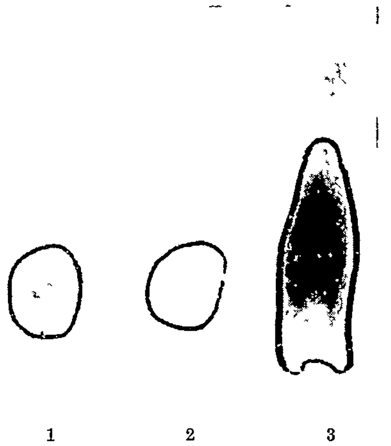

Fig 1 Chromatogram of gibberellic acid from culture of *Azotobacter chroococcum* 1 and 3, various concentrations of n-butanol extract, 2, 50 µgm of gibberellic acid

peroxide-free ether[2] Individual extracts were placed on Whatman No 1 filter paper and chromatograms developed in four systems butyl acetate saturated with water[3], benzene / pyridine / 3 M - ammonium hydroxide (3 6 1)[4], benzene/acetic acid/water (10 2 5 5)[5] and n-butanol/formic acid/water (4 1 5) Best separation of indole-3-acetic acid from gibberellic acid was obtained in butyl acetate saturated with water Gibberellic acid remained at the start ($R_F = 0\ 1$) while indole-3-acetic acid moved nearer the front ($R_F = 0\ 8$).

The last-named system, however, has the drawback of forming 'tails' behind the spots of both acids From the point of view of shape and sharpness the system with acetic or formic acid is more advantageous as dissociations of indole-3-acetic acid and gibberellic acid are suppressed and sharp round spots are obtained In the formic acid system the R_F values of the two growth substances are very close, which represents a handicap in elution of the spots from the chromatogram and in biological testing of the eluate

Detection of gibberellic acid on chromatograms was carried out with 3 per cent sulphuric acid in methanol followed by heating to 60° C for 5 min [4], or with 0 5 per cent aqueous potassium permanganate[5] By adding 0 05 per cent ferric chloride to the methanolic sulphuric acid the reagent can be used for detecting both indole-3-acetic acid and gibberellic acid Indole-3-acetic acid gives a violet-red colour and displays no ultra-violet fluorescence while gibberellic acid shows a blue-green ultra-violet fluorescence but gives no colour reaction in visible light

The position of the spot of the substance tested which gives a colour reaction with both reagents corresponds in all the four systems used to the R_F of a standard solution of gibberellic acid- Fig 1 shows a chromatogram developed for 8 hr in benzene–pyridine–3 M ammonium hydroxide (3 6 1) in the downward direction We used the system described by Bird and Pugh[5] to demonstrate that the *Azotobacter* strains contain mostly gibberellic acid (gibberellin A_3) rather than gibberellin A_1 The chemical detection of gibberellic acid was confirmed by a biological test Undetected spots corresponding to gibberellic acid on the chromatograms were eluted with distilled water and a part of the eluate was tested on etiolated sections of maize leaves and mesocotyls, as described by Boyarkin and Dmitriyeva[6] The test is specific for gibberellins Indole-3-acetic acid, if present in the solution tested, has a slight inhibitory effect A positive significant influence of the eluate on longitudinal growth of the maize leaf was established

The positive influence of the biological test, together with the results obtained by paper partition chromatography technique, indicates the presence of gibberellic acid in *Azotobacter* cultures The amount of gibberellic acid in the supernatant of a 10-day-old culture varied under the above cultivation conditions between 2 and 5 µgm /ml In a 17-day-old culture, where the source of carbon had been exhausted, the amount of gibberellic acid rose to 20 µgm /ml It is assumed that the presence of gibberellic acid together with indole-3-acetic acid in *Azotobacter* cultures can have a connexion with the frequently noted effect of *Azotobacter* cultures or their filtrates on germination of seeds or growth of seedlings

So far as is known from the literature the presence of gibberellic acid has been demonstrated in cultures of *Fusarium moniliforme*[7] and other *Fusarium* species[8], of some non-identified Actinomycetes[8] and of the soil yeast *Torula pulcherrima*[9] The finding described here supplies evidence for the existence of gibberellic acid in *Azotobacter chroococcum* Beij. It appears that gibberellic acid is more prevalent among micro-organisms than was believed, even if in lower concentrations than those found in *Fusarium* cultures

I am indebted to M Podojil of the Institute of Biology for a gift of a mixture of gibberellin $A_1 + A_3$

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Nutritional Requirements for the Induced Formation of Malic Enzyme in *Lactobacillus arabinosus* (17-5)

In 1948, Korkes and Ochoa[1] described an enzyme preparation from *Lactobacillus arabinosus* which catalysed the conversion of L-malic acid to lactic acid and carbon dioxide They called it malic enzyme. The activity of this enzyme was found to be very low in organisms grown on ordinary medium When malic acid was also included in the medium during growth, the ability of the organisms to break down malic acid was found to have increased about fifty times They also demonstrated that the presence of the growth medium and glucose was essential to the formation of the enzyme

The growth medium of *L arabinosus*, like that of other lactic acid bacteria, is very complex It contains hydrolysed casein, a mixture of inorganic salts, glucose, and special supplements of amino-acids, vitamins, purines and pyrimidines Gale has shown[3]

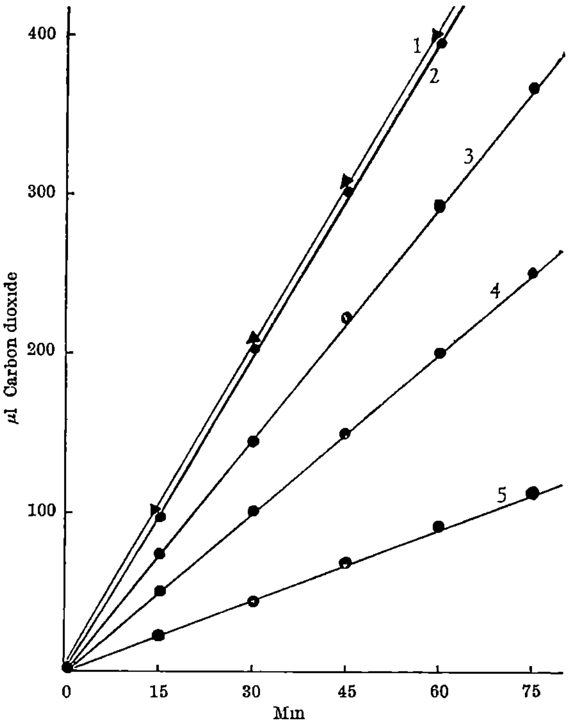

Fig 1 The effect of nutrition on malic enzyme induction The samples contained 0 04 *M* L-malic acid, *p*H 6 0, manganese ions, 0 1 *M* phosphate buffer and 5 0 mgm dry weight cells, suspended in phosphate buffer Curve 1, complete medium, curve 2, supplement containing eleven amino-acids and a purine/pyrimidine mixture, curve 3, supplement containing eleven amino-acids only, curve 4, supplement containing purine/pyrimidine mixture only, curve 5, control, contained no supplement In all cases the incubation mixture also contained 1 per cent glucose, manganese ions and phosphate buffer

that the nutritional requirements for optimum growth of organisms may differ from those necessary for the formation of a particular enzyme, and in a recent review Lichstein[4] has directed further attention to this phenomenon It was interesting, therefore, to find out which constituents of this complex medium may be more directly involved in the formation of malic enzyme

The activity of malic enzyme in intact cells of *L arabinosus* was determined manometrically, by recording the evolution of carbon dioxide from malic acid, at *p*H 6 0 and 30° C using small Warburg flasks

In order to separate the requirements of induced enzyme formation from those of growth, it was necessary to use resting cells in these experiments The organisms were collected from the ordinary growth medium by centrifugation, washed and re-suspended in phosphate buffer (*p*H 6 0) The cell suspensions were incubated for 90 min with malic acid (adjusted to *p*H 6 0 with sodium hydroxide), glucose, manganese ions and the supplement to be tested That no net growth occurred during the incubation period was judged from optical density measurements After the incubation period, chloramphenicol was added to the incubation mixture to prevent the formation of more enzymes. The cells were again separated by centrifuging, resuspended in buffer containing chloramphenicol, and their ability to dissimilate L-malic acid was determined manometrically

In the initial experiments, the supplement used in the incubation mixture was made up of 5 per cent vitamin-free casamino-acids and a purine/pyrimidine supplement, and the results showed that enzyme formation had been stimulated to the same extent as in the control which contained the complete medium The investigation was therefore limited mainly to testing the effect of various amino-acids, purines and pyrimidines in promoting the formation of malic enzyme The vitamin-free casamino-acids were then replaced by a mixture of the 19 amino-acids known to be present in hydrolysed casein Finally the amino-acids were appropriately deleted and substituted until it was found that a mixture of the following eleven amino-acids could promote malic enzyme synthesis to the same extent as the complete mixture of nineteen acids

L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, DL-phenylalanine, L-tryptophan, L-tyrosine and DL-valine, the final concentration of each amino-acid in the supplement being 25 mgm per 100 ml The purine/pyrimidine supplement consisted of adenine sulphate, guanine, thymine and uracil, the final concentration of each base being 10 mgm /100 ml

When tested separately the amino-acid mixtures proved more effective than the purine/pyrimidine supplement in promoting the synthesis of the malic enzyme in this organism But only when added together was the enzyme formed to the same extent as in the presence of the complete growth medium (Fig 1)

It was interesting to find that the vitamin supplement showed no effect in promoting synthesis of the enzyme, especially since a large number of vitamins have to be provided if this organism is to grow successfully in a synthetic medium. Ochoa and others had also demonstrated the inability of cells starved of biotin to form malic enzyme It is possible, however, that washed suspensions of normal cells contain enough vitamins to permit the formation of this induced enzyme

This work was carried out with a grant from the Department of Scientific and Industrial Research

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VIROLOGY

Influence of Tomato Mosaic Virus Infection on the Hollow Fruit Condition of Tomatoes

INFECTION of tomato plants by tomato mosaic virus (T M V) may have a very considerable influence on the quality of the fruit harvested It has been demonstrated[1,2] that poor colouring and/or fruit bronzing may follow virus infection

Hollowness is the term used to describe a condition of the maturing fruit in which the pericarp becomes separated by a cavity from the seed-bearing axial placenta (Fig 1) This cavity may be either discontinuous or continuous, in the latter event separating the seed-bearing pulp entirely from the pericarp The hollow condition is especially a feature of tomato varieties yielding bilocular fruits, as the fruit wall is less rigidly supported by the carpels than is the case with the fruit of tri- or multi-locular varieties As a consequence of this, when hollowness is associated

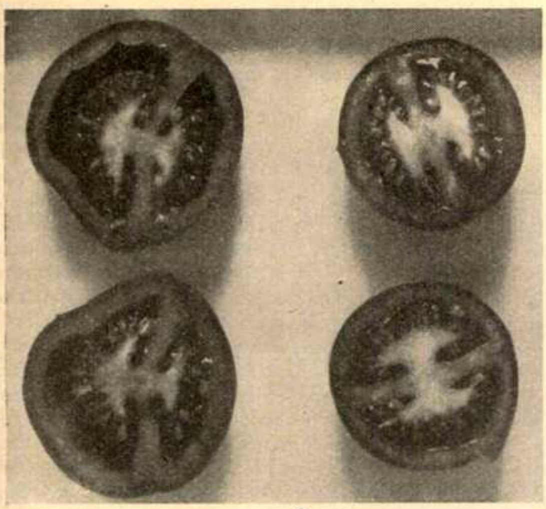

Fig. 1. Left, hollow bilocular fruit; right, normal bilocular fruit

with bilocular fruits, they frequently assume a flattened, angular appearance. This misshapen appearance, coupled with a lack of firmness and poor eating qualities, makes for low market value.

While investigating several aspects of the hollow fruit problem the relationship between this and tomato mosaic virus infection was examined. From a series of glasshouses, sparsely infected with virus, pairs of infected and virus-free plants were contrasted. Ten such random pairs were examined in each of twelve widely separated glasshouses. In all cases the numbers of hollow tomatoes borne on four consecutive trusses were compared with the total number of tomatoes on the same four trusses.

The results are presented in Table 1.

Table 1

Series	Plants	No.	Ratio sound/ hollow tomatoes	Standard deviation	Difference (per cent)
1	T.M.V. infected	10	6·45	± 0·96	66·2*
	Non-infected	10	19·35	± 3·95	
2	T.M.V. infected	10	6·97	± 1·50	37·4
	Non-infected	10	11·14	± 3·30	
3	T.M.V. infected	10	2·24	± 0·12	49·6*
	Non-infected	10	4·45	± 1·00	
4	T.M.V. infected	10	3·96	± 0·66	12·2
	Non-infected	10	4·51	± 0·34	
5	T.M.V. infected	10	2·99	± 0·54	51·5
	Non-infected	10	6·17	± 2·02	
6	T.M.V. infected	10	1·54	± 0·13	18·5†
	Non-infected	10	1·89	± 0·09	
7	T.M.V. infected	10	6·33	± 1·10	67·9†
	Non-infected	10	19·72	± 5·80	
8	T.M.V. infected	10	2·60	± 0·45	16·1
	Non-infected	10	3·10	± 0·18	
9	T.M.V. infected	10	5·03	± 0·72	37·9†
	Non-infected	10	8·11	± 1·08	
10	T.M.V. infected	10	4·67	± 1·20	53·5
	Non-infected	10	10·06	± 4·44	
11	T.M.V. infected	10	3·33	± 0·40	42·2†
	Non-infected	10	5·76	± 1·06	
12	T.M.V. infected	10	7·00	± 1·25	32·0
	Non-infected	10	10·30	± 4·93	

* Significant differences at $P = 0{\cdot}01$
† Significant differences at $P = 0{\cdot}05$

The standard deviations obtained from the data collected from non-infected plants are variable and frequently larger than those obtained from infected plants. It is likely that these high standard deviations indicate, in some cases, the fallibility of visual diagnosis of virus-infected plants. The more regular standard deviations occurring for infected plants, which are easily identified, support this theory.

Experimental methods utilizing inoculated plants would probably produce much more even data. Nevertheless, the results suggest that a relationship exists between tomato mosaic virus infection and the incidence of hollow fruit.

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Growth of Contagious Pustular Dermatitis Virus in Human Tissue Cultures

Contagious pustular dermatitis or 'orf' is a well-known disease of sheep which occasionally causes lesions, usually on the hands or face of people who work with sheep or their carcases. Such lesions may be difficult to distinguish from those caused by cowpox virus or by certain bacteria and fungi and a simple means of laboratory confirmation would be useful. Human infections may be confirmed by transmitting the virus to sheep and carrying out cross-immunity tests; but this is laborious and seldom used. A complement-fixation test using sheep material as antigen was used by Macdonald[1], but is not very sensitive. The virus will not grow in the developing chick embryo—which distinguishes it from cowpox virus—or in any of the smaller laboratory animals.

Plowright *et al.*[2] described the growth of contagious pustular dermatitis virus in sheep testis and kidney tissue culture. We have found that a stock strain of the virus grows well in lamb embryo kidney tissue culture in monolayers. Using a medium containing 10 per cent calf serum we have obtained average titres of 10^6 fifty per cent tissue culture infectious doses per 0·1 ml. of infected fluids on repeated passage at 36° C.

The identity of the virus was checked by rubbing tissue culture fluid into the shaved skin of a non-immune sheep and re-isolating the virus in tissue cultures from typical 'orf' lesions. Cross-immunity tests using three sheep were suggestive but not entirely satisfactory, because a reduced number of lesions developed in both challenged animals which had been vaccinated either with tissue-culture vaccine or the standard Burroughs Welcome vaccine. Cytopathic changes in lamb embryo kidney cultures included early rounding and swelling of the cells followed by clumping which superficially resembles the appearances seen with certain types of adenovirus grown in human embryo kidney cells. With heavy inocula of the virus, cytopathic effect was seen after 24 hr. and most of the tissue cells infected became detached from the walls of the tubes in three days. The virus appears to reach maximum titre in cells after three days and is then liberated into the medium.

Contagious pustular dermatitis virus which had been passed three times in lamb tissue was used to infect stationary cultures of human amnion, human embryonic kidney and human embryonic liver cells. In all types of cell the virus grew well, causing cytopathic changes similar, in unstained preparations, to those seen in lamb kidney cultures. The medium used for the growth of the virus in human amnion cells, which are widely used in virus diagnostic laboratories, contained 10 per cent calf serum.

Titrations of the virus in tissue cultures and direct isolation of the virus from experimentally infected sheep both suggest that human amnion cells may be the most suitable available method for isolating contagious pustular dermatitis virus from human lesions. In preliminary tissue culture experiments we have not been successful in demonstrating unequivocally the development of neutralizing antibody after infection in either sheep or humans. Using an antigen derived from infected lamb embryo kidney cultures we have shown that experimentally infected sheep develop significant rising titres, up to 1 : 64, of complement fixing antibody three weeks after infection. 'Orf' is locally common in certain parts of England and Scotland, but in our area it is rare and we have not yet had the opportunity to examine adequate specimens from the infection in man.

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AGRICULTURE

A New Method of Estimating the Dry-Matter Intake of Grazing Sheep from their Fæcal Output

In a series of investigations on dry-matter intake and fæces output of ewes, at different feed-levels, it was observed that a particular fraction of the fæces, readily dissolved by treatment with dilute acid, was closely related to the intake of dry matter. It was considered that this dissolved fæcal fraction would be unlikely to contain any high proportion of food residues already subjected to fermentation and digestion in the alimentary tract but was likely to consist largely of the more soluble parts of the metabolic fraction of the fæces. It has already been shown that this fraction bears a close relationship to dry matter intake[1,2].

Total collection of the fæces was employed[3] and 1 gm. of the milled dried fæces was allowed to stand in 150 ml. of 0·2 *N* hydrochloric acid (containing a small quantity of wetting agent) at room temperature for 18 hr. After filtering through a paper disk in a Gooch crucible the amount of this dissolved fæcal fraction was determined and the total daily production calculated.

Further work during 1959 and 1960 involved a range of feeds, including fresh and dried herbage, fed at different levels of intake to ewes and wethers. For eleven feeds, including grass–clover mixtures, each fed to several animals, the relationship between daily dry-matter intake and the daily dissolved fæcal fraction output in gm. for 64 observations was $y = 250 + 17{\cdot}8\,x$, y being the predicted dry-matter intake and x the dissolved fæcal fraction output.

In the cases of two other legume feeds, lucerne and a clover mixture, dissolved fæcal fraction outputs were higher than those for grass and grass–legume mixtures at comparable levels of dry-matter intake.

The errors associated with the prediction of other y values for two values of dissolved fæcal fraction output for means of various numbers of animals are shown in Table 1.

Table 1

No. of animals in the group for which the prediction is made	Dissolved fæcal fraction daily output (gm.) 50		100	
	S.E. of estimate	Percentage error	*S.E.* of estimate	Percentage error
1	73·5	6·4	78·1	3·9
5	33·9	3·0	43·0	2·1
10	25·0	2·2	36·4	1·8
20	18·9	1·7	32·5	1·6

These levels of precision suggest that this method should be suitable for estimating the free grazing intake of ewes, without the need to develop separate 'local' regressions for each type of sward grazed.

This method overcomes some of the difficulties associated with some 'fæcal index' regressions used in the past since it is based on the relationship of dry-matter intake and dissolved fæcal fraction output, thus avoiding the error involved in applying regressions of fæces composition on dry-matter digestibility, developed at one level of intake indoors, to studies of grazing animals at different levels of intake.

Where the basic relationship is between the total output of a fæces component and feed intake, and the regression does not pass through the origin, fæces composition at any one level of feed digestibility will vary with intake.

These investigations are being extended and this method applied, in observations on grazing ewes, with the aid of a grant from the Agricultural Research Council.

J. B. Owen

Department of Agriculture
(Animal Husbandry),
University College of Wales,
Penglais,
Aberystwyth.

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[1] Mitchell, H. H., *Bull. Nat. Res. Council*, 55 (1926).

[2] Hutchinson, K. J., *Austral. J. Agric. Res.*, **9** (4) (1958).

[3] Owen, J. B., and Ingleton, J. W., *Animal Production*, **3** (1) (1960).

Groundnut Variety, Mani pintar

Smartt, in a recent communication[1], mentions the very satisfactory performance of the groundnut variety, Mani pintar, in trials in Northern Rhodesia. In a series of trials on agricultural stations and on local farms in the Northern Region, Ghana, in 1960 the variety was outstanding, giving a mean yield 35 per cent higher than the local varieties, 24 per cent higher than previously released improved varieties and out-yielding all other recent introductions.

The variegated red and white testa characterizing the variety was found to be quite acceptable to the local farmer, who uses most of the crop for expression of the oil.

It is suggested that a variety with many excellent qualities and which has performed well in two widely separated African territories is worthy of widespread trial in other groundnut-growing areas.

I am grateful to Mr. Smartt for providing the original seed for my work.

J. McEwen

Central Agricultural Station,
Nyankpala,
P.O. Box 52,
Tamale,
Northern Region,
Ghana.

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[1] Smartt, J., *Nature*, **186**, 1070 (1960).

FORTHCOMING EVENTS

(*Meeting marked with an asterisk * is open to the public*)

Monday, October 9

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W C 1), at 5 30 p m —Dr B Mayo "The Dilemma of Determinism"

Royal Institute of Chemistry, London Section (joint meeting with the Ewell County Technical College and the Kingston Technical College Chemical Societies, at the Ewell County Technical College, Reigate Road, Ewell, Surrey), at 7 p m —Prof G E Coates "Organic Derivatives of Coinage Metals"

Tuesday, October 10

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N W 1), at 5 p m —Scientific Papers

Institution of Electrical Engineers, Measurement and Control Section (at Savoy Place, London, W C 2) at 5 30 p m — Mr W S Elliott "Trends in Computer Engineering" (Chairman's Address)

Institution of Mechanical Engineers, Automobile Division (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 30 p m.— "The Importance of the Design Stage" (Chairman's Address)

Oil and Colour Chemists' Association, London Section (at the Chemical Society, Burlington House, Piccadilly, London, W 1), at 7 p m —Lecture by Dr H M Talen (Paint Research Institute, Delft)

Royal Institute of Chemistry (joint meeting with the Brighton Technical College Chemical Society, at Brighton Technical College, Brighton), at 7 p m —Prof R L Wain, F R S "Chemical Control of Plant Growth".

Wednesday, October 11

School of Pharmacy, University of London (at 29–39 Brunswick Square, London, W C 1), at 3 p m —Sir Eric Ashby "Placebo and Analgesic in Education"

Geological Society of London (at Burlington House, Piccadilly, London, W 1), at 5 p m —Prof Arne Noë-Nygaard (University of Copenhagen) "Geology of the Faeroes" (Sixteenth William Smith Lecture)

British Coal Utilization Research Association (in the Conway Hall, Red Lion Square, Holborn, London, W C 1), at 5 30 p m — Mr L Rotherham "The Position of Coal in Electric Power Generation" (Tenth Coal Science Lecture)

Society of Chemical Industry, Food Group (at 14 Belgrave Square, London, S W 1), at 6 15 p m —Scientific Papers

Institute of Metal Finishing, Organic Finishing Group (at the British Institute of Management, 80 Fetter Lane, London, E C 4), at 6 30 p m —Mr D H Grover "Plastic Industrial Paints"

Society of Instrument Technology, Control Section (at Manson House, 26 Portland Place, London, W 1), at 7 p m —Mr H B Whitehouse and Mr M I MacLaurin "Process Control in Paper Mills"

Thursday, October 12

Institute of Refrigeration (at the Institute of Marine Engineers, The Memorial Building, 76 Mark Lane, London, E C 3), at 5 30 p m —Mr C M Brain Presidential Address

Institution of Electrical Engineers, Utilization Section (at Savoy Place, London, W C 2), at 5 30 p m —Dr H G Taylor "Research in the Field of Electricity Utilization" (Chairman's Address).

Radar and Electronics Association (in the Lecture Hall of the Royal Society of Arts, John Adam Street, Adelphi, London, W C 2), at 7 p m —Mr L F Matthews Space Communications Part 1 Systems and Equipment"

Royal Society of Tropical Medicine and Hygiene (at Manson House, 26 Portland Place, London, W 1), at 7 30 p m —Sir George McRobert "From Empire to Commonwealth" (Presidential Address)

Friday, October 13

Association of Applied Biologists (at the British Museum (Natural History), South Kensington, London, S W 7), at 10 50 a m — Meeting on "Mites of Economic Importance"

Saturday, October 14

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 3 30 p m —Dr J A Fraser Roberts "The Usefulness of the Blood Groups and some other Simply Inherited Characteristics in Anthropological Studies" *

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Assistant Lecturer (Grade B) in Mathematics—The Registrar, Flintshire Technical College, Connah's Quay, near Chester (October 13)

Assistant Lecturer in Zoology—The Registrar, The University, Nottingham (October 14)

Assistant Librarian (Acquisitions) (honours graduate with professional qualifications in librarianship and practical experience in an academic library)—The Secretary of University Court, The University, Glasgow, W 2 (October 14)

Lecturer or Assistant Lecturer (with an honours degree and practical experience of at least one branch of the food industry) in Food Science in the Proctor Department of Food and Leather Science—The Registrar, The University, Leeds 2 (October 14)

Tutorial Student (graduate or about to graduate) in the Department of Mechanical Engineering—The Registrar, King's College (University of London), Strand, London, W C 2 (October 14)

Postdoctoral Research Assistant, to work on high energy physics using the nuclear emulsion technique—Prof E H Bellamy, Department of Physics, Westfield College (University of London), London, N W 3 (October 20)

Scientific Assistant in the Chemistry Department, for the maintenance of physical instruments including spectrophotometers and radiochemistry equipment, duties will also include certain routine measurements and demonstrations—Head of the Department of Chemistry, Chelsea College of Science and Technology, Manresa Road, London, S W 3 (October 21)

Assistant Lecturer or Lecturer (preferably with a special interest in analysis or algebra) in Pure Mathematics—The Secretary, Birkbeck College (University of London), Malet Street, London, W C 1 (October 23)

Chemist, Chemical Engineer, or Physicist (with a university honours degree or equivalent in chemistry, chemical engineering or physics together with some years of research experience) in the Chemical Engineering Section, C S I R O , Melbourne, Australia, to undertake work of a fundamental nature in the fields of fluid dynamics, mass transfer and reaction kinetics—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W C 2, quoting Appointment No 608/15 (October 28)

Research Fellow or Senior Research Fellow in the Department of Parasitology, University of Queensland, for duties which will include the instigation of research work on pathogenesis of parasitic infections in domestic animals, and also a certain amount of undergraduate teaching—The Registrar, University of Queensland, Brisbane, Queensland, Australia (October 28)

Assistant Systematic Botanist (honours graduate in botany or agricultural botany)—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (October 30)

Laboratory Superintendent in the Department of Engineering, Fourah Bay College, University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (October 30)

Chair of Chemical Pathology—The Secretary, The University, Aberdeen (October 31)

Chemist (well qualified, with an interest in microbiology and enzyme chemistry), for research relating to the production of slipe wool—The Secretary, Wool Research Organization of New Zealand (Inc), P O Box 5115, Wellington, New Zealand, or the New Zealand Scientific Liaison Officer, Africa House, Kingsway, London, W C 2 (October 31)

Deputy (preferably with a degree in zoology) to the Director, Whipsnade Park, to assist the Director in the scientific and animal management work in the Park—The Secretary, Zoological Society of London, Regent's Park, London, N W 1 (October 31)

Lecturer or Assistant Lecturer in Anatomy at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (October 31)

Lecturers (2) (with special qualifications and experience in the food industry (other than the dairy industry) or in biochemical engineering) in Food Science—The Registrar (Room 22, O R B), The University, Reading (October 31)

Professor of Mathematics or Professor of Applied Mathematics at the University of Natal, Durban—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (South Africa and London, October 31)

Senior Lecturer and a Lecturer in Mathematics at the University College of Townsville, University of Queensland—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (October 31)

Lecturer or Assistant Lecturer in Mathematics at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (November 5)

Chair of Physics in Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (November 10)

Lecturer in Psychology in the School of General Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, November 15)

Lecturer in Philosophy at Newcastle University College, The University of New South Wales—The Agent-General for New South Wales, 56 Strand, London, W C 2, and the Appointments Section, Box 1, Post Office, Kensington, New South Wales, Australia (November 17)

Lecturer in Anatomy at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, November 18)

Lecturer/Senior Lecturer (graduate with an appropriate degree and with postgraduate research and/or field experience in agronomy) in Agronomy at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, November 30)

Assistant Professor in Pharmacology—Prof M F Murnaghan, University of Ottawa, Ottawa, Ontario, Canada

Experimental Officer (with a pass degree including chemistry or H N C in chemistry) in County Hall Laboratory, for duties which will involve research, control and development in a wide field of science—The Medical Officer of Health (D 1/N/2598/9), County Hall, London, S E 1

Head (with good qualifications in chemistry and teaching experience) of the Science Department—The Headmistress, Roedean School, Brighton

Keeper (with an honours degree and/or a museums diploma) of Ethnology—The Director, Liverpool Museums, William Brown Street, Liverpool 3

Laboratory Technician, for a research laboratory specializing in lipid analysis for metabolic studies—J A Balint, M B , M R C P , Division of Gastroenterology, University of Alabama Medical Center, Birmingham 3, Alabama, U S A.

Lecturer or Senior Lecturer in Education—The Principal, The James Graham College, Lawns House, Chapel Lane, Farnley, Leeds 12

Lecturer (preferably with experience in circuit theory and able to supervise research) in the field of Light Electrical Engineering—Head of the Department of Electrical Engineering, Imperial College of Science and Technology, London, S W 7

Lecturer (with an honours degree in chemical engineering or Corporate Membership of the Institution of Chemical Engineers, with experience in research and in the operation and supervision of full-scale chemical plant) in Chemical Engineering—The Registrar, The University, Nottingham

Master to teach Chemistry to all levels, including a substantial amount of sixth form work—The Headmaster, King's College School, Wimbledon, London, S W 19

Mathematicians (with an honours degree in mathematics, and preferably previous experience with computers) in the Computation Centre, National Research Council of Canada, for work which will involve the analysis and programming of problems which arise in the various scientific and engineering divisions of the Council—The Employment Officer, National Research Council, 100 Sussex Drive, Ottawa, Ontario, quoting Ref E R 210

Research Student in Inorganic Chemistry, for work concerned with inorganic polymers—The Professor of Chemistry, Westfield College (University of London), Hampstead, London N W 3

Research Students in the Department of Chemistry, for work on the inorganic chemistry of complexes in solution, polyacids, etc —The Registrar, Manchester College of Science and Technology, Sackville Street, Manchester 1

Technical Assistant (with a university degree or equivalent qualifications, and the ability to assist in the construction of research apparatus) in the Department of Applied Physics, to assist in research on subjective brightness—Head of the Applied Physics Department, Northampton College of Advanced Technology, St John Street, Clerkenwell, London, E C 1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

British Welding Research Association Sixteenth Annual Report, 1960-61 Pp 63 (London British Welding Research Association, 1961) [148

Royal Scottish Museum Annual Report, 1960 Pp 29 (Edinburgh Royal Scottish Museum, 1961) [148

Department of Scientific and Industrial Research Memoirs of the Geological Survey—Scotland The Economic Geology of the Fife Coalfield, Area II Cowdenbeath and Central Fife, including Fordell, Lochgelly, Cadham and Kirkcaldy By E H Francis, based on the work of J K Allan and J Knox Second edition Pp viii+152+3 plates (Edinburgh and London H M Stationery Office, 1961) 25*s* net [148

Gateways Guide to Courses of Further Education in Middlesex, 1961-1962 Part 1 Vocational Courses Pp 50+xiv+4 plates (London Middlesex County Council Education Committee, 1961) [148

The Twelfth Annual Report of the Wildfowl Trust, 1959-1960 By Hugh Boyd and Peter Scott Pp 185+24 plates (Slimbridge The Wildfowl Trust, 1961) 17*s* 6*d* net [148

Department of Scientific and Industrial Research National Physical Laboratory Notes on Applied Science, No 23 Radiometric Standards and Measurements By E J Gillham Pp iv+23 (London H M Stationery Office, 1961) 1*s* 9*d* net [148

Privy Council Medical Research Council Memorandum No 40 Ageing and the Semi-skilled—a Survey in Manufacturing Industry on Merseyside By Alastair Heron and Sheila M Chown, assisted by M S Featherstone and Catherine M Cunningham. Pp viii+59 (London H M Stationery Office, 1961) 5*s* net [148

Air Ministry Meteorological Office Scientific Paper No 10 Incidence of, and some Rules for Forecasting, Temperature Inversions over the North-East Atlantic By H C Shellard and R F M Hay Pp ii+21 (M O 696) (London H M Stationery Office, 1961) 3*s* net [148

Department of Scientific and Industrial Research , and Fire Offices' Committee Joint Fire Research Organization Fire Research 1960 Report of the Fire Research Board with the Report of the Director of Fire Research Pp viii+67+12 plates (London H M Stationery Office, 1961) 5*s* 6*d* net [148

Department of Scientific and Industrial Research Road Research Laboratory Road Research Technical Paper No 51 Factors Affecting the Amount of Travel By J C Tanner. Pp iv+26 (London H M Stationery Office, 1961) 2*s* net [148

Planning, Vol 27, No 454, (14 August 1961) Economic Planning in France Pp 205–240 (London Political and Economic Planning, 1961) 5*s* [148

Other Countries

Institut Royal des Sciences Naturelles de Belgique Tome 3 Bulletin No 2 Note Nomenclatoriale *Yunnanella* Graba *Yunnanellina* Grabu, *Nayunnella* Nom Nov (Rhynchonelles) P Paul Sartenaer Pp 3 Tome 37, No 3 Sur la Structure Submicr scopique de la Conchioline de Mollusques Par Charles Grégoire P 34+10 planches Tome 37, No 4 Contribution a l'Etude de Bostrychidae 27 Collection du Musée d'Histoire Naturelle Sencke berg a Francfort-sur-Main Par Jean-Marie Vrydagh Pp 23 Tom 37, No 5 Tendances Evolutives et Ornithosystematique Par Ren Verheyen Pp 27 Tome 37, No 6 Les Acariens Parasites Endopu monaires des Serpents (Entonyssidae Mesostigmana) Par Alex Fai Pp 135 Tome 37, No 7 Contribution à l'Etude des Bostrychida No 28 Etude des Types de Fahraeus, Désignation de Lectotype Par Jean-Marie Vrydagh Pp 10 Tome 37, No 8 Über Namen de Gattungen und Arten der Lycosidae (Araneae) Von Carl F Roewe Pp 19 Tome 37, No 9 Les Bryozoaires Devoniens de la Belgiqu Le Genre *Canutrypa* Bassler Par Edmond Dessilly Pp 12 + planches Tome 37, No 10 Contribution à l'Etude des Bostrychida No 30 Le Genre *Xylogenes* Lesne Par Jean-Marie Vrydagh Pp 1 Tome 37, No 11 Contribution à l'Etude de la Pigmentation che l'Homme (1re partie) Par André Leguebe Pp 29 Tome 37, No 12 *Piranheoxylon stockmansi* n gen , n sp , bois d'Euphorbiacees d l'Eocene de Belgique Par Nicole Grambast Pp 12+4 planche Tome 37, No 13 Un Trichoptere Nouveau d'Afrique Centrale *Macronema trilineata* Sp n Par Serge Jacquemart Pp 3 (Bruxelles Institut Royal des Sciences Naturelles de Belgique, 1961) [15

World Meteorological Organization Guide to Climatologica Practices Pp vi+94 (WMO–No 100 TP 44) 15 Swiss francs Guide to Climatological Practices, Supplement No 1 (June, 1961) Pp 16 (Geneva World Meteorological Organization, 1961) [15

Oak Ridge National Laboratory, Tenn , U S A Papers published i Journals and presented at Meetings, Reports Issued and Theses completed, during 1960, by ORNL Staff Members Pp v+107 (ORN Central Files Number 61–6–27) Oak Ridge, Tenn Oak Ridge National Laboratory, 1961) [158

United States Department of the Interior Fish and Wildlife Service Bureau of Commercial Fisheries Statistical Digest No 50 Alaska Commercial Salmon Catch Statistics, 1951–1959 By Robert R Simpson Pp ii+115 (Washington, D C Government Printing Office, 1960) 60 cents [158

Organization for European Economic Co-operation European Nuclear Energy Agency Catalogue of Courses on Nuclear Energy in O E E C Countries, 1961–1962 Pp 167 (Paris Organization for European Economic Co-operation, European Nuclear Energy Agency, 1961) [158

Institut Royal Météorologue de Belgique Publications Serie B, No 33 Un Hygrométre de Grande Sensibilité Par Dr S de Backer. Pp 10 Observations Ionospheriques—Station de Dourbes Aout 1960 Pp 26 Septembre 1960 Pp 26 Octobre 1960 Pp 26 (Bruxelles Institut Royal Météorologique de Belgique, 1961) [158

British Columbia Research Council 17th Annual Report, 1960–61 Pp 16 (Vancouver British Columbia Research Council, 1961) [158

Acta Neuropathologica, Vol 1 Fasc 1 (Organ of the Commission for Neuropathology and of the Commission for Comparative Neuropathology of the World Federation of Neurology) Pp 106+10 (Berlin und Wien Springer-Verlag, 1961) 38 D M [158

New South Wales Department of Agriculture Report of the Department of Agriculture for the year ended 30th June, 1960 Pp 140 - (Sydney Government Printer, 1961) [158

Sandia Corporation, Albuquerque Sandia Corporation Bibliography SCR–299 An Index of Tables and Charts for use in Gas Dynamic Studies By D E Randall Pp 32 75 cents SCR–255 Civilian Application Releases through 1960 By the Technical Information Division Pp 120 2 50 dollars SCR–288 Mach Tables for Real Gas Equilibrium Flow of Air in Hypervelocity Test Facilities with Total Temperatures to 10,000° K. By K L Goin (Sandia Corporation Monograph) Pp 82 2 dollars SCR–245 Table of Atomic Masses Data compiled by R L Heath Edited by J W Guthrie (Sandia Corporation Monograph) Pp 302 4 dollars (Albuquerque, New Mexico Sandia Corporation, 1961 Obtainable from Office of Technical Services, Department of Commerce, Washington, D C) [158

United Nations, New York Demographic Yearbook, 1960 (Special Topic Population Trends) Pp xi+621 (New York United Nations, London H M Stationery Office, 1961) [158

United States Department of Agriculture Leaflet No 494 The Armyworm and the Fall Armyworm How to Control Them Pp 8 (Washington, D C Government Printing Office, 1961) 5 cents [158

New York Public Library Russian Journals of Mathematics a Survey and Checklist Pp 10 (New York New York Public Library, 1961) [158

Bulletin of the Florida State Museum, Biological Sciences Vol 5, No 7 Subfamilies, Genera, and Species of Phytoseiidae (Acarina Mesostigmata) By Martin H Muma Pp 267–302 50 cents Vol 6, No 1 Postcranial Osteology of the Waterfowl By Glen E. Woolfenden Pp 1–130 1 60 dollars (Gainesville, Fla Florida State Museum, 1961) [158

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THE AMATEUR IN SCIENCE

IN an address on August 31 to Section L (Education) at the British Association meeting at Norwich, in which he surveyed some current programmes of research in education, Dr W D Wall, director of the National Foundation for Educational Research in England and Wales, commented that he was himself an amateur in research ; and he raised the question of the place of the amateur in research It is true that, in the physical sciences, the high cost of equipment has in itself largely eliminated the amateur and even the individual, but the position is perhaps too lightly accepted without due thought for the consequences It could be argued that the creative years of an individual scientist, in which his most important contributions are made, are those early years in which he is emerging from amateur or tyro to true professional status Again, it could be suggested that part of the value of mobility between one technology or one particular subdivision of a scientific discipline and another lies in the stimulus which a fresh outlook can supply.

To some extent this can be found in the research teams comprising scientists and technologists trained in different disciplines which are now commonly built up for the attack on problems which involve contributions from different disciplines, but it might be worth while to re-examine, under modern conditions, the contribution which the amateur could make, and the relations between amateur and specialist to-day, as well as the factors that most conduce to co-operation There could scarcely be a more serious reproach to the system of education in Britain than the admission of a leading educationist that the first task of institutions of further education is to awake the desire to learn, and in his presidential address to Section J (Psychology) Prof George Seth remarked that intellectual eagerness is by no means the most obvious characteristic of the university entrant of the mid-century Prof Seth was concerned particularly with the attitude of the student to knowledge, to learning and scholarship, and the disinterested search for truth If, as he pointed out, a student lacks concern for these matters, even the able student will be unfitted for the university environment, and it is also true that he may be handicapped in his subsequent professional as well as in his university career

It was therefore fitting that two of the sectional presidential addresses at Norwich should be concerned with aspects of amateurism or intellectual curiosity Prof Seth, who thinks that the universities may already be getting as many of the intellectual *élite* as they could expect, was much more concerned with the quality of teaching, and particularly the consequences if many of the brightest youngsters are taught largely by those who are intellectually less able than themselves Apart from this, he suspects that some of the teaching techniques in British universities have been ill-designed to encourage or to stimulate creative originality in their students He puts this fairly to the universities as in part their problem, though they may reasonably look to the schools for help What is looked for is imaginative freedom rather than conformity or preoccupation with satisfying the examiners, and Prof Seth rightly points out that such a quality can only flower in an atmosphere of freedom

Clearly there is here a line of research that should commend itself to the universities and to their psychology departments, as well as to institutions concerned with educational research, and the problem is urgent for if, in the immediate future, we must look to the schools to stimulate this quality, the universities should be striving to acquire teachers of sufficient number and quality to meet the need in all departments It is to the teacher that Prof Seth looks primarily, and he maintains that an active scepticism of traditional educational instruments, institutions and philosophy holds enormous possibilities for good A community that lives complacently on the inheritance of accumulated experience that we call tradition is already dead, however rich and splendid its past may have been, but Prof Seth also reminds us that, without that past, and without a clear and just appreciation of its meaning for us, our future advance will be feeble, lacking in impetus, uncertain and wavering in direction

There in some degree is expressed the case for co-operation between amateur and professional, and it might be held that the Earl of Cranbrook's presidential address to Section X on September 1 on "Science, Politics and Social Service" expounds that theme Insisting on the importance of the possession of a curious and inquiring mind, which is regarded as a main difference between man and other animals, Lord Cranbrook notes the bearing of intellectual curiosity and the scientific outlook on the advances in the seventeenth and eighteenth centuries He comments on the range of interest which marked the early Fellows of the Royal Society, and shows how out of such amateurism in the true sense arose the scientific societies of the eighteenth and nineteenth centuries As a result, the gradual advance towards universal compulsory education, which was fortunately faster than the gradual extension of the franchise, was accompanied by the growth of societies which encouraged men actively to do things for themselves as well as being educated passively by the educational methods of the day

Lord Cranbrook emphasized the majestic diversity of the thoughts and achievements of the great scientists of the sixteenth and seventeenth centuries, and pointed out that the ordinary educated man of those days could still make his contribution to science well on into the nineteenth century, and he does not accept as inevitable any divorce between science and the humanities He makes no ill-founded claims for political wisdom or judgment for the scientists, even for biologists or economists Scientists are just as ready as other men to fit themselves into the Procrustean bed of their own ideology and to study the humanities with all the blind prejudices of the most obscurantist politician The connexion between scientific and political thought to which he referred could only exist in the earlier stages of man's development, and the last great advance in scientific thought, which grew out of the Renaissance, built up a scientific discipline inapplicable to political or sociological problems In Lord Cranbrook's view, the scientist's training and method of approaching his problem no more fits him to be the arbiter of political thought than does that of the priest, the actor, musician or author

While Lord Cranbrook thus does not suggest that the scientist alone can put things right in the world, he certainly gives no support to the idea that the politician should know little or nothing of science or take pride in being, as Mr Harold Macmillan claimed at the Royal Society Tercentenary banquet, the only amateur remaining In the world of to-day, men and women in all spheres should understand what is happening and why, and the growth of leisure should facilitate this Moreover a well-balanced man or woman should be able to understand and enjoy, at least in part, both arts and sciences, very appropriately, since he was addressing the Assembly of Corresponding Societies, Lord Cranbrook pointed to opportunities which these societies even to-day could use to foster such understanding The development of communal life is indeed a step to the development of political life and understanding

Nor is this confined to the village or rural communities of Britain or Europe Perhaps the most important passage of Lord Cranbrook's address is that in which he discusses the bearing of our experience on what is happening in the under-developed countries The major scientific societies of those countries are unlikely to pass through the stage of consisting mainly of amateurs, as did the Royal Society and other learned societies in Europe in the seventeenth and eighteenth centuries They will consist mainly of professional people, and the developing schools and universities of these countries may have to concentrate on producing professional men and technicians In the immediate future medical men, veterinary surgeons, engineers, agricultural advisers and teachers are the primary need rather than research workers, but even so, it will be important that the general body of the population should understand something of the meaning and purpose of science

Since these professional scientists are likely to be preoccupied in professional practice or the training of technologists and technicians, Lord Cranbrook suggested that it will be the more important that a body of amateurs should be built up to study the natural history, archæology and local history of these countries He visualized a series of Associations for the Advancement of Science, from which these peoples can learn something of the pleasures as well as of the meaning and purpose of science, and he sees a very clear parallel between technical development and knowledge of natural history in Britain 150 years ago and in the less highly developed countries to-day It was as we passed from being a mainly agricultural to a mainly industrial community that men of all classes were busily engaged on finding, describing and cataloguing the living things around them

Lord Cranbrook thus looks to something of a renaissance of the local scientific society both in Britain and in the lesser developed countries, and in this he has the support of Sir Cyril Hinshelwood, who, in a notable address last autumn to the Manchester Literary and Philosophical Society, referred to the part which such societies could play in promoting understanding between the arts and scientists Moreover, Lord Cranbrook recognizes that something can be done to help under-developed countries on the social side also through the technical assistance which is now being increasingly given He did not refer specifically to either the British or the United States schemes for voluntary service by young people overseas, but, of the young people from Britain who are going to the under-developed countries in increasing numbers on medium-term engagements as technicians or teachers and for short periods to assist generally in social work, he emphasizes that it is important that they should all realize how Britain developed and how our experience might be useful to others

Perhaps the Voluntary Service Overseas scheme may have particular potentialities in this connexion , but for any effective assistance to be given in this way by those who proceed overseas under that scheme or any scheme of technical assistance, there is one essential condition Something more than knowledge is required—an inquiring mind and the eagerness to learn which mark the amateur and characterize the true professional are needed Unless both are nurtured and encouraged by schools and universities alike, it is idle to expect that Britain will maintain the excellence which has characterized her scientific work and much of her technology in the past, and still less that she will make the contribution to the leadership of the emergent nations in welfare or in scientific and technical development which her experience and her tradition so richly qualify her to render These two addresses at Norwich have pointed to opportunities and they have issued a challenge which should be accepted not only by the schools and the universities but also by many of the professional institutions, learned societies and the local societies in which professional and amateur have often come together and co-operated on terms of mutual respect and understanding Moreover, the same challenge was issued, but in another context, quite as clearly by the Master of Sidney Sussex College, Cambridge, Dr David Thomson, in speaking of what the universities expect from the schools Thus he referred to the subtle and imperceptible threshold between pupil and student, and the absence of evidence that everyone can reach or cross this threshold, and he urged that it is the supreme duty of a sixth-form teacher to lead his charges to this threshold and encourage them to venture beyond, independent of teacher or text-book A similar task, different only in level, confronts every teacher in further education Britain's economic and industrial future, no less than the extent to which she can rise to her opportunities of leadership, alike depend largely on the effectiveness and vision with which it is discharged

MATHEMATICAL TABLES

Table of Values of the Function

$$w(z) = \mathrm{e}^{-z^2}\left(1 + \frac{2i}{\sqrt{\pi}}\int_0^z \mathrm{e}^{t^2}\,\mathrm{d}t\right)$$

for Complex Argument

By V N Faddeyeva and N M Terent'ev Edited by V A Fok Translated from the Russian by D G Fry (Mathematical Tables Series, Vol 11) Pp v + 280 (London and New York Pergamon Press, 1961) 100*s* net

Tables of Airy Functions and Special Confluent Hypergeometric Functions for Asymptotic Solutions of Differential Equations of the Second Order

By A D Smirnov Translated from the Russian by D G Fry (Mathematical Tables Series, Vol 7) Pp vii + 260 (London and New York Pergamon Press, 1960) 100*s* net

Bessel Functions

Part 3 Zeros and Associated Values Edited by F W J Olver Prepared under the direction of the Bessel Functions Panel of the Mathematical Tables Committee (Royal Society Mathematical Tables, Vol 7) Pp lx + 79 (Cambridge At the University Press, 1960) 50*s* net

Tables of the Riemann Zeta Function

By C B Haselgrove, in collaboration with J C P Miller (Royal Society Mathematical Tables, Vol 6) Pp xxii + 80 (Cambridge At the University Press, 1960 Published for the Royal Society) 50*s* net

Representations of Primes by Quadratic Forms

Prepared by Hansraj Gupta, M S Cheema, A Mehta and O P Gupta Edited by J C P Miller (Royal Society Mathematical Tables, Vol 5) Pp xxiv + 125 (Cambridge At the University Press, 1960) 45*s* net

ANY suggestion that the advent of electronic computers may lead to the cessation of further publication of mathematical tables is refuted by this collection of new tables The collection is particularly interesting, since the first two are of Russian origin, and allow some sort of comparison with British tables

The first volume deals with a function $w(z)$, which at first sight would appear to have little practical value But if we write $z = x + iy$, then both $w(x)$ and $w(iy)$ are simply related to integrals of exp $\pm\, t^2$, and when $x = y$, $w(z)$ is connected with integrals of the Bessel function $J_{\pm\frac{1}{4}}(t)$ The function itself may be expressed in the form

$$w(z) = \frac{i}{\pi}\int_{\infty}^{\infty} \frac{\exp - t^2\,\mathrm{d}t}{z - t}$$

It occurs in scattering theory, in the propagation of electromagnetic waves, and in the breadth of spectral lines These tables give $w(x + iy)$ for $0 \leqslant x,y \leqslant 5$, to 6 figures, with differences, and at a tabular interval of 0 1 The tables themselves are clear to read, but it is a pity that not more information is available about their checking, and the elimination of errors The publishers apologize for the 'messy' character of the introductory pages The apology is very appropriate

The second volume, by Smirnov, deals with Airy functions These functions satisfy differential equations of the form $y''(x) + x^a y(x) = 0$ They appear frequently in all sorts of numerical methods, and their tabulation in detail is very desirable This collection gives $y(x,a)$ and certain derivatives and differences, for $| x | \leqslant 6$ at intervals of 0 01, and for 16 rational values of a between $-\frac{3}{4}$ and 2 Lagrangian interpolation is possible to within 10^{-3} on average The tables are beautifully printed, and are presumably copied photographically from the Russian original The preliminary explanatory material is less attractive, and the volume is rendered less satisfying than it could be by the inclusion of some so-called "confluent hypergeometric functions", which are quite distinct from the usual function $F(a,b,x)$ of that name

The remaining three volumes are all part of the Royal Society series of mathematical tables Here we meet an element of distinction, previously missing All three introductory sections are alive, well informed and interestingly written In particular, the volume on Bessel functions—which is No 3 in the series, and follows earlier volumes in 1937 and 1952—reads like a detective story It is an intriguing sign of the influence of electronic computers that in the end, as part of the checking process, 18 years of work by desk machines was repeated in 12½ hours on the computer But zeros of transcendental functions are notoriously difficult to evaluate Here again are given the first fifty zeros of J_n, Y_n, J_n' and Y_n', together with those for the gradient of the spherical Bessel functions, for $n = 0(\frac{1}{2})20\frac{1}{2}$ The layout is good, and the methods of testing and checking are clearly stated This is a first-rate volume

With the Riemann zeta function we enter the realm of pure mathematics There is still no proof that all the non-trivial zeros of $\zeta(s)$ in the critical strip for which the real part of s lies between 0 and 1, are on the critical line bisecting this strip But this seems to be the case so far as calculations have been carried This volume gives the real and imaginary parts of $\zeta(\frac{1}{2} + it)$ and $\zeta(1 + it)$ for $t = 0$ (0 1) 1000 to 6 decimals Immense tabulation on a computer was needed But the results are worth it

Finally, we have Gupta's tables giving various representations of prime numbers p not exceeding 100,000 If $p = a^2 + Db^2$, where $D = 5$, 6, 10 or 13, the values of a and b are given (if they exist) for all p So also is the least value of k and corresponding n, where, for the same p and D, $kp = n^2 + D$ These tables are easy to read, and should give much pleasure to those mathematicians interested in the theory of quadratic fields The tests used by the authors are impressive The typing of the tables in a form suitable for direct photo-copying was admirably done on a card-controlled typewriter C A COULSON

ORIGIN OF THE NORTHERN RHODESIAN COPPER DEPOSITS

The Geology of the Northern Rhodesian Copperbelt

Edited by Dr. F. Mendelsohn. Pp. xvi + 523. (London: Macdonald and Co. (Publishers), Ltd., 1961.) 84*s* net.

IN November 1902 W. C. Collier shot a roan antelope in the bush while prospecting near the Luanshya River: close to the body of the buck he found outcrops carrying copper and pegged a claim. Twenty-nine years later Roan Antelope became the first of the great Northern Rhodesian copper mines to come into production. To-day the Copperbelt supports a community of more than a quarter of a million and produces nearly one-fifth of the free world's copper.

Something of the scientific effort behind this great expansion is revealed by the twenty-three authors of the monograph under review. The majority are members of the geological staffs of the Anglo-American Corporation and Rhodesian Selection Trust, but there are also contributors from the British South Africa Company and Union Minière du Haut-Katanga. Since the ore deposits on the Copperbelt are almost wholly confined to a series of sediments of presumed Pre-Cambrian age, the Lower Roan Group of the Katanga System, the first necessity for exploration was a geological map showing the extent of these rocks. Its production was aided by aerial photography, and the recognition of individual deposits has been facilitated by a variety of geophysical and geochemical prospecting techniques.

Economic mineralization on the Copperbelt is concentrated in a zone about 100 miles long and up to 40 miles across. This is, however, but a fraction of the 500-mile arc of folded Katanga beds which stretches through Northern Rhodesia and the Congo into Angola, covering more than 80,000 square miles and forming one of the world's greatest base-metal provinces. Structurally, the Copperbelt is dominated by the north-west trending Kafue anticline, which brings up a central belt of pre-Katanga rocks and is flanked to the north-east by the Mufulira syncline and to the south-west by a series of *en echelon* downfolds on the flanks of which Bancroft, Nchanga, Chibuluma, Nkana and Roan Antelope Mines are situated. The ore-bodies occur in rock of varying lithology: in the Mufulira basin they are predominantly arenaceous, to the south-west more commonly argillaceous.

Lively controversy has surrounded the origin of the ores. On one hand their great lateral extent, their persistence at certain stratigraphical horizons, their intimate connexion with sedimentary features of the host rocks, the inferred relation of sulphide zoning to facies, the evidence that much of the mineralization is pre-folding, the absence of any obvious deep-seated source for the mineralizing solutions and the difficulty of envisaging movement of large volumes of such solutions for great distances through sediments of low permeability have suggested a sedimentary syngenetic origin. Lean copper deposits known to occur in the Pre-Katanga rocks have, it is suggested, been eroded, transported in solution to the basin of deposition and precipitated during the sedimentation of the Lower Roan Group. Subsequent redistribution has taken place during the folding and metamorphism of the sediments. On the other hand, there are many features more readily explained in terms of epigenetic hypogene mineralization. These include the relation of the deposits to the central region of the Katanga geosyncline and to fold axes, the presence of local mineralization in Pre-Katanga rocks immediately beneath ore-bodies in the Lower Roan Group, the geochemistry of the sediments, suggesting that they have undergone widespread metasomatism, and the inadequacy of the metamorphic temperatures capable of producing rocks of the greenschist facies to account for certain observed mineralogical features.

In many cases the evidence recorded from different mines is conflicting. At Roan Antelope ore has been proved over a strike length of about 7 miles and to a depth of nearly 4,000 ft. in a stratigraphically controlled ore-body with an average thickness of 25 ft.: at Nchanga it transgresses stratigraphical boundaries through more than 300 ft. of varied sediments. At Chibuluma and Roan Antelope the mineralization is unrelated to the folding: at Nkana South the ore-body is confined to the highly contorted and fissile portions of carbonaceous shales. At Mufulira mineralization is regarded as pre-dating slumping: at Mindola it appears to post-date faulting. It is scarcely to be wondered at that the various contributors fail to reach agreement as to the genesis of the ores, though more space is devoted to the sedimentary syngenetic theory than to any other. It is perhaps more surprising that no attempt is made to reconcile the puzzling association of criteria suggesting both syngenesis and epigenesis in some mechanism such as long-continued infiltration of ascending solutions into the basin of deposition as well as into the growing pile of sediments.

Supergene alteration persists to great depths on the Copperbelt, and some of the conflicting evidence may be a consequence of failure to recognize the full extent of secondary sulphide enrichment. More detailed investigation of this problem is evidently needed. Equally essential is the study of the relation of mineralization to both sedimentary and tectonic features in the base metal province of the Katanga geosyncline as a whole rather than in the small portion of it which forms the Copperbelt. Political factors, unfortunately, make this a more difficult task to-day than at any time in the past fifty years. When it can be undertaken it should prove both scientifically rewarding and commercially profitable.

J. H. Taylor

SCIENCE IN POETRY AND VERSE

A Book of Science Verse

The Poetic Relations of Science and Technology. Selected by W. Eastwood. Pp. xvi + 279. (London: Macmillan and Co., Ltd., 1961.) 21*s* net.

THIS is a pleasant and successful anthology of the treatment of 'science'—the term is given a wide construction—in poetry and in verse. Mr. Eastwood, who is vice-principal of Reading Technical College, starts on an optimistic note. He points out in his introduction that "the separation of science and poetry does not go back much further than the late eighteenth century", that "poet and scientist are alike concerned with the ordering of experience, and to both the imagination is important" . . . "a poetry which ignores science and its applications is, in the modern world, divorced from life" . . . "There is no future for a culture which cannot reconcile the poetic and scientific impulses, for both are necessary to the human spirit'' These statements, apparently so simple, do in fact raise issues of vast importance, an appendix of selected pronouncements by Wordsworth, Peacock, Herbert Spencer, A N Whitehead, I A Richards and C Day Lewis provides material for further argument But the greatest difficulty of all goes unsolved the basic and ever-increasing difference in the vocabularies of scientists and poets John Donne could use astronomy, alchemy, metallurgy, optics and a little geometry, because knowledge had not yet given them a specialized diction A glance at technical glossaries—say, of computers or guided missiles—offers little hope for their use in metrical structures.

Among the poems, or parts of them, that we should have expected are those from Lucretius, Chaucer, Shakespeare, Donne, Milton, Butler of *Hudibras*, Akenside, Erasmus Darwin, Wordsworth, Shelley, Tennyson, Kipling, but Mr Eastwood has cast his net far more widely, and with effect It is improbable, for example, that the common reader will know Blackmoor's *Anatomy and Revelation*, Young (dullest of poets) on *Scientific Theology and Religious Naturalism*, Hood's *Craniology* He will find an extract from Blake's *Vala* and perhaps regret that he does not find more passages from, let us say, *Jerusalem*

But it is in the poetry of our own century, and in particular of the past twenty years, that Mr Eastwood's harvest is richest, Hart Crane's admirable *The Bridge*, Kathleen Raine's *The Human Form Divine* and *Rock*, Patrick Dickinson's *Jodrell Bank*, Robert Conquest's *Guided Missile Experimental Range*, John Wain's *Poem Feign'd to have been by an Electronic Brain*, and much else A reviewer is prompted, inevitably, to speculate on what he himself might have added or omitted 'Q's parody of Sir Patrick Spens is good only in parts Surely there was a case for including W J Turner's *Geological*, perhaps his *Formal Lyric*, Wilfred Ashley's *Goods Train at Night* possibly the magnificent epitaph in terms of the railway that rests in the south transept of Ely Cathedral And while we have a poem on a tractor—there must be many written in the Soviet Union, too—why have we no poem on motor-cars?

T R HENN

A NEW LOOK IN PHYSICS TEXT-BOOKS

Physics

(Physical Science Study Committee) Pp viii+656 40s Laboratory Guide for Physics Pp vii+87 10s (Boston, Mass D C Heath and Company, London George G Harrap and Co , Ltd , 1960)

THIS book represents a new kind of physics course It is a year's work intended for American high-school pupils aged about 16 years, who may have done no physics previously and who need not necessarily be doing mathematics concurrently It is excellent by any standards, authoritative, well planned, clearly written, and in this final (or more probably, current) edition, which is the outcome of drafts already tested in schools, well produced It is unlikely, at least in the near future, that many British teachers will find themselves using such a course, but this is a book which every physics teacher ought to read He will find it stimulating and instructive, it will also possibly provoke some disagreement—and it is about time that teachers in Britain had something as provocative as this to shake up their ideas

The Physical Science Study Committee in the United States has organized this course down to the finest detail, covering text-book, laboratory guide, apparatus, films, tests, and background reading, and also the teacher who is, as Prof Zacharias has said, only one of the range of teaching aids Indeed, experienced teachers are being re-trained on full-time courses lasting about a term to prepare themselves to work this syllabus This almost savours of 'The Method', but both British and American physicists who have been to these courses assure me that after a short initial phase of scepticism they have been imbued with the ideas of the Physical Science Study Committee It should also allay the misgivings of those who feel that the concepts of modern physics can only be presented to schoolboys in a dogmatic manner, which is the chief basis for any disagreement with the Committee's course, for it is intended to be taught by people who have been converted to the belief that it can be done soundly, scientifically, and as undogmatically as anything can be done at school—and who understand how

The text-book is in four parts, each the work of a group composed of university physicists and school-teachers The first part deals with the measurement of time and distance, motion, vectors, the concept of mass, elements, atoms and molecules Part 2, on optics and waves, explains wave propagation very fully, and gives a detailed treatment of interference and diffraction, it also discusses the particle behaviour of light Part 3, on Newtonian mechanics and gravitation, deserves a very careful study by those who are suspicious of dogma Newton's third law (which, incidentally, is not called that) is obtained inductively (as Newton found it) from experimental observations on the conservation of momentum The really honest reader will find himself wondering whether he has not been teaching dogmatically for years Part 4, on electricity and atomic structure, is written in rationalized M K S units, sooner or later science teachers in Britain will have to be as adaptable as teachers in other countries who must have undergone the discipline of mastering them on the job recently But the work on electromagnetism is made crisper than usual by the elimination of the vector H It is possible that the treatment may be revised in places Great emphasis is laid, for example, on Millikan's experiment for e, performed with small plastic spheres This has been criticized as taking a lot for granted, and I believe the Committee was rather worried about its soundness, but the latest report on it was reassuring Atomic structure is dealt with entirely in terms of energy-levels, the existence of stationary states being justified by an appeal to elementary wave-mechanics The Bohr atom itself, which looks as if it may at last be finding its way into British school syllabuses, has been dispensed with in this book

Many of the experiments in the laboratory manual are to be commended as they show what can be done with quite simple apparatus These include the determination of molecular size, the examination of spectra and the determination of wave-length, the relation between inertia-mass, gravitational-mass and weight, the verification of Kepler's law of equal areas, and the determination of the mass of the electron

Heat, as it is usually taught, does not come into the course at all, and, in what is intended for a one-year course, the compilers have had to be rigorously selective in other directions as well They have concentrated on what they believe to be fundamental and what can be well taught They report that the course has proved itself in practice, and the tests given to the pupils are certainly exacting I was able to obtain a specimen set, and I can only say that I should not care to tackle them after a hard day spent determining coefficients of expansion

Prof Francis L Friedman, of the Massachusetts Institute of Technology, describes the development of the text-book in an appendix, and lists the 200 or so contributors to the programme They must be proud of their achievement G R NOAKES

MATERIALS FOR REACTORS

Reactor Handbook, Vol 1

Materials Edited by C R Tipton, Jr Second edition, revised and enlarged Pp xv+1207 (New York Interscience Publishers, Inc , London Interscience Publishers, Ltd , 1960) 260*s*

THE first edition of this handbook was published by the United States Atomic Energy Commission before the 1958 Geneva Conference, at a time when little atomic energy information had been released It is not surprising, therefore, that considerable expansion and revision of the data have been necessary in such a rapidly expanding field and with the declassification of much of the earlier material In particular, both liquid and gaseous reactor materials have now been included and the number of authors contributing extended to some 130 reactor specialists The majority of the data has been drawn from U S Atomic Energy Commission reports, many of which are still classified, with generally only brief mention of British and other European work It is claimed that the subject-matter is current to about the spring of 1958, which would suggest that little of the information released at the 1958 Geneva Conference is included

It is only possible to indicate briefly the scope of a volume of 1,200 pages which covers a wide range of materials In a general introductory section, brief surveys are made of radiation damage and of the health and safety aspects involved in handling the special materials in the nuclear energy industry About a third of the book is devoted to the production and properties of fuels, which include those based on uranium, plutonium and thorium metals both in solid and liquid form and as ceramics, aqueous solutions and molten salts A most comprehensive list of canning and control materials is considered and the moderators reviewed are hydrogen-containing compounds, graphite and beryllium-base materials of interest The properties of both gaseous and liquid metal coolants are detailed together with compatibility data relevant to reactor operation A section on shielding materials covers their physical and mechanical properties and behaviour under irradiation The volume concludes with a selected list of references dealing with the constitution of various binary alloys, which is in addition to a large number of equilibrium diagrams of technological interest distributed throughout the text

Certain aspects of reactor technology of somewhat limited interest are described in considerable detail, whereas other problems, the elucidation of which has occupied a great deal of effort, are not dealt with adequately For example, swelling of uranium metal fuels which can severely restrict their life under certain operating conditions, is barely mentioned and the section on radiation damage in beryllium could justifiably be expanded This also applies to the problems associated with pressure vessel steels, which could be considered in much greater detail The section on magnesium alloys only briefly mentions the magnox series which were developed by British workers and adopted as the canning material for the first seven Central Electricity Generating Board nuclear power stations and ignores the zirconium-bearing *ZA* alloy which the French have developed successfully for their reactors One is left with the impression that the handbook was prepared to satisfy the interests of United States reactor technologists up to 1958 rather than to present a more comprehensive and general survey of reactor materials Nevertheless, it is a valuable reference book and contains a wealth of useful information

B W MOTT

ENZYMES AND CANCER

Chemistry of Enzymes in Cancer

By Prof Franz Bergel (A Monograph in American Lectures in Living Chemistry) Pp xi+122 (Springfield, Ill Charles C Thomas, Oxford Blackwell Scientic Publications, 1961) 44*s*

THIRTY years ago biochemists were hopefully seeking for qualitative differences in metabolism between normal and tumour cells which might explain their radically different biological behaviour That search is presumably over, and we are left with only a few quantitative differences, most of them of dubious relevance to the problem of the causation of cancer

Prof Bergel starts from the working hypothesis that these differences, which are the outward signs of changes in the deoxyribonucleic acid constitution of the cells, are related to the abnormal properties of cancer cells, and that if the metabolic differences could, by any therapeutic means, be removed, then the cells would revert to normal behaviour

In Part 1 he outlines the three major kinds of hypothesis which attempt to relate enzymes to cancer (1) the 'faulty' respiration theory of Warburg, (2) the generalization of Greenberg that all tumours possess a similar enzymic constitution, (3) the 'deletion' theory, due chiefly to Potter, according to which the cancer cell has lost certain protein constituents, probably concerned in the synthesis of enzymes, and he criticizes each in the light of recent biochemical developments There follows a detailed discussion of the present state of knowledge with respect to certain selected groups of enzyme systems, namely, those concerned with nucleic acid catabolism, which are deficient in tumours, those concerned with protein and amino-acid catabolism, which are deficient at least in some tumours, and the enzymes of carbohydrate breakdown, where, among many other differences, phosphorylase is deficient All these changes are in the direction of providing an increased potential for cell division

Part 2 deals with the possibilities of acting on tumour cells *in vivo* in such a way as to correct the faults in their metabolism and restore them to normality The final chapter gives several examples in detail, the emphasis being on 'restitution' therapy, in which enzymes deficient in the tumour are injected into the animal and effects observed on the rate of growth of the tumour Cessation of growth has been observed as a result of injection of both xanthine oxidase and ribonuclease

This is not a book for the general reader, it assumes a biochemical background, and an intimate knowledge of a vast literature Prof Bergel almost accepts (p 81) that the fundamental difference between normal and cancer cells must lie in the chemistry of their deoxyribonucleic acids (DNA), and that the changes in enzyme concentration which are observed must necessarily result from this change in the DNA, through mechanisms the study of which is still in its infancy It follows that attempts to correct enzyme imbalance by 'restitution' therapy cannot restore cancer cells to normality, though its continued application could conceivably control their harmful activities The attempt to "repair biochemical lesions residing in the DNA" (p 82) lies far in the future Enzyme therapy obviously depends on the possibility of introducing macromolecules into cells The only mechanism so far suggested for this process is pinocytosis (the engulfing of small quantities of extra-cellular fluid by cells), a phenomenon about which little is known with certainty, Prof Bergel agrees that it is "still a subject of speculation and research" (p 58)

The subject-matter is presented with exemplary clarity, and the book excellently produced and well indexed It will obviously be read with the greatest interest by all those concerned in cancer research

L H STICKLAND

PHYSICAL OCEANOGRAPHY

Physical Oceanography

By Prof Albert Defant Vol 1 pp xvi+729 Vol 2 pp viii+598 (London and New York Pergamon Press, 1961) £10 10*s* per set of two volumes

IN these two volumes Prof Defant has attempted the seemingly impossible task of covering single-handed the whole field of physical oceanography That he has succeeded with such thoroughness and in such minute detail reflects the wide range of his lifetime's work in this subject In almost every chapter, references are found to significant original contributions by Prof Defant himself, and the text is often illuminated with examples drawn from the results of the *Meteor* expedition of 1925–27, in which he took part

Each volume is in two parts , the first part of Vol 1 deals with the properties of sea-water and related topics Following a brief chapter on the dimensions of the oceans and the topography of the sea-floor, the next five chapters, occupying some 200 pages, deal with the physical and chemical properties of sea-water, the distribution of temperature, salinity and density in the ocean and their variation with time, and the temperature–salinity relationship and its connexion with mixing processes These are followed by two chapters on the water budget of the Earth, and ice in the sea

The second part of Vol 1 is devoted to dynamical oceanography, the movement of sea-water in the form of ocean currents After considering the forces acting on the water, and the basic hydrodynamic equations, the theory of currents in homogeneous water is developed This is followed by a discussion of the conditions at the boundary surfaces of water masses, and of currents in a non-homogeneous ocean Special consideration is given to currents in straits, and after a brief discussion of the effect of wind on the mass distribution and density currents, the remainder of the volume, amounting to 150 pages, is devoted to an account of the general circulation of the oceans

In Vol 2, Prof Defant is concerned with periodic movements in the ocean The first part deals with the theory of surface waves, methods for their measurement and analysis, and theories of their growth and propagation Waves in shallow water and near coasts, long waves in canals and standing waves in enclosed basins are also considered The second part of this volume is an account of the tides and tidal currents The tide-generating forces, the theory of tides, and the methods of harmonic analysis of tidal observations are described first, followed by a detailed account of the tides in the Mediterranean and the shallow seas Tides in estuaries are next dealt with, then follows an account of tides in the deep oceans, particularly in the Atlantic There is a chapter on Earth tides and the effect of friction due to tidal currents, and the volume is concluded with a substantial chapter of 50 pages on internal waves

The manuscript of this book was completed in 1945, but post-War difficulties prevented its publication until 1961 Several revisions had been made by then, and also the text had been translated from German to English Inevitably there is some unevenness in the revision , some topics have been brought up to date as far as 1957, others have been relatively neglected The claim in the preface that "the oceanographic literature has been considered in its entirety to the end of May 1957" seems exaggerated The first chapter on the structure of the sea-floor is out of date, and post-War revisions have left some confusion Three different values are quoted for the maximum depth in the Pacific Much recent work on the topography of the North Atlantic, and the geophysical structure of the Earth's crust beneath the oceans and continents could have been included In the chapter on properties of sea-water, no mention is made of its electrical conductivity, the precise measurement of which is used extensively nowadays as a substitute for salinity determination Post-War work in the United States on forecasting ice conditions is not referred to in the chapter "Ice in the Sea" Recent measurements of radiocarbon concentration in sea-water and its implications in estimating the time-scale of the deep circulation are not considered, and much more recent work on the generation and propagation of surface waves could have been mentioned Besides this lack of uniformity in up-to-date revision, the text is marred by a number of misprints, especially in authors' names But Prof Defant disarms criticism with his quotation from Newton, that these volumes "may be read with forbearance, not so much with the view to censure, as to remedy their defects" , and indeed these errors and omissions seem minor in comparison with the vast quantity of information presented here

Physical oceanographers may consider themselves fortunate to have so much of Prof Defant's knowledge and experience made available to them in these volumes, even at the price of ten guineas

J C SWALLOW

Priests and People

A Study in the Sociology of Religion By Conor K Ward (Social Research Series) Pp xi+182 (Liverpool Liverpool University Press, 1961) 25*s.* net

THIS book is the fruit of fifteen months field work by a team of sociologists in a Roman Catholic parish in Liverpool It provides, therefore, a very thorough examination of the life and activities of the parish and makes available much material which can be used in assessing the effectiveness of a parish in an urban setting This is perhaps where the chief interest of the book lies for the general reader For more than a thousand years the territorial parish with its church and resident clergy has been the normal centre of Christian life and in the past it developed into very much more than a centre for worship only Around the parish church was collected the educational, social and sometimes political life of the area But with the coming of the Industrial Revolution all this was changed and the parish ceased to be the centre it had been

The question has been raised whether the system of territorial parishes is the most effective expression of Christian community in an industrial society Ought the Church not to be training its man-power for specialist ministries, industrial chaplaincies, etc , rather than for parochial work ? Such questions are constantly being argued and strong opinions are expressed Dr Ward's book makes it abundantly clear that more sociological research is a first essential for finding effective answers for these and other problems It would be dangerous to make generalizations from this study of one parish Few parishes will be as socially monochrome as this one was Studies of other parishes would be useful to test how far the situation here described is typical One suspects that the social cohesion of Liverpool with its history of religious intolerance might not be so true of parishes in other parts of Britain Yet Dr Ward has made one thing clear Though changes must be made to adapt the parish more effectively to its urban environment, it will not be superseded in the foreseeable future J ROBINSON

Handbuch der Astronautik, Lieferung 1–6

Herausgegeben von Prof Karl Schutte und Hans K Kaiser Band 1, Heft 1 Pp 1–32 Band 1, Heft 2 Pp 33–64 Band 1, Heft 3 Pp 65–96 Band 1, Heft 4 Pp. 97–128 Band 1, Heft 5 Pp 129–160 Band 1, Heft 6 Pp. 161–192 (Konstanz Akademische Verlagsgesellschaft Athenaion, Dr Albert Hachfeld, 1958 und 1960) 1 50 dollars each part

THIS *Handbook of Astronautics* will consist of 4 volumes, each made up of about 12 sections, the individual sections being issued separately and each containing 32 pages Of the proposed 48 sections it is the first 6 which are the subjects of this review There is no editorial introduction to explain the aims or scope of the *Handbook*, but the intention appears to be to cover the whole subject of astronautics, from the details of rocket engines to the niceties of space law, in a series of articles of about 20 pages each, which begin from first principles and presumably in later issues will go into the subjects more fully The issues under review contain 8½ articles Willy Ley writes on the history of the idea of space flight, W Schaub on "Gravity and Its Conquest", dealing with the fundamental dynamics, J. C Cooper on "Flight-space Law", and A. J Zaehringer on solid-propellant rockets H Stumke describes how to calculate the flight performance of unmanned rockets in considerable detail, S J Smith and G P Sutton review heat-transfer problems in liquid-propellant rocket motors, R P Haviland outlines the applications of satellite vehicles, and J Eugster investigates the dangers to space travellers from cosmic rays and other radiations Finally W Alveimann and W Lohse begin to discuss the theory of rocket engines, but suffer a premature cut-off in mid-sentence These articles form sound and useful introductions to their subjects, and the *Handbook* as a whole promises to be valuable as a work of reference and for teaching purposes, though it remains to be seen whether the choice of subjects for articles will be well balanced The book follows the unsatisfactory dual-language convention, with about half the articles in English and half in German Since most readers are not equally fluent in both languages, the value of the book is, for them, halved The separate sections have attractive paper covers, but are presumably intended to be bound together, since they are apt to finish in mid-sentence, so the total cost of the 1,500-page *Handbook* will be 72 dollars plus binding costs D G KING-HELE

A Statistical Model for Chemists

By Edward L Bauer Pp x+156. (New York Academic Press, Inc , London Academic Press, Inc (London), Ltd , 1960.) 4 75 dollars.

THE author in his preface describes this book as "a manual for the working chemist" and no more exact definition of this excellent little handbook could possibly be given Shorn of all complicated mathematical functions and lucid in its approach, this volume supplies the chemist with typical examples taken from ordinary laboratory data, and indicates those benefits relating to economy, accuracy and interpretation which can accrue from applications of the statistical method

The relation of the Gaussian normal distribution curve to the 'Student's t' concept of Gosset is clearly described, the latter function being of most general use to the chemist, who is usually concerned with the averages of small sample results

Conventional statistical terms such as precision, accuracy, average deviation, variance, standard deviation, etc , are all concisely defined. Fixed, random and mixed models of the analysis of variance technique are illustrated by reference to three chemists carrying out certain analytical observations Most of the tests of significance used in the book are based on the analysis of variance method, but attention is directed to the simplifications resulting from the use of range rather than variance in the calculations Chapter 6 contains an interesting exposition of regression analysis involving correlated variables, and is illustrated by the effects of unknown 'blank' titres, etc , in quantitative analyses. Theories of sampling, control of routine analyses and factorial experimentation are all given fairly full treatment

The volume ends with a collection of useful statistical tables, for example, for use with F and t functions, computation of confidence limits, etc.

This book should prove a most useful elementary introduction to the amateur statistician, particularly in the chemical field D. T LEWIS

ARSENIC CONTENT OF NAPOLEON I's HAIR PROBABLY TAKEN IMMEDIATELY AFTER HIS DEATH

By Dr STEN FORSHUFVUD, Dr. HAMILTON SMITH and Dr. ANDERS WASSÉN

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IT has generally been deduced from the report of the post-mortem dissection dated St Helena, May 6, 1821, and signed by the British medical officers, Thomas Shortt, Archibald Arnott, Charles Mitchell, Francis Burton and Matthew Livingstone, that Napoleon I's death the preceding day was due to extensive cancerous lesions of the stomach

The view that Napoleon died of 'cancer' was not accepted by Francesco Antommarchi, the man who actually performed the autopsy, who had been Napoleon's household physician for the last twenty months, and was the only physician on St Helena with a pathologist's training He maintained that the hepatitis the Emperor had suffered from for a long time was the cause of death Furthermore, the Emperor's enlarged, tender liver, jaundiced complexion and yellow conjunctivæ had also been diagnosed as signs of severe hepatitis by Barry O'Meara, Napoleon's household physician during his first three years in exile, and John Stokoe, who had attended Napoleon for a week in January 1819

Consequently, apart from Dr Arnott, who assisted Antommarchi during the last few weeks when the morbid picture was especially baffling, those medical men who personally had attended Napoleon during his illness refused to admit that he had died from cancer

Many medical writers with doubts about the cancer diagnosis have attempted to identify Napoleon's disease on St Helena by analysing compilations of his signs and symptoms This has produced surprisingly disparate results Thus, when exiled on St Helena, Napoleon is alleged to have had, or suffered from the sequels of, the following diseases peptic ulcer, intestinal ulceration, various liver inflammations, undulant fever, malaria, dysentery, rheumatoid arthritis, heart failure, congenital extremely slow blood circulation, epilepsy, tuberculosis, pleurisy, severe hormonal imbalance leading to obesity and impotence (dystrophia adiposogenitalis), syphilis, gonorrhœa, intoxication from defective teeth (so-called focal infection), gout, piles, and a constitutional predisposition to severe constipation which, it is said, was fatal owing to auto-intoxication and poisoning by laxatives

If a suitable selection is made from the variety of signs and symptoms manifested by Napoleon on St Helena, it is a simple matter to make out a convincing case for every one of these diagnoses in turn But if what, after all, was a fairly unchanging disease pattern on St Helena is taken as an entity, and allowance is made for all the signs and symptoms and their interrelations, then one cannot escape the impression that all the pieces form an orderly picture of two highly characteristic syndromes, namely, the chronic and acute types of arsenic poisoning

Napoleon's condition was rather poor during the Hundred Days However, his health improved while he was being taken into exile on board H M S *Northumberland*, and Napoleon's general health remained good during the initial period on St Helena when, with his sole companions and collaborators, the Chamberlain Las Cases and his son, he lived in a simple cottage on the estate, 'The Briars'

Soon after moving to 'Longwood', which became his permanent residence, Napoleon's ailments again became manifest He turned moody, had various aches and pains, the lower legs swelled up and would not carry him, exanthemata broke out, particularly on the legs, his sleep was abnormal with either insomnia or somnolence diarrhœa and constipation alternated, headaches occurred with increasing frequency and severity

His indispositions at first lasted only a few days at a time, his mental powers returning undiminished in the intervals Nevertheless, members of his staff noticed marked changes in his expression and increasing difficulties in locomotion Napoleon's legs were swollen and often collapsed under him

On May 1, 1816, Napoleon had an attack lasting for a whole week Las Cases mentions that he complained of weak legs, headache, hypersensitivity to light, and felt cold and shivery, his facial expression had changed markedly, he spoke sluggishly, and was morose and taciturn Napoleon believed himself to have gout, suggesting that his feet were swollen and painful Though Napoleon's teeth were perfectly sound, O'Meara recorded on June 16, 1816, that his patient had toothache Later it turned out that the toothache was due to 'scurvy', that is, stomatitis On July 26, 1816, Napoleon experienced the first stab of that pain in the hypochondrium which was a constant source of distress through the rest of his life After a number of brief attacks of malaise, Napoleon fell ill for six weeks in succession on October 1, 1816 A troublesome cough was now added to the list of symptoms, he had pustules on the lips as well as in the oral cavity and throat. Napoleon was tormented by an insatiable thirst and had become perceptibly hard of hearing Lassitude alternating with restlessness was a characteristic feature He always felt cold and liked to sit near the fireplace He could scarcely walk, because the swollen and weak legs would not carry him The gums swelled up and the teeth became loose Diffuse nausea with a predisposition for spasmodic vomiting without actual regurgitation of food were also noted Napoleon had a relapse with the same manifestations as before on December 3 and 4, 1816, and his companions now became aware of his very jaundiced complexion On December 14, 18 and 28 there were further attacks the symptoms of which included spastic involuntary movements and unconsciousness on several occasions According to the head valet Marchand, one of these bouts was combined with dysentery (that is, severe diarrhœa) For the first time in two months Napoleon went out of doors on January 26, 1817 Apart from brief indispositions in May June and July, Napoleon's health seemed to remain comparatively good until September 25, 1817, when a period of illness set in which lasted approximately a whole year The signs and symptoms were on the whole the same as before, but new features had been added For example, in Dr O'Meara's case notes for October 1817, we read on the third a swelling was palpable on the right side near the liver, on the ninth the pains in the legs and neighbourhood of the liver had increased and pains in the shoulder supervened, on the eleventh the patient was seriously distressed by tachycardia Afterwards severe constipation developed, the skin and conjunctivæ were yellow and the appetite was poor The vomiting tendency was now accompanied by stomach-ache The stomatitis was very distressing and on several occasions caused the cheeks to swell Constant phenomena were 'febrile attacks' at nightfall and profuse sweating at daybreak The tongue was coated, the pulse often very rapid and irregular

Although Napoleon now had no physician in attendance—the British authorities had ordered O'Meara away from 'Longwood' on July 25, 1818—his health underwent a gradual improvement in the latter half of that year Some symptoms nevertheless persisted for another few months, such as lancinating hypochondrial pains and painful and weak legs Often the feet were ice cold and had to be warmed with hot towels, and the ex-Emperor frequently sat before the fire with his feet in a flannel bag

In the final months of 1818 it seemed as though Napoleon was well on the way towards full recovery Once more he began to take interest in his literary work and in his garden He started taking regular exercise, a sign that his legs would support him again In the beginning of November 1818 Bertrand, le Grand-Marechal du Palais, wrote in his diary· "   le malheur a passé sur son caractère, comme sur un roc, sans l'abattre et sans y laisser de traces"

But Napoleon again became seriously ill towards the end of December 1818 The manifestations included tachycardia, chills, fever, and the old trouble from the legs In the night between January 16 and 17, 1819, his life was threatened, he lost consciousness several times, and Dr Stokoe was summoned (He had been asked to become the Emperor's household physician the week before) In his bulletins, Stokoe stated that he had found the patient in a state of extreme exhaustion, his complexion was yellow and he had pains in the region of the liver and in the shoulder There was excruciating headache, vertigo and fainting fits Some improvement took place when the patient began to sweat profusely Similar attacks recurred the next night and the next night but one Stokoe issued three bulletins about Napoleon's health, and these so displeased the Governor, Sir Hudson Lowe, that Stokoe was constrained to leave St Helena So on January 22, 1819, Napoleon again found himself without a physician His health nevertheless improved and in the latter half of 1819 he was busily at work every morning from 4 a m to 10 a m supervising the extensive landscaping projects he was undertaking in his garden He could now walk with a stick, had recovered his former good temper, and conversed pleasantly and cheerfully with his companions Apart from a few brief indispositions, this state of good health lasted for fifteen months

However, a new long period of uninterrupted ill-health commenced on September 18, 1820 This period was marked by at least six intercurrent attacks of violent illness with severe and acute symptoms, but during the intervals of remission Napoleon's health improved day by day even though some of the symptoms persisted During these periods of convalescence he could be up and about and also, albeit with tottering steps, go for short strolls in the garden

According to Dr Antommarchi's case notes, the acute attacks were accompanied by the following symptoms headache, dyspnœa, weak, rapid and irregular pulse, ice-cold feet and legs, gastric pains, loss of appetite; somnolence that seemed refractory to treatment, yellow conjunctivæ, yellow complexion, excessive paleness, dark rings under the eyes, coated tongue, burning thirst, tachycardia, tremor, nervous dry cough, vertigo, vomiting, sensation of heat in the viscera, pain in the legs and from the liver, sternum and shoulder regions, constipation and diarrhœa, spasmodic contractions of triceps, remarkably weak legs, restlessness, nightmares, exanthemata, loose teeth, bleeding gums, insomnia, hardness of hearing, extreme hypersensitivity to light, impaired vision, very severe general exhaustion

On March 22, 1821, Napoleon's disease changed character, with constant severe vomiting supervening This was doubtless because he had been prescribed and—without knowing it—taken tartar emetic Accordingly, antimony poisoning was now an added complication A remission lasting for two and a half weeks occurred in the beginning of April 1821, and Napoleon took this opportunity to write his last will and testament

Late at night on April 24, 1821, there was a severe relapse with typical manifestations of acute antimony poisoning Additional relapses occurred over the next few days However, a moderate improvement seems to have taken place on May 3 In the afternoon of that day Napoleon unknowingly took a large dose of calomel Thirty minutes before midnight he had copious stools with the appearance of tar Additional evacuations of blood occurred on May 4 He died on May 5, 1821, after lying absolutely motionless for approximately 24 hr

At the post-mortem examination the stomach was full of black blood and the gastric mucosa was very badly corroded These findings were probably sequels of the calomel given on May 3 rather than of arsenic On the other hand, the following signs of arsenic poisoning were encountered enlarged and hardened liver, enlarged spleen, fluid effusions in the pleuræ, bronchial and mediastinal lymph nodes degenerated and in suppuration, loss of all body hair, large fat deposits under the skin and in the abdomen

Napoleon was not embalmed Yet, when his coffin was opened in 1840, those present were astonished at finding him extremely well preserved

In the light of the foregoing, it seems that on St Helena Napoleon suffered from chronic arsenic poisoning with intervening periods of acute arsenic poisoning In such circumstances, we deemed it interesting to apply the activation technique in a study of Napoleon's hairs from the period on St

Helena Through the courtesy of M le Commandant Henry Lachouque, the great French expert on Napoleon's life and the organizer of the permanent Napoleonic exhibitions on St Helena and in the French Army Museum, hair taken from Emperor Napoleon I's head, probably on the day after his death, has been made available to us

The hair sample thus obtained was sent to the Department of Forensic Medicine at the University of Glasgow, where it was examined using an activation analysis technique (Smith, 1959) as follows

The hair sample was weighed (1 72 mgm) and sealed in a polythene container It and a standard arsenic solution sealed in a silica ampoule were irradiated by thermal neutrons for one day at 10^{12} neutrons/cm^2/sec in a nuclear reactor at the Atomic Energy Research Establishment. Harwell Thereafter the sample was returned and the arsenic extracted with added carrier arsenic by a modified Gutzeit technique The activity from the hair sample was compared with that from the standard arsenic sample and the arsenic content of the hair was calculated

The value found for the sample of hair was 10 38 parts per million This is high by comparison with the normal mean arsenic content of about 0 8 p p m Unfortunately, it was not possible to make any distribution studies as no further hair samples were available It is impossible to tell from the value alone whether the arsenic was evenly distributed (as expected in continuous exposure) or located in one point (as would be the case in a single large exposure)

This investigation shows the great advantage of activation analysis when only very small quantities of sample are available

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TEACHING MACHINES

So many members were interested in the symposium on "Teaching Machines" in Section J (Psychology) at the British Association meeting at Norwich on September 4 that the 'house-full' notice had to be used Introducing the subject, the chairman, Prof H Kay, pointed out the theoretical and practical implications of teaching machines It is likely that their use in research will contribute considerably to our understanding of learning processes Psychologists recognize that hitherto they have carried out extensive experiments on animal behaviour but have neglected the study of human learning Here is a definite opportunity to examine in ideally controlled conditions the variables which are influencing learning On the practical side there is not only a desperate national need for teachers, particularly in science subjects, but also an increasing problem of how to impart technological information on a world-wide scale The urgent need is for new forms of communication, teaching machines represent one such form

Dr J Annett, of the Department of Psychology, University of Sheffield, outlined the main requirements for a teaching machine It is a device not only for imparting information but also for sensing that this information has been received and understood It is a two-way communication channel designed at every stage to check the progress of the student and, ideally, to modify its instructions on the basis of the students' responses This may be achieved by several methods Perhaps the simplest is the small step or linear-type programming developed by Prof Skinner at Harvard, where a series of statements and questions are built up and are repeatedly modified on the basis of the actual success of students in mastering them Eventually a sequence is established so that when this programme is presented to other students nearly all the problems of subject-matter presentation have been anticipated Such a programme is time-consuming to prepare, is lengthy in so far as there are many items with short steps between them, but is efficient and requires little use of 'gadgets' for presentation

An alternative system is known as intrinsic programming Here there is a main line of communication, and provided a student can respond correctly he can proceed smoothly along this route But if he makes a mistake he is directed to a branch line, where further explanations are given before returning him to the main sequence Dr Annett explained that in the type of machine constructed at the Department at Sheffield this system had been achieved by a sequence of '*A*' frames and '*B*' frames If any one of the '*A*' frames is misunderstood, a '*B*' frame is selected and the point is explained in greater detail This type of machine is flexible enough to cope with students differing considerably in intellectual ability A machine might operate on a 'constructed response mode' where the student first makes his own answer and compares it with the answer which is then supplied by the machine, or on an alternative procedure, where the machine first supplies a multi-choice solution and the student has to select the correct response. The advantages of these procedures are being investigated.

Mr. Leslie Reid, lecturer in psychology in the University of Aberdeen, discussed and actually produced the simpler forms of teaching machines which he has developed and is using at present in his research work in Scottish schools. His programmes have followed Skinner's principles with an accent on eliminating frustration and difficulties. From this point of view, teaching machines preserve the teacher–pupil relationship by ensuring that pupils are consistently rewarded, and not punished, for their responses. This is achieved primarily by the ordering of items in a suitable sequence, such that the transition from ignorance to knowledge is not a bumpy and apparently unmapped journey, but a smooth and logical progression in which a pupil can be expected to achieve success. Mr. Reid explained how a pupil working through a programme achieves insight through a gradual understanding of his subject; by contrast, that insight which a pupil achieves by a sudden flash of comprehension—the so-called 'eureka' phenomenon—is mainly a product of bad teaching.

Mr. Gordon Pask, of System Research, explained the principles of an adaptive teaching machine. Unlike a programmed machine the adaptive device itself 'learns' about the student's behaviour and modifies the training routine to suit each individual throughout the learning process. In teaching a manual skill the designer has to select certain parameters. These refer both (1) to the students' behaviour, such as the response latency, and (2) to the instructor's behaviour, such as the amount of 'cue' information with each category of problem. It will be observed that the parameters in (1) define measures of the student's behaviour which an instructor might make, and those in (2) consider the ways in which the instructor might manipulate a training routine. Thus the designer is constructing a computing device which measures the performance and then by an adaptive servo-mechanism adjusts the parameters of (2) in order to increase to a maximum the performance measure. Well-designed machines adopt a competitive strategy when the student is doing well and a co-operative strategy when he is making no improvement. Mr. Pask went on to describe how adaptive teaching machines might be used for group or classroom instruction. Here students are carrying out exercises in scientific inference and act either as a problem poser or a problem solver. The student coming quickest to the right conclusion using the minimum of evidence is most successful. A miniature economy is imposed on the group, and the machine controls the group's behaviour by modifying the parameters of the game. The most useful parameters seem to be 'cost' of communication, 'cost' of evidence, 'gain' for successful solution, and a bias on the process of role assignment.

Mr. K. Austwick, of the University of Sheffield, presented "An Educational Viewpoint" on the subject. Mr. Austwick has conducted preliminary experiments at schools on the teaching of mathematics with a simple linear programmed procedure. Preliminary results are encouraging and suggest that work learned by this method is well retained over a period of time. Programming of school and university courses is now under way. If such procedures were widely adopted they could lead to modifications in school design since they do not require the formal arrangement of a classroom. The possibilities of such machines to handicapped children, isolated children, personnel in the Services, and adults keeping up to date in a subject were brought out. On the other hand, the machines could not take over the 'human' role of the teacher, though they might give the teacher more time and opportunity to show his humanity.

The discussion was brisk enough and ranged from the university teacher anxious to discover how far machines could take over the part of tutor to a self-styled 'plain local family doctor' who knew they could not. These are early days for the teaching-machine movement, but it was apparent that we have here an educational development which is already arousing considerable interest and some opposition—both happy indexes. In a remarkably short time this type of instruction has now used 'hardware' ranging from simple pencil and paper booklets to electronic gadgets controlled by a full-scale computor. Systems are already being produced for public sale by both the publisher and the electronics industry. It is clear that the results of research are needed to give some guidance to this diversity, but research efforts in Britain are, as usual, on a small scale. To the academic psychologist this subject represents a further development in the field of communication systems, and it now offers another opportunity for studying how man can most efficiently receive and store information. But the pace and detachment of academic research are confronted by the urgency of the demand for instruction. The need in Britain for scientific and technical teaching is obvious enough, but is trivial in comparison with other areas of the world. Part of the impetus behind automated teaching is that universally, in so many subjects, there is so much demand for knowledge. There is every indication that this demand will continue to increase, and that it cannot be fulfilled by conventional methods.

H. KAY

BREATHING

UNDER the chairmanship of the Section's president, Sir Bryan Matthews, a symposium on "Breathing (External Respiration)" was held on September 4 by Section I (Physiology and Biochemistry) during the recent British Association meeting in Norwich. Four papers were presented, covering varied topics connected with respiratory physiology. Two of these had a clinical bias, and concerned the distribution of ventilation and flow of blood in the lungs, and the effects of oxygen on breathlessness. The other two demonstrated the application of respiratory physiology to aviation, one concerning the design of an aircraft breathing system, and the other describing the results of research into the effects of positive acceleration on the mechanics of respiration.

The symposium was opened with the paper “Variation of Ventilation and Blood-Flow in Different Parts of the Lungs in Health and Disease”, presented by Dr P Hugh-Jones, physician at the Hammersmith and King's College Hospitals Starting by showing a model of the air passages in the lungs, and demonstrating how a two-lumen tube could be used to collect expired air from the two lungs separately, Dr Hugh-Jones went on to describe the limitations of X-rays as aids to studying lung function Chest radiographs were shown to illustrate this, where there was a complete dissociation between the X-ray appearance and the subject's exercise tolerance Two new techniques were then described which could give details of local lung ventilation and blood supply, full tribute being paid to the advances of the physicists which had made them possible In the first, the subject is merely required to take in a quick breath of air containing a minute trace of radioactive oxygen (oxygen-15), and then to hold his breath for about 30 sec Counters arranged to look at different areas of lung tissue through the chest wall sense the arrival of the oxygen-15 in their fields and then follow its disappearance as it is washed away in the pulmonary circulation The rate of rise of the counting-rate is a measure of ventilation, and the rate of the ensuing fall in counting-rate is a measure of the local blood-flow The 2-min half-life of oxygen-15 is a disadvantage of the technique, but was immaterial at Hammersmith, where it is prepared on the spot in the Medical Research Council's cyclotron Other gases with longer half-lives might be used elsewhere where such facilities did not exist, for example, radioactive isotopes of argon or krypton Carbon dioxide made from oxygen-15 had been found to give better results when the technique was used for studying pulmonary blood-flow

The second technique described by Dr Hugh-Jones was that of regional gas analysis, using a mass spectrometer which could provide almost continuous analysis of up to four gases simultaneously Samples were taken through a 0 5-mm diameter tube which could be inserted into any chosen part of the lung during bronchoscopy. This allowed a study to be made of ventilation and blood-flow in any small area of the lungs, and information obtained in this way would be of value not only to the experimental physiologist but also to the physician and surgeon

The second paper, “Physiological Requirements in the Design of Aircraft Breathing Systems”, was presented by Squadron-Leader J Ernsting of the Royal Air Force Institute of Aviation Medicine The variation in properties of the atmosphere with increasing altitude was described and it was shown that the two main problems to be overcome at a flight-level of 40,000 ft were an air temperature of − 55° C, and an air pressure of 1/5 of an atmosphere. Ideally, aircrew and passengers should be provided with sea-level cabin conditions at any aircraft altitude, but the weight penalty of the structure needed to withstand the required pressure differential is too great, so that the physiologists reach a compromise with the engineers, and a cabin pressure equivalent to an altitude of 8,000 ft is chosen Though this is acceptable, the possibility of a failure in the pressurization system must be allowed for, and with this in view the physiological effects of decompression had been studied In a civil air-liner, window or valve failure will result in slow loss of pressure over several seconds In military aircraft, on the other hand, a smaller cabin, together with the necessary provision of aircrew escape systems, makes an explosive decompression, where ambient pressure is reached in 1/10 sec or less, a real possibility Dr Ernsting went on to describe the effects of explosive decompression, first, those due to the low final pressure, anoxia and decompression sickness, and secondly, those due to the rapid change in pressure Protection against anoxia could be provided by the breathing of oxygen between 10,000 ft and 40,000 ft, and the breathing of oxygen under pressure at higher altitudes Above 50,000 ft, the pressure required is so great that counter pressure has to be applied to the rest of the body to make breathing possible, and since at 63,000 ft, the vapour pressure of water at body temperature is equal to the barometric pressure, some form of pressure suit is required to prevent vaporization of body fluids Experiments on aircrew were described with a short film in which explosive decompression from 8,000 to 38,000 ft was followed by short periods of impaired function, even when the decompression was followed by breathing 100 per cent oxygen within 5 sec It had been found that the subject must already be breathing at least 40 per cent oxygen at the time of decompression if impairment of function were to be avoided

Dr Ernsting concluded by discussing the serious consequences of explosive decompression on enclosed volumes of gas in the body If the lungs were shut off from the outside air, serious or even fatal lung damage might occur Masks had to be designed which would allow free escape of air when decompression took place but which would immediately allow oxygen to be breathed under pressure The sudden expansion of 200 ml of gas in the gut to 1 litre was less serious but still very uncomfortable

The third paper, “Breathlessness its Alleviation by Oxygen in Health and Disease”, was presented by Dr J E Cotes of the Medical Research Council Pneumoconiosis Research Unit at Llandough Hospital Pointing out how appropriately this subject followed the centenary of the birth of J S Haldane, he went on to discuss the 12-month mortality figures of a group of 67 men severely disabled with breathlessness, and the curious finding that those whose symptoms were improved on breathing oxygen fared far worse than those on whom oxygen had no effect If the reason for this were known, then more might possibly be done for these patients

Dr Cotes then discussed the development of our knowledge of the effects of oxygen, and showed that as a respiratory stimulant, while lack of oxygen was of less importance than excess of carbon dioxide, it did play a small part at rest, and with increasing exercise it played an increasingly important part The administration of oxygen improved tolerance of exercise, and prolonged the time for which heavy exercise could be carried out Oddly enough, 50 per cent oxygen was better than 100 per cent oxygen in this respect, due possibly to the adverse local effects of too high oxygen tensions on the brain, and it was suggested that 50 per cent oxygen might be the better gas for respirators when work has to be carried out in unfavourable environments A small cylinder of 50 per cent oxygen should allow the wearer to run a mile in 3½ min A portable respirator was demonstrated, and sets like this were of great value in improving the tolerance of exercise by disabled men, not only subjectively, but also objectively in treadmill tests The increased work which could be carried out was associated with a reduction in pulmonary ventilation, the limit to capability of work being reached when the resulting ventilation reached the subject's voluntary maximum

Dr Cotes was unable to say why the breathing of extra oxygen should produce these effects, but many possibilities had been excluded, such as secondary changes in the pulmonary circulation, blood lactate-levels, or in the stiffness of the lungs This was the limit of present knowledge on the subject, but it was hoped that the mechanisms operating would soon be elucidated

The fourth and final paper in the symposium was given by Flight-Lieutenant D H Glaister, also of the Royal Air Force Institute of Aviation Medicine. under the title "Some Effects of Positive Acceleration on Respiratory Mechanics" Describing the interest physiologists have had in the effects of posture on breathing, Dr Glaister went on to show how the human centrifuge could be used to extend data beyond the 1*g* which normally limited laboratory experiments Positive acceleration was described as that acting in the same sense as gravity on a man standing on Earth's surface, and is measured in terms of this being 1*g* The postural change from lying to standing was thus equivalent to a change from 1*g* transverse acceleration to 1*g* positive acceleration

Circulatory effects prevented many experiments being carried out above 4 or 5*g*, since the degree of relaxation required to produce accurate respiratory measurements resulted in blackout or even unconsciousness Most comparisons were therefore made between 1 and 3*g* As positive acceleration was applied the diaphragm was pulled down by the weight of the abdominal contents, and at 3*g*, about 500 ml of air were drawn into the lungs Despite this, the vital capacity of the lungs fell by 300 ml, due partly to an increase in stiffness of the chest wall, and partly to a rise in intra-abdominal pressure which limited the full descent of the diaphragm The basic mechanics of respiration were then described, and it was shown how the various properties of lung and chest wall could be isolated and measured Results of experiments using these techniques had shown that no change occurred in the stiffness of the lung, nor in the air resistance of the passages leading to it, at least when the subjects were submitted to positive accelerations of 3*g*

The work done on the lungs had been measured by recording continuously the changes in their volume and the pressure gradient across them during quiet breathing This was shown to be unchanged at least up to 3*g* The total work done by the muscles of respiration. however, had to rise under the effects of increased positive acceleration, due to the stiffening of the chest wall and to the raised intra-abdominal pressure The overall efficiency of the respiratory process thus fell during positive acceleration Dr Glaister concluded by pointing out that while this lowered efficiency was probably of little moment at the lower *g*-levels, and with a subject at rest, it would impose a marked limitation to tolerance of exercise during prolonged positive acceleration

The symposium concluded with a brief discussion on the four papers D H GLAISTER

THE NORMAN LOCKYER OBSERVATORY

By Prof G K T CONN
Department of Physics, University of Exeter

SIR NORMAN LOCKYER founded and endowed the Hill Observatory at Salcombe Regis, Sidmouth He was its first director It is now the Norman Lockyer Observatory and has been, since 1948, intimately associated with the University of Exeter Lockyer was a remarkable man His discovery of helium in the Sun's spectrum in 1868 was made as a "private citizen", for he was not appointed to the Science and Art Department at South Kensington by Disraeli until 1875 On the formation of the Royal College of Science he became director of the Solar Physics Laboratory and professor of astrophysics When the Solar Physics Laboratory at South Kensington was transferred to Cambridge in 1913 he set about building a new observatory on the superb site at Sidmouth, at the age of seventy-seven Lockyer was the founder editor of *Nature* in 1869 and remained responsible for fifty years He was succeeded as chairman of the Observatory Council by Sir Richard Gregory, his successor as editor of *Nature* Indeed, both Lockyer and Gregory took great interest in the affairs of the old University College of the South West, now the University of Exeter, and it was Gregory's initiative that led to the intimate association of the Observatory with the University, "a possession which is unequalled in any but the older universities"

The peninsula Salcombe Regis is relatively free from industrial haze and Lockyer chose a site with a superb horizon and a very low noise-level, it is now of some 44 acres The buildings consist of offices and laboratories with a well-equipped library, a store room. a cottage and three domes The McLean dome houses a 12-in refracting telescope with an objective prism of 20°, mounted with a 10-in visual refractor on a Grubb equatorial mounting with electric control (The donor, Sir Francis McLean. was the co-founder of the Observatory and became known to the public as the first airman to fly under the Thames bridges in 1912) The Kensington telescope is a 9-in refractor with an objective prism of 45°, made by Henri Frères, and giving great transparency in the near ultra-violet, on a Cooke equatorial with Russell control, a 10-in Cooke visual telescope is mounted alongside The Mond photographic telescope consists of a four-camera, astrographic equatorial by Cooke with three Zeiss triplet lenses of apertures 17, 14 and 10 cm and one Zeiss anastigmat, aperture 7 cm The mounting is designed to allow an uninterrupted circumpolar motion at all declinations, the clock drive is fitted with electric control There are various pieces of equipment which have not hitherto been set up. for example, a 30-in reflecting telescope and a 21-in siderostat

Work began in 1919, the first paper, published in the *Monthly Notices of the Royal Astronomical Society* in 1920, being "Spectroscopic and Magnitude Observations of Nova Cygni III, 1920" Some 90 research papers have been published, about 40 in the period 1920–36 and 45 during 1936–56, when D L Edwards was director (see p 112 of this issue) The work has been largely on stellar spectroscopy Since 1936 the principal lines of work have been the development of methods of measuring stellar gradients and colour temperatures, using the McLean prismatic camera, and a photographic survey of galactic clusters made with the Mond telescope New ground has been broken in recent years with the initiation of photometric research on the twilight luminescence of atmospheric sodium The Kensington telescope has been used to study the irregular variable star, γ-Cassiopeiæ, there are some 460 spectrograms of this star in an almost unbroken sequence from 1921 to 1956

The Observatory was, and is, maintained as a non-profit making corporation through endowments and funds provided by donors Since 1948 the management has been vested in the Council of the Corporation on which the University of Exeter has a definitive majority The funds available have never been lavish Rapidly rising costs-since the War, together with the development of sophisticated experimental techniques, have meant that the position of the Observatory as an effective centre of original research has become progressively more difficult and indeed critical Against the background of developments in satellites and in space research, the extensive recent progress of studies of the upper atmosphere and the revival of interest in astronomy and astrophysics, such a difficult position presents an obvious challenge The site is large enough for astrophysical work and for an extensive programme of research and teaching in geophysics, a growing interest of the University's Department of Physics, and of geomagnetism, an interest of the Department of Mathematics

The first immediate change of activity for the Observatory will follow from the interests of Dr K Weekes and Mr A Nichol Conditions in the high atmosphere can be studied by various means using both radio and optical techniques Up to the height of the greatest electron concentration in the F_2 region it is possible, in principle, to infer the distribution of ionization from routine soundings Above the peak of the F_2 region, artificial satellites may be used, or Thomson scattering of radio waves and optical experiments An examination of the radio waves emitted by satellites may be used to infer the integrated electron density between the receiver and the satellite This is relatively insensitive to the distribution of electrons, but as the satellite changes height in the course of a transit, the distribution at satellite height may be inferred The most valuable direct means of studying the distribution of electrons in the high atmosphere is by the Thomson scattering of radio waves by free electrons Examination of experiments in the United States to heights of about 700 km suggests that useful results should be obtained to a height of about 2,000 km It is commonly believed that the emission lines of OI at 6300 and 6364 Å are excited by recombination in the F region The correlation between the optical intensities and radio measurements of ionization clearly must be examined Moreover, it is possible, with careful techniques, to measure the line-width of the OI lines and so obtain a direct measure of the temperature of the atmosphere at the height of emission of these lines

To a classical astronomer nowadays anything below a fixed star is no doubt to be termed 'terrestrial', just as to many people any study above roof-level is termed 'astronomical' The future is seen to lie in the wedding of optical with radio techniques in the fields of astrophysics and geophysics and of geomagnetism Moreover, the vitality of the Observatory has suffered from its relative isolation The University did indeed, so Gregory said, acquire "a possession which is unequalled in any but the older universities" The means are there to train students and research workers in the old and in the new techniques of exploring space The raw material of research is to hand The Observatory began as Lockyer's hobby in his old age it has acquired a reputation in astrophysics and an affectionate regard in the south-west of England By acquiring additional eyes we shall see more and see farther An old man's hobby has become a young University's opportunity.

LONG-RANGE RADIO COMMUNICATION BY SATELLITE MICROWAVE DIPOLES

AN article under this title detailing the West Ford Project was published in *Nature* of September 23, p 1237 At the eleventh General Assembly of the International Astronomical Union held in Berkeley, California, on August 24, two resolutions were unanimously adopted concerning that Project and similar space experiments that might have harmful effects on astronomy At the recent General Assembly of the International Council of Scientific Unions in London, a resolution similar to Resolution No 1 of the International Astronomical Union was adopted, which refers to the whole of science, rather than to astronomy in particular

The following is the text of the resolutions adopted by the International Astronomical Union

Resolution No 1 *Viewing* with great concern the grave danger that some future space projects might seriously interfere with astronomical observations in the optical as well as in the radio domain,

and *believing* that a degree of contamination of space which at the present time would be hardly detectable, might, if long-lived, well be disastrous to future observations with improved techniques,

and *maintaining* that no group has the right to change the Earth's environment in any significant way without full international study and agreement,

the International Astronomical Union *gives* clear warning of the grave moral and material consequences which could stem from a disregard of the future of astronomical progress,

and *appeals* to all Governments concerned with launching space experiments which could possibly affect astronomical research to consult with the International Astronomical Union before undertaking such experiments and to refrain from launching until it is established beyond doubt that no damage will be done to astronomical research

Resolution 2 The International Astronomical Union expresses its appreciation that the plans for Project West Ford have been publicly announced well ahead of proposed launching and of the United States Government's official policy* that further launchings will be guided by the principle that such projects shall not be undertaken unless sufficient safeguards have been obtained against harmful interference with astronomical observations

Nevertheless the International Astronomical Union views with the utmost concern the possibility that the band of dipoles proposed in Project West Ford might be long-lived, and it is completely opposed to the experiment until the question of permanence is clearly settled in published scientific papers with adequate time being allowed for their study The International Astronomical Union is opposed to any experiment which might hamper future developments in astronomy

If a short lifetime for the dipoles and the harmless nature of the experiment can be assured, and if Project West Ford is carried out, the International Astronomical Union regards it as essential that the fullest observations of, and experiments on, the properties and behaviour of the band of dipoles be carried out by all possible means The observations and experiments should be performed and analysed according to the highest scientific standards and with the best equipment available, bearing in mind that signals which are barely, or not, detectable to-day will probably cause serious interference with future scientific research because of the development of more sensitive equipment

The observations and experiments to be made on West Ford are likely to be difficult to perform, and will, in many ways, be similar to those carried out by the authorities responsible for operating West Ford Moreover, much specific information such as precise and up-to-date ephemerides will be required The International Astronomical Union will attempt to arrange for rapid and full co-operation among astronomers making observations and calculations, and to provide for world-wide dissemination of their results conforming to accepted standards of scientific research

The International Astronomical Union welcomes the position[*] taken by the Government of the United States that any decision on later experiments of the West Ford type will be taken in the light of the results obtained from the presently proposed experiment To enable the International Astronomical Union to obtain the necessary data, it requests the Government of the United States to grant full privileges to a group of astronomers, acceptable both to the Government and to the Union, to co-operate with West Ford authorities in performing quantitative experiments to determine the properties of the proposed belt of dipoles, its changes with time and location, and its impact upon present and future astronomical research

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[*] Letter of August 11, 1961, from Dr J B Wiesner to Dr L V Berkner

THE SWEDISH SEED ASSOCIATION

THE title traditionally given in English translations to the Sveriges Utsadesforening does not perhaps fully indicate the scope of the scientific work in this pioneer research station

Throughout its development the Swedish Seed Association has had strong support from both farmers and scientists, and when the seventy-fifth anniversary of the Association was celebrated in July 1961, King Gustav VI Adolf welcomed a large attendance of members living within reach of each of the eight branch stations as well as growers and technicians who are directly in touch with the work at the Svalof headquarters

Founded in 1886 as a co-operative association of farmers on the initiative of Birger Welinder, who farmed at Svalof, it reflected a big increase in interest in crop-husbandry problems During the extension of the arable cropping area in the middle of the nineteenth century Swedish farmers had come to realize that the severity of the winter, the relatively long days and the short summers made demands which could not be met by even the best of the foreign varieties.

From the beginning the pioneer plant breeders at Svalof fought hard to convince the large body of farmers and seed growers of the importance of using the best scientific techniques in the improvement of crops As the programme developed under Profs Hj Nilsson, N H Nilsson-Ehle, A Åkerman and other distinguished scientists and agronomists it became clear that improved crop varieties could best be obtained through an active programme of research some of which must be of a fundamental nature

Cytogenetic Research

Practical breeding work is undertaken at Svalof on the herbage crops, oil and fibre crops, and the root crops for fodder production as well as on the four main cereals

For this breeding work material is provided mainly from three sources

(1) By the introduction of valuable genes from primitive forms or wild species, or from cultivated varieties with special characteristics One of the most marked features at Svalof is the importance attached to collaboration with crop improvement and other research stations in foreign countries Valuable plant material has been obtained from a wide range of countries either by exchange or by plant collecting expeditions

(2) By inducing mutations The Swedish workers are effectively using this technique to increase the genetic variability of the cultivated varieties and breeding material with which they are working

(3) By increasing the chromosome number through auto- and allo-polyploidy, thus offering new possibilities for the development of new genotypes Especially promising are the polyploids of red and white clover, rye and turnips (Oil rape is an important crop in Sweden. By crossing artificially induced polyploids of turnip rape and different types of kale, new high-yielding and hardy varieties of winter rape are being developed)

Research of a more fundamental nature is in progress to ascertain the causes of reduced fertility in artificial polyploids and to investigate different methods of using polyploids in breeding programmes

In the development of polyploids the work at Svalöf has benefited greatly from the close co-operation of Prof A Muntzing at the Institute of Genetics situated a few miles away in the University of Lund, and similarly with Prof Å Gustafsson on induced mutations in barley and other crops Mutations have been induced in smooth-stalk meadow grass which have brought a return to cross-fertilization as the means of reproduction in place of apomixis

In this way the range of genetic variation has been greatly increased

The nature of yield in some species is being investigated genetically and physiologically with the aid of special growth chambers

Biochemical Investigations

Great importance is attached to the breeding of new varieties of improved quality and in this the chemical laboratory is closely concerned

More fundamental studies are concerned with the enzyme changes associated with the ripening of seeds In a country extending as far north as northern Iceland, and as far south as Northumberland, the ripening of grain and other seed crops is of special importance

Cereal Breeding

The breeding of spring oats at Svalof has been of inestimable value to Britain

Victory, Eagle, Star, Sun II and Blenda all came from Svalof, and these represent some of the best-known varieties grown in Britain during the past fifty years (A peculiar rhythm of growth is needed in oat varieties grown around Lake Malaren, where severe drought conditions are likely to occur in spring, and the most successful varieties are those in which the shooting of the tillers is delayed for a period)

Barley varieties in Sweden cover a wide range of climatic conditions, and Svalof has been successful in breeding early six-row barleys for middle and northern regions as well as the varieties for southern Sweden, of which Ymer and Freja have been successful in Scotland and England

Barley has been used as a convenient subject for experiment at Svalöf to find how the mutation process may be directed towards the production of desirable mutants Much is being learnt by the association of cytogenetical analysis of the mutations with a close observation of field characters in barley

Herbage Crops

The ecotype concept was largely developed in Sweden, and it is not surprising that special attention has been given to the interesting range of regional types of red clover growing between southern Sweden and the northern limits of the crop Day-length reactions are being studied in relation to growth rhythm, time of flowering and to the stage at which reserve nutrients are stored Of notable success is the work on resistance to stem nematodes in red clover

The Filial or Branch Stations

Most of the new varieties introduced in recent years have been the result of collaboration between the main station at Svalof and one or more of the eight 'filials' or branch stations At Ultuna (Uppsala), for example, some of the main work on red clover, lucerne and peas has been undertaken Vegetable crops, if one excepts the important pulse crop of dried cooking peas, are not at present included in the programme of work of the Association

Seed Certification and Distribution

To relieve the Swedish Seed Association of the task of multiplying and distributing seed of the Svalof varieties, the General Swedish Seed Co was established in 1891 In Sweden, the seed trade is organized differently from Britain, and the Seed Co at Svalof finds it necessary to produce large tonnages of seed This is done partly on the farms owned by the Company

The Swedish Seed Association co-operates closely with the Central Government Seed Testing Station in the production of State certified seed, and all the seed which leaves Svalof is of certified grade

From the beginning the Swedish Government has given monetary support to the plant-breeding work, and this at present amounts to about £135,000 a year Other sources of income are the profits from the General Swedish Seed Co at Svalof, research grants and donations from foundations and private organizations, and fees for analyses made in the laboratories.

Links with Britain

By the year 1919 Svalof was already well established, and it was natural that representatives of the National Institute of Agricultural Botany, founded in January 1919, should visit Svalof to take advantage of the unique experiences gained there in crop improvement work In the party representing the Council of the National Institute of Agricultural Botany was Prof R G Stapledon, then newly appointed to Aberystwyth The value of the ecological approach to variety testing, and to the collection and use of local varieties and ecotypes developed in Sweden was particularly appreciated by the visitors from Britain The ecotype concept was to be further developed by Stapledon at the Welsh Plant Breeding Station, and by J W Gregor in Scotland

Exchange visits between Svalöf and the crop improvement stations in Britain have been numerous in recent years during the directorship of Prof E Åkerberg, and they have undoubtedly been of great benefit to both countries

All workers in crop improvement, whether agricultural botanists, plant breeders, farmers or seedsmen, will join in congratulating the Swedish Seed Association on the remarkable achievements of the past seventy-five years and in sending good wishes for the future

F R HORNE

OBITUARY

Prof. Eric Bradshaw, M.B.E.

PROF ERIC BRADSHAW died suddenly at his home in Marple, Cheshire, on August 15 He was aged fifty-two and was professor of electrical engineering and director of the Electrical Engineering Laboratories in the Faculty of Technology of the University of Manchester at the Manchester College of Science and Technology

Eric Bradshaw was the son of Lady Bradshaw and the late Sir William Bradshaw, and was educated at King's School, Grantham, and in the Department in which he later became professor He was a student under Prof Miles Walker and graduated in the University of Manchester with a degree of B Sc (Tech) with honours in 1930, and with the degree of M Sc (Tech) in the subsequent year Following his graduation he spent some time with the British Thomson-Houston Company at Rugby

He became a lecturer and afterwards special lecturer in high-voltage engineering at the Royal Technical College, Glasgow, during 1933–44 While in Glasgow he was responsible for the inception there of the high-voltage laboratory and gained his Ph D at the University of Glasgow In 1944 Eric Bradshaw moved to his old Department in the College of Technology, Manchester, as senior lecturer in high-voltage engineering, he was appointed professor of electrical engineering in October 1952

After the War he was a pioneer in the organization of courses at a very high level for the senior engineering staff of the newly nationalized electricity supply industry and was responsible for many such courses at Electricity Hall, Buxton, during the years 1947–52 Most of these courses, changing their content from time to time but retaining Bradshaw's form of organization, eventually became a standard part of the educational activities of the Central Electricity Authority (later the Central Electricity Generating Board) and the Area Boards During the same period he founded, in 1948, the *Bulletin of Electrical Engineering Education*, and continued thereafter as organizer and editor until his untimely death This journal to-day circulates to between four and five hundred universities, colleges and other subscribers throughout the world

Bradshaw's published work and interests lay in four categories In the field of units, symbols and nomenclature, he was one of the authors of a well-known symposium of papers to the Institution of Electrical Engineers on the M K S system of units, was the author of a book on the same subject, and served on British standards and international electrotechnical committees During his career at Glasgow and his early years at Manchester he published work on high-voltage measurements using oscillating electrode systems Immediately afterwards his attention turned to problems of the power supply industry, and he was responsible for several devices for the direct measurement of rate of change of frequency, one of which was published by the Institution of Electrical Engineers His latest interests lay in the field of electric railway traction He was responsible for the design and building at Manchester of a track performance calculator for British Railways, the details of which were published in a paper for the Institution of Electrical Engineers, latterly this work had extended to analogue devices for determining short-time thermal ratings of traction equipment

He was known throughout the length and breadth of Britain for his work in furtherance of higher education, many were the advisory committees, colleges and universities to which he gave his time Many indeed, furthermore, are the individuals, both within and without his profession, who have reason to be grateful for his kindness of heart Eric Bradshaw was incessant in his activity on behalf of cases of adversity and hardship which came his way, this particularly, but in no way exclusively, applied to any student who through no fault of his own was likely to be incapable of pursuing his studies

Eric Bradshaw was a keen amateur musician, played with the orchestra organized from time to time in the College of Science and Technology, and generously gave his time and support to musical events in the College, particularly those centred around the organ Perhaps his greatest lay interest was in the history of the Industrial Revolution He was intensely interested in the development of railways and canals, and frequently lectured to societies and schools on these subjects He married Joyce Ena Smith in 1935, by whom he is survived

C Adamson

NEWS and VIEWS

The Norman Lockyer Observatory

Mr D R Barber

Mr D R. Barber retired on September 30 from his post as superintendent of the Norman Lockyer Observatory at Sidmouth, after twenty-five years service (see also p 108 of this issue) He was appointed as assistant to the late W J S Lockyer in September 1936 and on Lockyer's death in December continued with the new director, D L Edwards, until 1956, when Edwards died, Barber then became superintendent Edwards and Barber introduced precise methods of photographic photometry into the evaluation of stellar spectra From 1938 they applied the Greenwich colour temperature technique in a modified form to the measurement of spectrophotometric gradients and their work on the changes in the continuous spectrum of γ-Cassiopeiæ (*N L O Comm*, 58, 62, 64) is well known About this time Barber built the first of a series of night-sky photometers, designed to measure the green, OI, line of the air-glow This instrument was taken to the Lick Observatory, Mount Hamilton, on Barber's appointment to a Martin–Kellogg fellowship in the University of California (1940–41) With this he demonstrated for the first time a definite correlation between the intensity of the green line and geomagnetic activity (*Nature*, 148, 88 (1941))

During 1941–45, Barber worked at the Kodak Research Laboratories in England, and on his return to the Observatory he became interested in the sodium *D*-line emission in the twilight and night skies, which he studied by photographic and photoelectric methods He established the presence of highly polarized twilight emission following solar flare and geomagnetic activity (*J Atmo Terrestr Phys*, 10 172, 1957), and a dependence of the intensity of emission on lunar phase, presumably because of tidal movement of the upper atmosphere at the level of sodium emission, 70–110 km (*Intern Astro Union Draft. Rep*, Berkeley Meeting, August 1961, *Rep Comm*, 21, *Luminescence du Ciel*) Lately Barber's interests have broadened to include solar–terrestrial relationships, certainly he has already obtained fascinating and remarkable results Those on the effects of living organisms will certainly keep him occupied in his retirement

John T Tate Medal of the American Institute of Physics

Dr P. Rosbaud

Dr Paul Rosbaud has been awarded the first John T Tate International Medal for distinguished service to physics The Medal is of gold and has been established in order to recognize distinguished service to physics by individuals who are neither residents nor citizens of the United States 1,000 dollars accompany the award The late Prof J T Tate, who died on May 27, 1950, was dean of the College of Science, Literature and Arts at the University of Minnesota, and served as research professor of physics, chairman of the Governing Board of the American Institute of Physics, and managing editor of the American Physical Society.

Dr Rosbaud before and during the Second World War was science editor for the Springer Company, a Berlin publishing house. As an editor, he is credited with being the driving force behind the creation of new journals and the improvement of existing periodicals, as well as being responsible for the publication of many books by scientists. Born in Graz in 1896, he received his doctorate in Berlin and then carried out research in X-ray crystallography. He joined the Springer publishing house in the 1930's and succeeded in sending its valuable scientific books to safety before the bombing of Berlin. His extraordinary courage and acquaintanceship with friends in high places, who were uncommitted to the Nazi régime, enabled him to keep in touch with scientists in occupied Europe during the War and to boost their *morale*. He had two French war prisoners, who were physicists, staying with him on *parole*, and enabled them to work and keep in touch with scientific developments. They are now prominent atomic scientists.

During the War he risked his life and freedom by communicating with Allied scientists via the Norwegian underground and neutral channels and by being the confidant of German physicists who opposed the decline of science under the Nazis. After the War, he became editor for Butterworth Scientific Publications (London), and later served as scientific director of the Pergamon Press. Since 1956 he has been European editor for Interscience Publishers and a consultant to other publishing companies.

Council for Scientific and Industrial Research New Appointments

Five new members of the Council for Scientific and Industrial Research were recently appointed, and took office on October 1. They are Mr. L H Bedford, director of engineering, Guided Weapons Division, English Electric Aviation, Ltd, Mr G B R Feilden, managing director, Hawker Siddeley Brush Turbines, Ltd, Prof E R H Jones, Waynflete professor of chemistry, University of Oxford, Prof O A Saunders, professor of mechanical engineering, Imperial College of Science and Technology, London, and Mr H C. Tett, chairman and managing director, Esso Petroleum Co, Ltd. Three members who have completed their five-year term of office retired on September 30: the former chairman, Sir Harry Jephcott, chairman of Glaxo Laboratories, Ltd, Prof C E H. Bawn, professor of inorganic and physical chemistry, University of Liverpool, and Sir Willis Jackson, at present director of research and education, Associated Electrical Industries (Manchester), Ltd. A fourth member, Sir Walter Drummond, who was also due to retire on September 30, has been re-appointed for a period of three months. The present constitution of the Research Council is: *Chairman*, Sir Harold Roxbee Cox, *Members*, L H Bedford, Prof B Bleaney, Prof C F Carter, Dr J W Cook, Frank Cousins, Sir Walter Drummond, G B R Feilden, Prof E R H Jones, Vice-Admiral Sir Frank Mason, Prof. O. A Saunders, Dr C J Smithells, H C Tett, Lewis T Wright, *Secretary*, Sir Harry Melville.

Ergonomics in Industry

Delegates from industry, the trade unions, universities, research associations and Government departments attended the three-day Conference on Ergonomics organized by the Department of Scientific and Industrial Research in London during September 1960. The Proceedings of the Conference have now been published and include all papers and a verbatim report of the delegates' discussions (*Proceedings of Conference on Ergonomics in Industry*, 27–29 *September*, 1960, published for the Department of Scientific and Industrial Research by H M S O. Price 12*s* 6*d*. By post, 13*s* 4*d*.) Ergonomics, the scientific approach to the problem of fitting the job to the worker, is aimed at improving industrial efficiency by designing equipment so that its operation is within the mental and physical capacities of the average person. The purpose of the conference was to bring ergonomics to the notice of senior people in industry and to provide an opportunity to discuss its wider implications, in particular where ergonomics fits in with the interests of production engineering, equipment design, work study and industrial health.

Mental Health Book Review Index

The *Mental Health Book Review Index*, which is sponsored by the World Federation for Mental Health, the International Council of Psychologists, the American Foundation for Mental Hygiene, and the Research Centre for Mental Health, New York University, lists references to signed book reviews appearing in 150 journals in the English language, of which 133 are still current and publish reviews, regularly or occasionally. The indexing begins with the volumes current in January 1955. Books are listed with references to three or more reviews when they appear in psychological, psychiatric, or psychoanalytical journals, when the journals represent at least three fields in the behavioural sciences as a whole, when at least three of the journals originate outside the United States, or when the books reviewed are in foreign languages. The bibliography of books in the sciences of human behaviour and the field of mental health which results from this compilation of book reviews is intended for information only, and neither inclusion nor omission of a title implies a definitive selection. In addition to the 2,062 titles listed since the first issue of the *Index*, about 6,900 titles of books with at least one review are on file until they can be listed under these criteria. The *Index* is published annually under the auspices of the American Foundation for Mental Hygiene, the most recent (6, No 11, 1961) being published in July.

Diet of Sheep

Sheep are highly selective in their grazing habits, picking and choosing between the different plants available in a mixed sward, and even between stem and leaf on individual plants. It is only during the past few years that the extent and importance of such sheep preferences in influencing both the diet of the animal and the botanical composition of the pasture have been appreciated. G W Arnold, of the Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, has recently described preliminary results of research on the grazing behaviour of merino sheep on the southern tablelands of New South Wales (*Rural Research in the Commonwealth Scientific and Industrial Research Organization*, 36, June 1961). In one experiment, a seeds mixture of lucerne and *Phalaris tuberosa* was sown in rows and grazed continuously by three-year-old merino wethers. The sheep selected leaf in preference to stem where this was physically possible. Furthermore, they invariably chose the leaf or stem material containing most nitrogen. In another experiment, a direct relationship was found between the amount of pasture available and the time spent by sheep in grazing. In spite of a progressive increase in the time spent, the sheep were unable to maintain their live-weights as grazing became more time-consuming. The animals grazing longest suffered the least loss of live-weight. This suggests that other sheep stopped eating because of fatigue before their appetites were fully satisfied. Such differences between individual sheep in their capacity to graze for longer periods on poorer pastures could have practical implications, particularly if the character should prove to be heritable.

Geological and Geographical Research in the U.S.S.R.

A review of geochronology research in the Soviet Union is given by D. I. Shcherbakov (*Priroda*, 2, 9, 1961). The scale appended gives, for example (in million years), Upper Cretaceous 100, Carboniferous 320, Cambrian 570. The oldest Pre-Cambrian 3,400–3,500. A. I. Perel'man (*Priroda*, 1, 41, 1961) is continuing his discussion of geochemical landscapes by applying geochemical studies to the previous periods of the Earth's history, thus contributing to a new science, named by A. A. Saukov "historical geochemistry". R. V. Teis (*Priroda*, 4, 31, 1961) provides a short summary of the work on oxygen isotopes in the Soviet Union, especially of the isotope palæothermometry as applied to the calculation of the temperature of ancient seas as determined by the date derived from sedimentary rocks of the Russian platform and those of the Crimea. Isotope methods were also used to determine the geochemical history of atmospheric oxygen especially during the process of photosynthesis. The Lake Balkhash, in spite of being situated in a semi-desert zone and prone to evaporation, has a very low salinity, namely, 2.84 gm./l. According to M. N. Tarasov (*Priroda*, 2, 72, 1961) this may be due to the infiltration or seepage of the Lake waters into the fringing rocks surrounding the numerous bays of this Lake. Other suggestions and hypotheses are also discussed.

Angara–Yenisei Hydroelectric Schemes

A short review of these schemes has recently been prepared by P. M. Dmitrievsky (*Priroda*, 1, 25, 1961). Both the Rivers Angara and Yenisei will have 'cascades' of six dams, providing power for hydro-electric stations. The largest of these, situated at Bratsk, on the River Angara, was begun in 1955. Its head is more than 100 m., power, 4.5 million kW., and annual output, 21.7 milliards kW./hr. The annual output of the Angara set will be 70 milliards kW./hr., and of the Yenisei scheme 120 milliards kW./hr. Completion of some units is expected by 1965. The area served by these two schemes is rich in coal, iron ore, aluminium ore and salt.

High-Temperature Induction Furnaces

The design of laboratory high-temperature induction furnaces has been examined in both theoretical and practical aspects by P. H. Dundas, of the Carbon Research Laboratory, Chemical Engineering Department, Imperial College of Science and Technology, London. Dundas has attempted to simplify an otherwise complex electrical engineering analysis, so that the research physicist can avoid wasting much time and energy over trial and error methods when faced with problems of high temperature that can be satisfied only by the use of induction heating. An article in *Research Applied in Industry*, published by Butterworths, 88 Kingsway, London, W.C.2, provides a new approach to the use of induction heating as an important high-temperature tool (14, No. 7, July 1961).

Histoplasmosis

The discovery of benign histoplasmosis in 1945 spurred interest in a disease that had been considered rare and invariably fatal. During the ensuing years, a great deal of basic information has been gathered on the epidemiology of histoplasmosis. Most of this knowledge has been gained through the development and use of immunological tests, case history studies, and the screening of soil samples and animals for the presence of *Histoplasma capsulatum*. In a review of existing information (*Mycopathologia et Mycologia Applicata*, 14, August 1961), Dr. Libero Ajello, of the Communicable Disease Centre, Mycology Unit, Public Health Service, U.S. Department of Health, Education and Welfare, describes how the culture of internal organs of domestic and wild animals has revealed the presence of *H. capsulatum* in different parts of North America, Central America, South America and Africa. It shows also that this fungus apparently cannot multiply in internal organs of birds, the body temperature of which is higher than 40° C. Epidemiological investigations almost invariably revealed that *H. capsulatum* was present in the soil of the region where epidemics occurred. Sometimes the soil containing *H. capsulatum* was transferred by human activities from infected localities through non-infected air, lessening the possibility that the spores of the fungus have been transported by air from the central endemic areas (Ohio–Mississippi–Missouri river valley) to localities far removed from these regions. In the same journal, Henry C. Sweany considers the diagnosis of histoplasmosis and shows that the disease has such a clinical variability that it may simulate not only tuberculosis, sarcoidosis, lymphomas and certain severe anæmias, but it may also occur together with any one of them, which makes the diagnosis all the more difficult. In 25 per cent of the cases studied, it was associated with tuberculosis.

The Australian Mathematical Society

The Australian Mathematical Society met in Brisbane, at the University of Queensland, during May 24–26, and held a joint session with the Australian and New Zealand Association for the Advancement of Science on May 29, 1961. Sixty members attended. The new Council elected was as follows: *President*, Prof. T. G. Room; *Vice-Presidents*, Prof. A. L. Blakers and Prof. L. S. Goddard; *Publications Secretary*, Dr. G. E. Wall; *Treasurer*, Prof. C. S. Davis; *General Secretary*, Prof. H. O. Lancaster. A successful summer research institute was held at the Australian National University, Canberra, during January and February 1961, with Prof. T. M. Cherry as director. A second institute meeting will be held in Canberra, in January 1962, with Prof. E. S. Barnes as director.

Mathematical Reprints

The reprints of the Chelsea Publishing Co., 50 East Fordham Road, New York, 58, have made available a large number of scarce or unobtainable mathematical classics The most recent batch illustrates the wide variety which has been selected Salmon's *Higher Plane Curves* (4 95 dollars) and Bell's revision of Frost's *Curve Tracing* (3 50 dollars) are museum pieces, no longer recommended reading but held in warm affection by all who know them *Projective Geometry* (4 95 dollars) is a translation of the second part of the 1935 book by Schreier and Sperner, the first part having already been issued in translation by the Chelsea Co , under the title of *Modern Algebra and Matrix Theory*, this completes the version of a work which, if now a little out of date, is still recommended by the experts as a suitable introductory university text on algebra and algebraic geometry Then an omnibus volume (6 dollars) contains two monographs by Weyl, *Das Kontinuum* and *Mathematische Analyse des Raumproblems*, Landau's tract (1916, second edition 1929) on function theory, which inspired so many undergraduates in the 'twenties, and the famous Riemann dissertation, on the foundations of geometry, with Weyl's notes The books are well produced and stoutly bound, and many libraries, private and public, may be glad of the opportunity to fill gaps reasonably cheaply

The International Society for Stereology

The International Society for Stereology was established during May 1961 Stereology is the science of three-dimensional interpretation of flat images, such as sections and projections, that is, the extrapolation from two- to three-dimensional space The methods range from the evaluation of shadows through serial sections to statistico-geometrical analysis Stereology finds applications in microscopical anatomy, electron microscopy, geology, metallurgy, aerial mapping, astronomy and cosmology The Society is holding its first congress at Key Biscayne, Florida, during April 24–26, 1962 Further information concerning the Society and the congress can be obtained from Dr Hans Elias, Department of Anatomy, Chicago Medical School, 710 South Wolcott Avenue, Chicago 12, Illinois

Postgraduate Courses at the Imperial College of Science and Technology

Information contained in a booklet (*Shorter Postgraduate Courses*) issued by the Imperial College of Science and Technology relates to shorter postgraduate courses to be held at the College during the autumn term of the session 1961–62, and advance information on those to be held during the spring and summer terms The courses included comprise not only those specially arranged but also a selection of lecture courses forming part of the normal departmental syllabuses, where these are likely to be of interest to students as separate courses and where it is possible to accept part-time students The booklet is divided into two sections covering part-time courses and short full-time courses Further information on all these courses can be obtained from the Registrar, Imperial College of Science and Technology, Prince Consort Road, London, S W 7 Details of full-time postgraduate courses extending over one year are given in a separate booklet, *Postgraduate Courses*, which is obtainable from the Registrar, price 2*s*

B.B C Research Scholarships

Each year the B B C awards research scholarships (valued at £500 per annum) to university graduates in electrical engineering or physics, to enable them to work at any university in the United Kingdom for a higher degree in those fields of telecommunications or physics which have an application to sound or television broadcasting This year, three scholarships have been awarded to Mr J Elliott (1 year), of the Imperial College of Science and Technology, London, who has been working under Prof C Cherry and Mr J Roberts, to Mr K L Hughes (2 years), of the University of Birmingham, for work under Prof D G Tucker and Prof J T Allanson at Birmingham, to Mr B J Tilley (2 years), of the University College of Swansea, who will work at Swansea under Prof W Fishwick and Mr J MacCormack

University News: Southampton

The title of reader in botany has been conferred on Dr J M Lambert

Loughborough College of Technology

Dr G F Reynolds has been appointed reader in analytical chemistry at Loughborough Previously, Dr Reynolds was a principal scientific officer in the Chemical Inspectorate at the War Office

Announcements

Dr Sigvard Eklund, of Sweden, has been appointed director-general of the International Atomic Energy Agency, in succession to Mr Sterling Cole, whose term of office expires on November 30

Sir Willis Jackson has been elected president of the British Association for Commercial and Industrial Education

Stafford Beer, founder of the Department of Operational Research and Cybernetics, United Steel, has now become managing director of SIGMA (Science in General Management), Ltd SIGMA was recently set up to offer professional services in operational research to industry and Government Roger Eddison is now director of operations of SIGMA Previously Mr Eddison was manager of the Plans and Methods Department in NAAFI

Mr C T Melling, a deputy chairman of the Electricity Council, has been appointed a member of the Advisory Council on Research and Development of the Ministry of Power, in succession to Sir Josiah Eccles

Mr L H Welch, recently chief electrical engineer to the London Electricity Board, has been appointed as industrial relations consultant to the British Electrical and Allied Industries Research Association

Sir Cyril Hinshelwood, past president of the Royal Society, will deliver the 1961 Eddington Memorial Lecture in Cambridge at the Arts School Lecture Theatre, Bene't Street, on November 10, at 5 p m The title of the lecture is "The Vision of Nature"

The Natural Rubber Producers' Research Association is holding a symposium under the general title, "Fundamental and Practical Approaches to Ageing Protection", at its laboratories at Welwyn Garden City during November 2–3 Attendance will be by invitation only Further information can be obtained from the Natural Rubber Bureau, 19 Buckingham Street, Adelphi, London, W C 2

The Association of Universities of the British Commonwealth has issued invitations to its member universities to appoint delegates to the ninth quinquennial Congress of Universities of the Commonwealth, which will be held at the University of London during July 15–19, 1963, and which will mark the jubilee of the founding of the Association

COAL SCIENCE

THE biennial International Conferences on Coal Science, which began in The Netherlands in 1955, moved on the most recent occasion to France: the fourth Conference was held at Le Touquet during May 30–June 2. Tradition was followed in the extent of local co-operation, and the Mayor, Dr. Jules Pouget, received delegates and their wives at the Town Hall. A tribute should be paid, for the excellent organization and remarkable hospitality, to the French sponsors, the Centre d'Études et Recherches des Charbonnages de France, particularly to Prof. M. Letort (a director-general of the Centre), who presided, R. Loison, the French member of the International Committee, and A. Ploix, who undertook all the details of organization. The conference was fortunate in gaining Mr. Berkx's experience from the previous meeting which he organized in The Netherlands.

Interest continues to increase; the recent Conference included a delegation from the U.S.S.R., and Spain, Brazil and Czechoslovakia were represented. On the other hand, delegates from India and East Germany, who attended in 1959, were absent. The papers presented originated from ten countries. Titles of more than one hundred were submitted, and this number was only reduced with some difficulty to about 50 by narrowing the field—to papers of direct relevance to the chemistry, physics and carbonization of the coal substance.

Since authors are submitting their papers to journals of their choice and proceedings of the Conference will not be published, the 53 papers presented will all be mentioned briefly, grouped under the three main headings used in the sessions. An independent summary by A. Ladam in the French language will be found in a late-1961 issue of *Chimie et Industrie*. A numbered list of references so far available has been appended.

Chemical Properties

P. H. Given[1] reviewed his proposed molecular model for coals in the light of more recent work on dehydrogenation, nuclear magnetic resonance, and other evidence; he concluded that the general features of the model had been confirmed, but indicated changes in structural detail that appeared desirable.

H. A. G. Chermin attempted to deduce from measured densities and elementary analyses the mean size and proportion of aromatic units in coals, and directed special attention to the accuracy possible. Corrections to previous attempts were made and quite good agreement with X-ray measurements of mean size was found.

H. S. Rao, P. L. Gupta, F. Kaiser and A. Lahiri considered the assignment of the band at 1,600 cm $^{-1}$ in the spectra of coals. This band could have one or all of several origins, and the results of reducing the coals with lithium aluminium hydride, since this did not cause a change in intensity in the band, were considered to discount assignment to a carbonyl vibration. On a related subject was an investigation, by L. Czerski and L. Czuchajowski, of the same spectral band in polycondensates of *o*-, *m*- and *p*-dihydroxybenzenes with formaldehyde. When the *para*-derivatives were oxidized with ferric sulphate the 1,600 cm $^{-1}$ band increased in intensity, possibly owing to formation of hydroxyl/carbonyl chelate structures, supporting earlier assignments of this band in coals to such structures.

M. T. Monaghan and K. Littlewood attempted to use trimethyl-acetoamminohydrazide hydrochloride (Girard's reagent *T*) to extract carbonyl-containing substances from coals as water-soluble derivatives (see p. 128 of this issue of *Nature*). M. Weclewska described the effect on eight coals of exposing them to a variety of organic vapours. Conclusions were drawn as to the variation with rank of free and hydrogen-bonded hydroxyl groups. P. H. Given, M. E. Peover and W. F. Wyss had examined the chemical reactions and properties of some petrological constituents of coals. Moderate differences in hydroxyl content and reducibility were found, the general molecular pattern being similar for all the macerals studied. B. K. Mazundar, S. Ganguly, N. Gopal De and A. Lahiri had investigated the effect of phosphoric and sulphuric acids on coal structure; they found that a constant proportion of the total carbon in the coal (92 per cent) was retained in the chars produced when the products were carbonized. Since extensive dehydrogenation did not result, an apparent condensation between the alicyclic structures and the remainder of the molecule was postulated to explain the inhibition of tar formation caused by the treatment. J. D. Brooks and T. M. Spotswood[2] discussed the reaction of carbonized coals with bromine and with sodium in various forms; the results were consistent with the presence of polycyclic hydrocarbon structures.

Several papers were concerned with oxidation or oxidation products. H. E. J. Brusset compared humic acids extracted from low-rank coals with those produced synthetically from polyphenols by oxidation; he adduced support for an analogy between these classes of material. Oxidation of coal in alkaline medium at 260° C. was described by J. E. Germain, J. Guillon and P. Delattre. The high yields of benzene carboxylic acids obtained were extracted with methyl ethyl ketone and subjected to chromatography. Fractions were characterized according to the number of carboxyl groups per benzene ring; increase in rank of coal was accompanied by a decrease in the mean number of groups per ring (about 4) and an increase in total yield of these acids. G. J. Lawson and S. G. Ward reported a further examination of the resolution of sub-humic acids (secondary oxidation products of coal) partly by means of ion-exchange resins. Several known acids were isolated, including some previously reported (for example, glycollic and mellitic acids) and two aliphatic acids containing nitrogen (nitrilotriacetic acid and oxalylglycine). B. Juttner[3] described the oxidation of coke with nitric acid followed by sodium hypochlorite and extraction with butanone. The yield of mellitic acid increased sharply between 600° and 700° C. carbonization temperature, and then fell slightly. A related topic was a description of the use of ozonolysis in research on coal tar, by P. G. Copeland. Oxidized pitch fractions and model substances were further decomposed with alkaline peroxide and/or permanganate in pyridine and the products were identified. Mechanisms were established from the work with models, but the products from coal tar pitch had not yet been identified

Reaction of coals with alkaline solution at 200°–300° C was found, by M Ihnatowicz[4], in many cases to increase greatly the agglutinating values observed when the products were later heated A strong hydrogenating action accompanying hydrolysis was indicated

Possibly the most novel work described was that of L A Herédy and M B Neuworth on a depolymerization of bituminous coal at only 100° C The coal was allowed to react with a labelled phenol–boron trifluoride system, depolymerization was recognized by substantially increased solubility in phenol Models were used to elucidate the reactions taking place With coal, cleavage of aromatic-aliphatic linkages and also interchange of aromatic units with phenol were both involved Some of the structural units were thus characterized in terms of analysis and molecular weight, and evidence of bridges other than *via* methylene groups was obtained, possible model structures for coal that would fit the evidence obtained were discussed E E Donath adduced the presence of polynuclear aromatic units in pitches from coal hydrogenation from a study of the ease of change in hydrogen content

Two papers considered processes that may occur during the formation of coals G Huck, J Karweil and K Patteisky[5] showed in an experimental study that only dynamic pressure is likely to accelerate increase in rank, and that spores are unsuitable as indicators of the temperature of formation I A Breger considered the 'coalification' of wood in uranium-bearing sandstones It seems that nuclear radiation has caused dehydrogenation and condensation, leading to effects similar to those produced by biochemical coalification

P Dath, H Renson and E H Grand'ry reported work on the influence of rank and petrological composition on the behaviour of coals in the Audibert-Arnu dilatometer and came to the conclusion that both are important A Ihnatowicz, M Lason and M Zyla had shown by treatment of coal with alcoholic potash and with pyridine, together with hydroxyl determinations and adsorption measurements, that the two treating agents used altered these properties in a similar manner Evidence that this was due to a mechanical loosening of the structure was obtained J D Brooks, J F Stephens and H Silberman[6] had examined the X-ray diffraction patterns and functional groups in chars from carbonized salts of brown coal The 002 band was shifted, and it was suggested that in brown coals this band may possibly arise from an interchain *d*-spacing Phenolic groups in the salt form were less-easily eliminated by heating than in the untreated coals B Roga surveyed Polish and international classifications of coals and suggested modifications

Physical Properties

S Ergun and M Mentser carried out investigations on the X-ray scattering patterns of ten anthracites and *meta*-anthracites Those of higher rank gave rise to three-dimensional reflexions, indicating that coal can graphitize during metamorphism Nuclear magnetic resonance techniques were applied by J Smidt, W van Raaijen and D W van Krevelen to show that the mobility of the protons in coals changes with rank, and that it shows a characteristic variation with temperature for the coking vitrinites The effect, on earlier published conclusions, of this variation was discussed. Pitches were also studied M Jacubowicz and J Uebersfeld described results obtained with several coals when studied by double magnetic resonance The nature of the interactions (dipolar, etc) between the electronic and the nuclear magnetic moments was determined, and a better understanding was achieved of the electron spin resonance ascribed to free radicals Some possibilities for studying adsorption were also mentioned

Two papers were concerned with the specific surface and heat of wetting of coals and carbonized coals S Prégermain, S Durif and P Chiche studied the adsorption of water, methanol and nitrogen on carbonized coals, and their low-angle X-ray scattering *Inter alia*, a relation was found between the ease of graphitization and the development of the internal surface L Robert and S Prégermain found a quantitative relation between heat of wetting in methanol, per unit surface of the carbonaceous solid, and its oxygen content, extrapolated to zero oxygen content the heat evolved is still considerable, and the chief effect, therefore, is to raise the estimated surface areas of high-rank coals and chars while leaving those of low-rank coals little changed J W Phillips reported on the self-adhesion of coal during briquetting without a binder The physical properties, and the structure revealed by photomicrographs, of the briquettes were linked with the stress and strain relations during the application of pressure with additional shear strain D H Everett, E Redman, A J Miles and D H Davies reported adsorption isotherms on chars made from polyvinylidene chloride Important differences in hysteresis, relevant to the mechanism of coal carbonization, were discussed

Reflectances of vitrinites carbonized at a series of pressures were reported by M -Th Mackowsky An attempt was made to relate the changes found with the chemical changes known to occur during heating L A Gilbert had determined refractive indices and absorption coefficients of polished surfaces of coals, in the ultra-violet region, by a polarized light method This was the first application of this method to coals and yielded valuable results All the vitrains used showed a strong aromatic-type absorption around 2600 Å S Ergun and J T McCartney[7] reported the refractive indices of ultra-thin sections of coals and graphite in the visible region and extinction coefficients in the ultra-violet and visible regions The results were tentatively considered to lead to conclusions regarding the aromatic units in coals similar to those derived from X-ray investigations

E de Ruiter, R Leutner and H Tschamler reported the electrical resistance of powdered coals under pressure and of their products of extraction by pyridine in relation to temperature Little change in the energy of electronic activation for coals was observed between 80 and 90 per cent carbon content, values of this energy were similar for the residues of extraction of solvent, but greater for the soluble products by extraction The results for the extracts were considered to accord with conclusions from X-ray diffraction

Thermal Decomposition

No less than four papers were concerned with the plastic softening of coking coals in the early stages of heating Rheological properties during the initial softening had been studied by H R Brown and P L Waters[8] They found that the temperatures of initial softening of a wide range of Australian coals were almost independent of rank within the range of 82–89 per cent carbon content, and that the softening was initially reversible, but maximum fluidity and solidification temperature were dependent on rank A medium-rank coal behaved as though the flow were Newtonian until the onset of active decomposition A mean activation energy of about 70 kcal /mole was estimated J C Macrae and K S Murthi[9] had determined, in a special cone viscometer, the variations of fluidity at a constant temperature below the active decomposition point, they interpreted these in relation to the microscopically observed porosity of the char after cooling and the amount of chloroform extract obtainable from this char The duration and magnitude of fluidity were found to be highly dependent on temperature, and the increasing fluidity before the maximum to be directly related to the dilation of the mass with gas bubbles, the amount of chloroform-soluble material present did not change materially over the same period and therefore was considered unlikely to produce this increase

I G C Dryden and W K Joy[10] had investigated the provenance of the fusible chloroform-soluble material in heated coals, by comparing similar materials obtained in three independent ways They concluded that this fusible material is partly present in the unheated coal and partly formed or disengaged during heating, the proportion originally present increasing with increase of rank up to 100 per cent in prime coking coals Evidence was also presented leading to the conclusion that the portion originally present is also more resistant than the remainder to thermal decomposition The plasticity developed was considered, on limited evidence, to depend critically on the concentration of fusible material—not entirely in agreement with the conclusions of Macrae and Murthi A D Dainton demonstrated by static beam-bending methods that visco-elastic behaviour persisted in the semi-coke from a coking coal above the commonly accepted point of solidification for the plastic coal, that is between 480° and 800° C

Development of the fissures in coke that result from shrinkage, following resolidification of the plastic mass, was studied by H E Blayden, H C Wilkinson and G E Oatley in relation to the temperature distribution in an experimental coke oven A clear correlation between shrinkage and fissuring was observed, and these authors also concluded that semi-cokes may show visco-elastic behaviour G H Taylor[11] reported the appearance in both reflected and transmitted light of coals at various stages in carbonization Colour photomicrography was an important feature of the techniques used Development of an optical mosaic structure from the plastic vitrinite was observed The behaviour suggested that the resolidification of coke is analogous to a crystallization process H Pichler and D E Lee[12] reported the effects of extracting a bituminous coal for various times with tetrahydrofuran The consequent changes in plastic behaviour, dilatometer curves, volatile matter and other characteristics were described W F Landers and J E Goodman had investigated the effects of temperature and gas pressure on the tar obtained from a lignite Increased susceptibility to decomposition of the tar liberated between 500° and 600° C was evident, and this was further increased by pressure

S Černič reported an investigation, with the help of labelled pyrite, of the behaviour of organic and inorganic sulphur-containing constituents of a series of coals during carbonization Evidence was obtained that inorganic and organic forms of sulphur were interchanged to some extent, according to the rank of coal, during the thermal decomposition The effectiveness of carbonizing in hydrogen, ammonia and pre-heated steam, in reducing the sulphur content of the resulting coke or char, was also investigated, steam proved the best

Heats of coking formed the subject of three papers, the definition used must be considered when making comparisons D Fitzgerald reported coking heats for an anthracite and several low-rank coals An isothermal fluid bed calorimeter was used for the measurements The poor agreement between results of other work was critically discussed C Meltzheim, J Lahouste and A Boyer had made a direct comparison of two methods of measuring the heats of carbonization, and found them in satisfactory agreement The net heat of carbonization over the whole range of temperature, for a number of coals, was always exothermic, the value varying with rank in a complex manner in the region of 30–170 (± 40) cal /gm C Kroger and J J. Das[13] had investigated the net heats of carbonization of individual macerals by direct calorimetry The heats were not directly related to the yields of volatile products A relation between the decomposition heat up to 500° C and the hydrogen/oxygen ratio and the carbonyl content of the coals investigated was, however, found At 1,000° C vitrinite appeared to give the lowest heat values of the three macerals studied

The rate of evolution of volatile matter from coal, during carbonization in a fluid bed with sand as heating medium, was interpreted by G J Pitt on the assumption that the coal is a complex mixture, each component of which decomposes independently by a first-order reaction He showed that this assumption can lead to the linear change with logarithm of time observed, and with this assumption deduced from the results a distribution of activation energies (35–75 kcal /mole, maximum at 50–55 kcal /mole)

A continued investigation of the pyrolysis of model compounds believed to resemble coal was reported by D W van Krevelen and H W den Hartog[14] Polycondensates in which aromatic and non-aromatic structures (or oxygen atoms) alternated, prepared with labelled formaldehyde, were pyrolysed, from the weight, and analysis of and distribution of radioactivity in the products, conclusions were drawn about the influence of structure on the degree of (temperature) overlap in tar and gas evolution and on the weight loss–temperature curves, and it was concluded that C—O bonds decomposed more readily than C—C bonds

In the next group of four papers, the work described had been directed towards the establishment of relations between volatile products of the pyrolysis of coal and the chemical structure of the coal itself G Claxton described work on the genesis of aromatic hydrocarbons using gas-liquid chromatography In a coke oven, the peak evolution of non-aromatic hydrocarbons occurred earlier in the heating process than the peak for benzene formation, significant effects of the heating-rate were observed In laboratory experiments more detailed information was obtained A Ladam, R Ferrand, P Payen and A Boyer had studied the constituents of low-temperature tars (550° C) from vitrinite and exinite concentrates, using vapour phase chromatography Normal paraffins and olefines arose almost entirely from exinite, and the results suggested that as much as 10 per cent of the exinite used may consist of chains with about 30 carbon atoms, correspondingly, aromatic compounds from exinite tar were more highly alkyl-substituted than those from vitrinite tar W Waddington had compared the relative abundance of isomeric forms in tar with that to be expected from the postulated thermal reactions of a proposed structural model He suggested that the results obtained support the view that coals contain in part a 6 alkyl-4 propyl-2 methoxy phenol type of structure, this may be gradually converted to other structures by thermal/pressure reactions during coalification C Kroger and R Brucker[15] reported results from high-vacuum pyrolysis of coals and discussed these in relation to the coal structure Products in the range 300°–575° C were examined by gas chromatography and other methods Rate constants and activation energies for the evolution of hydrogen, many simple hydrocarbons and the carbon oxides were estimated, and from these in turn, by means of allocation of reactions to structural elements on certain assumptions, quantitative parameters for 17 elements of structure, such as the proportion of aromatic carbon in the coal, were deduced

There proved to be a shortage of time for formal discussion, and on the other hand many delegates remarked on the value of spontaneous extra-mural discussions Further experiment is clearly necessary in an attempt to correct this lack of balance It is hoped that, since these conferences continue to produce more novel material than the average delegate can comfortably digest, further innovations will be made at the next conference, to be held at Cheltenham during 1963

I G C DRYDEN

[1] Given, P H , *Fuel*, 40, 427 (1961)

[2] Brooks, J D , and Spotswood, T M , *Proc Fifth Bien Carb Conf U S A* (1961)

[3] Juttner, B , *Brennstoff-Chem* (in the press)

[4] Ihnatowicz, M , *Prace Glównego Instytutu Górnictwa* (in the press)

[5] Huck, G , Karweil, J , and Patteisky, K , *Brennstoff-Chem* (in the press), *Freiberger Forschungsheften, A* 229 (1961)

[6] Brooks, J D , Stephens, J F , and Silberman, H , *Proc Fifth Bien Carb Conf , U S A* (1961)

[7] Ergun, S , and McCartney, J T , *Fuel*, 40, 109 (1961)

[8] Brown, H R , and Waters, P L , *Fuel* (in the press)

[9] Macrae, J C , and Murthi, K S , *Fuel* (in the press)

[10] Dryden, I G C , and Joy, W K , *Fuel* (in the press)

[11] Taylor, G H , *Fuel* (in the press)

[12] Pichler, H , and Lee, D H , *Brennstoff-Chem* , 42, 241 (1961).

[13] Kröger, C , and Das, J J , *Brennstoff-Chem* , 42, 223 (1961)

[14] Van Krevelen, D W , and den Hartog, H W , *Brennstoff-Chem* (in the press)

[15] Kroger, C , and Brucker, R , *Brennstoff-Chem* , 42, 245 (1961)

BIOCHEMICAL ENGINEERING

A SYMPOSIUM on "Biochemical Engineering" was convened by the Institution of Chemical Engineers on May 30 , it is doubtful whether any two of the participants, lucid as they were on their own topics, would have agreed on a definition of the title Though, on one hand, one may assume unanimity of outlook in chemical engineers, the term 'biochemist' has been used by many disciplines, from the quantitative biologist to the organic chemist by way of the medical analyst Moreover, in the fields where engineers encounter Nature in the raw, ought not such a title to be amplified to 'biological chemical engineering'? It is easy to see how confusing such exercises in labelling can be, though there is always a stimulation of interest and research by collecting certain topics together under a high-sounding title, however arbitrary

Nevertheless, many industries are now based on the controlled use of biological processes (Strangely enough, the largest industry of this type, brewing, was already developed more or less to its present size before the terms 'biochemist' or 'chemical engineer' existed) It was the production of antibiotics by fermentation, especially under the high pressure of the Second World War, that brought a variety of biological interests (botanists, zoologists, pharmacists, agriculturists, entomologists—for there were very few true biochemists, by any definition, available at the time) face to face with the engineer (rarely of the 'chemical' variety) The difficulties of communication were considerable and were overcome the hard way It was also realized that similar problems existed in the food preservation industries and the sewage and effluent disposal services To help with these problems, the establishment of courses in 'biochemical engineering' were proposed—seldom, however, with any clear idea of what the graduates should do afterwards, although the vague idea of a superman who could control both sides of the industrial scene may have been in the minds of some proponents The problems were considerable, for whereas most of the engineers' deficiencies were conceptual and philosophical, most of the biologists' were factual and mathematical Making good the former must have seemed easier or, perhaps, they were not so apparent and the new discipline became, at least for a time, the protégé of chemical engineering

Two difficulties then faced the new dispensation first, that with normal chemical engineers at a premium, the supply of new graduate output could not hope for many years to affect the position ; second, that with the enormous rate of growth of each parent topic, no person could hope to keep up to date in both biochemistry and chemical engineering It was clear, therefore, that for many years to come the two functions of engineer and biologist would have to be realized by specialists in each discipline working side by side as separate entities It was with this aim of bringing together the two groups that this symposium was called

In the opening paper Dr D J D Hockenhull reviewed the formal aspects of the production of economically desirable products from micro-organisms He stated, as an axiom, that the primary function of a living organism is to reproduce itself It does this by a complex interlinked system of biochemical reactions on the nutrients supplied to it Interference with these processes may lead to the accumulation of intermediates or of alternative products derived from them Industrially important examples of such interference were unbalanced nutrition (acetic acid), deficient aeration (alcohol), mineral deficiency (citric acid), use of trapping agent (glycerol) or of foreign precursors (penicillin) or inborn error of metabolism (lysine) It was pointed out that these 'primary colours' of metabolic interference, in Dr G L Solomon's words, were most usually applied in combination to-day to obtain the best results A more detailed account of the biosynthesis of penicillin was then used to illustrate the concept of controlled fermentation In the early days, it used to be sufficient to choose an appropriate medium, inoculate it with the organism and leave the fermentation to proceed under set conditions until the time for collecting To-day, it has been found that strict control of *p*H value, sugar-level and the rate of supply of nutrient are all essential for maximal yield Such control brings fresh problems for the engineer in the design of suitable equipment Sensing devices capable of withstanding the conditions of sterilization and of operating satisfactorily in fermentation broths are needed It is particularly important in this context that the biochemist and the engineer should have a true appreciation of each other's problems

The engineering problems associated with large-scale fermentations are mainly those of designing a bacterium-tight fermenter and supplying it with large quantities of sterile air Then there are the associated problems of finding the best methods of aerating and agitating the fermentation broth to promote maximum yields of antibiotic and elimination of the foam that frequently occurs

Other papers were concerned with more specific problems The highly aerobic fermentations, as used for production of penicillin and streptomycin, present their own problems of aeration and agitation Not only must the air supply be sufficient to provide all the oxygen the organism requires but it must also strip the fermentation broth of carbon dioxide which becomes toxic if allowed to build up to a high concentration Agitation is necessary, first, to break the air into small bubbles, thus presenting a large surface area to the broth, and, second, to provide the shear action that aids mass transfer Other functions of agitation are to keep solids and mycelium in suspension to ensure that the fermentation broth is of uniform composition Good mixing is also important for close control of temperature Efficient agitation also breaks down the mycelium and prevents it growing into large aggregates or clumps

Two papers were presented on the separate aspects of aeration and of agitation of fermenter broths Prof P H Calderbank, in his third paper on physical rate processes in industrial fermentations, examined the rate of mass transfer from the liquid to solid particles suspended in mixing vessels An attempt was made to simulate a mycelial suspension by the use of screened ion-exchange resin particles The rate of mass transfer was assessed by introducing the acid form of the resin into a dilute sodium hydroxide solution and noting its rate of change of *p*H with time under the desired conditions of agitation and aeration A serious restriction in transfer occurred if the solid were allowed to aggregate into large clumps The process of liquid-to-solid mass transfer seemed to be rate-determining in many industrial fermenters

Dr N Blakebrough considered aeration and agitation from a different point of view, using a photographic technique developed to examine patterns of flow in a mixing vessel The importance of work on flow-patterns, for large-scale operations, is not so much to find out the actual distribution of velocity but rather to confirm that the broth in all parts of the fermenter is being agitated so that no stagnant zones exist

Both Prof Calderbank and Dr Blakebrough approached their problems as chemical engineers They had used bench-scale apparatus with low-viscosity Newtonian liquids, very different from the viscous non-Newtonian broths often used in industrial fermentation practice and requiring agitation and aeration in vessels of, say, 10,000 gal capacity It is to be hoped that, in future, more work will be directed towards the study of non-Newtonian liquids, and that some indications will be obtained on the methods of scale-up needed for predicting with confidence the degree of mixing in a large fermenter

In aerobic fermentation of pure cultures, sterility is a special problem as the volumes of air involved are large For example, a fermenter of, say, 10,000-gal capacity could be fed with 1,000 cu ft /min air In industrial practice absolutely sterile air can rarely be obtained A compromise is made between the effectiveness of the sterilizing treatment and the detrimental consequences of an infection For design purposes, a penetration of 1 in 10^{12-13} has been suggested , that is to say, out of every 10^{12-13} organisms in the air presented to the sterilizing device only one should pass through into the fermenter

In laboratory-scale work the most common way of sterilizing air is to pass it through a cotton-wool plug or an absorbent filter On the large scale, filters of glass wool, slag wool or powdered carbon are generally used Some form of air heater may also be used The main interest in Mr R Elsworth's paper lay in the demonstration that sterilization by heat to kill infecting organisms can be considered as a simple chemical reaction the rate and extent of which can be predicted from an equation of the Arrhenius type When absolute sterility is not essential, the information presented in this paper can be most useful for determining the time/temperature relationship necessary for a desired penetration

In the laboratory, experimental work to improve the performance of a fermentation is normally carried out with shaken conical flasks , this is the cheapest way of finding out the effects of variations in the strain of organism or the medium used to grow it, for replicate cultures are easy to set up However, the characteristics of aeration and agitation in shaken flasks are very different from those in conventional deep fermenters Intermediate development work is therefore also carried out in bench-scale fermenters having a capacity of about 1 gal , and in pilot-plant units of several hundred gallons capacity

Dr A Parker presented a paper on equipment for such laboratory and pilot-plant scale fermentations After a brief review of the literature of this subject, he divided his paper into two parts, the first dealing with laboratory-scale vessels of 5-l capacity and the second with 220-gal pilot-plant fermenters The wide diversity of engineering problems associated with the design of fermenters clearly illustrated the relationship between the engineering designer and the biochemist using his equipment. Another point made was the great similarity in fermenter design regardless of its size Particular attention was needed in the design of agitation-shaft glands, the techniques of aseptic sampling and the transfer of seed cultures Rates of oxygen transfer, as measured by the sulphite method, were given for the fermenters described, under various conditions of agitation and air flow Sulphite oxidation-rates could not necessarily be correlated with actual fermentation results, but they did serve as a useful index of performance and were also helpful in comparing different agitation systems.

Aeration processes for the biological oxidation of waste waters were described by Mr A L Downing In effect, the activated sludge process is a very dilute fermentation The whole process must be carried out as cheaply as possible Various devices used for aerating the waste liquors were described, such as the induction of air through a Venturi tube and the use of a submerged impeller and sparger The effects of bubble diameter and depth of liquid through which the bubbles travel were also studied in tap water containing 5 p p m anionic surface-active agent The effects of surface-active agents on mass transfer were found to depend on the method of aeration As would be expected, the rate of absorption was roughly inversely proportional to the bubble diameter, and the oxygen absorbed per unit consumption of energy tended to decrease with increased depth of liquid

The shortness of the Symposium imposed very strict scheduling and handicapped free interchange of opinions The discussion was held back until several principal papers had been given, and this led to some 'cooling off', which was also encouraged by the separation of question and response There was a tendency for many of the contributions to be too restricted in scope and lacking in general philosophical content Perhaps this is a way of saying that they were too specialized for one-half of the audience Again, a contribution on equipment design, or even some account of the problems associated with it, even to such special details as design of valves, sampling devices, probes and electrodes, might have brought a little more practicality and scope for argument

The meeting, although demonstrating clearly that biologists and chemical engineers speak rather different languages even when working on the same processes, did indicate that the barriers are not impassable Moreover, who knows, in crossing the perilous mountains between the two disciplines, one might come across that 'abominable noman', the biochemical engineer The proceedings of the symposium are to be published in *Transactions of the Institution of Chemical Engineers* D J D HOCKENHULL

COSMIC RAY RESEARCH IN LATIN AMERICA

THE third Latin-American conference and course on cosmic ray physics was held in Mexico during June 26–July 5 It was arranged by Profs M S Vallarta and J Roederer on behalf of the Latin-American Council for Cosmic Ray Research under the auspices of Unesco, the Mexican Nuclear Energy Commission, and the U S Space Science Board

The meetings were held at the University of Mexico, famous for the elegance of its remarkable architecture, and at the headquarters of the Nuclear Energy Commission There were present some 25–30 representatives from Argentina, Bolivia, Brazil, Chile, Mexico, Peru, the United States and the United Kingdom The course consisted partly of original contributions and partly of lectures of a review nature The topics covered in the lectures included the nature and origin of the galactic cosmic rays and solar-produced high-energy particles, the intensity variations of the galactic radiation and their relation to solar activity and the structure of the interplanetary magnetic field, the motion of primary cosmic rays in the geomagnetic field, the properties and origin of the Earth's radiation belts, and solar radio astronomy

The Latin-American countries between them operate a chain of neutron monitors covering a wide belt of latitude from Mexico in the north to Ellsworth in Antarctica, and a good deal of the original work reported at the meeting was concerned with the analysis and interpretation of the data from these stations

In particular, the remarkable cosmic ray intensity variations during July 1959 and November 1960 (when there were examples both of decreases in the intensity of galactic particles and of injection of additional low-energy particles by the Sun) have been very carefully analysed by the Argentinian, Bolivian and Brazilian groups In discussing the events of November 1960 the Argentinian workers arrive at the conclusion that the main intensity increase starting at 1930 U T on November 12 was due to the envelopment of Earth by a cloud of magnetized solar plasma containing trapped solar particles generated by the 3+ flare which began at about 1330 U T on that day They also conclude that the presence of the magnetic field associated with the plasma was responsible for producing the decrease in intensity of the galactic cosmic rays observed at this time They attribute the increase in intensity of solar particles between 1330 and 1930 U T, prior to the main increase, to leakage from the trapping field in the plasma

The Bolivian group, which operates the extremely well-appointed laboratory at Chacaltaya (altitude 5,200 metres above sea-level) includes in its cosmic ray activities the study of both large air showers and intensity variations related to solar processes Included in the work reported by this group was an account of the rapid, short-lived intensity decrease observed on November 13, 1960, by means of its neutron monitor, which has the very high counting-rate of $\sim 5 \times 10^5$ per hr Immediately after the magnetic storm sudden commencement at 10 hr 21 min U T on that day, the cosmic-ray intensity started to decrease and shortly after 1300 hr U T it dropped suddenly, remaining 3 or 4 per cent below the general prevailing level for about 1¼ hr, after which it returned suddenly to the pre-drop value Analysis of data from other stations showed that this phenomenon happened on a world-wide scale, and the Bolivian group concludes that this kind of modulation of the galactic cosmic-ray intensity could only be produced if the solar plasma contained a very well-defined region of relatively high magnetic field strength

The work of the cosmic ray group in Santiago at the University of Chile, reported by Prof G Alvial, is principally concerned with the investigation of the primary cosmic rays using nuclear emulsion techniques, and he described work which has been carried out on charge determination of heavy nuclei in a stack of *G*-5 emulsion exposed at 131,000 ft over Minnesota Charge determination by two methods gives agreement to better than one unit One particle has been found with $Z = 30$

Mrs R Gall, of the Institute of Geophysics, University of Mexico, gave an account of the work carried out there by the theoretical group on the trapping of charged particles in the geomagnetic field and on the relation between the radiation belts and the inner allowed region of Stormer Work is also being carried out on the motion of cosmic ray albedo particles in Earth's field and on the changes in threshold momenta resulting from the secular variation in the geomagnetic field At some points on Earth's surface these changes have amounted to as much as 25 per cent during the past hundred years

This course, which is a biennial event, provides, in addition to the presentation of original work and review lectures, a forum for the discussion and co-ordination of future programmes in cosmic-ray research in the participant countries The two previous courses were held in La Paz, Bolivia, and San Carlos de Bariloche, Argentina, and it is intended that the next one should be held once again in Bolivia In a report of this length it has only been possible to select a few representative aspects of the work reported by the Latin-American groups

H Elliot

PHARMACEUTICAL CONTROL IN BRITAIN

In his chairman's address to the British Pharmaceutical Conference, held at Portsmouth during September 18–22, Dr D C Garratt states a case for a more effective control of the quality of medicinal substances Drugs not governed by official standards should not become available to the public except under a notification scheme, similar to that already in force for pesticides, so that their clinical characters and standards of purity, with appropriate methods of analysis, would have prior approval Standards for all medicines should be enforced by testing in regional laboratories specialized for the purpose and maintained by the Ministry of Health For reputable approved firms, the contractor's own inspection organization could be given the initial responsibility for compliance of goods with specification The pharmaceutical analysis involved should be admitted as a distinct discipline, by the institution of special training courses and recognized qualifications

At present, control analysis is too often rendered pointless by insufficient attention to the precision of the methods involved, and for a full realization of the potentialities of such techniques as, for example, ultra-violet spectrophotometry, a national collection of standard specimens of authentic materials of known impurity content is essential Authority for the enforcement of those standards should be incorporated in an Act of Parliament

It should be every analyst's concern to consider what can be done to reduce costs of examinations, for example, by using equally effective, but cheaper, solvents, by applying rapid instrumental techniques or, where possible, by replacing costly bio-assay investigations by simpler paper chromatographic techniques

Dr Garrett suggests that the public would receive better protection if the control of drugs were divorced from the present Food and Drugs Act and incorporated in a new Medical Substances and Preparations Act built around Sections 11, 12 and 13 of the Pharmacy and Medicines Act Imported drugs should be subject to stringent examination by analysts of acceptable status Such status might be conditional on the possession of a special diploma incorporated as a requirement of competence in the proposed Medical Substances and Preparations Act Examinations for such diploma qualifications would be conducted by those chemical or pharmaceutical institutions approved by the Ministry of Health

THE LABORATORY OF THE GOVERNMENT CHEMIST

The very varied work done in the Laboratory of the Government Chemist during 1960 has recently been reported* The Laboratory, which forms part of the Department of Scientific and Industrial Research, exists to provide a service to any Government department that requests it on matters broadly concerned with chemistry, and more particularly with chemical analysis The Laboratory is the oldest Government chemical institution in the Commonwealth It was created in 1842 by the Board of Inland Revenue, principally to assist the revenue authorities in detecting and preventing the adulteration of tobacco with worthless additives The special responsibilities which the Laboratory retains to H M Customs and Excise are highlighted by the number of samples received from this authority Of a total of 327,460 samples examined and reported on by the Laboratory during the year, no less than 280,125 were submitted by H M Customs and Excise These samples cover a wide range of dutiable commodities such as wines, beers and spirits, sugar, textiles, fuel oils, etc

The statutory duties of the Government Chemist under the Food and Drugs Act, the Fertiliser and Feeding Stuffs Act, and the National Health Act, involve the Laboratory in such work as the analysis of orange juice, dried milk, fertilizers, pesticide chemicals and formulations thereof, pharmaceuticals and drugs, canned foods Examples of the variety of this work are the determination of glycogen in oysters and the iodine content of school dinners

An organization which exists to provide an advisory service must carry out a considerable number of routine analyses as, for example, on the variety of materials purchased by various Government departments , this work varies little from year to year

Important work from the public health point of view concerns the examination of water and sewage Much attention is given to the control of fluoridation in municipal supplies (where this is practised) The control of micro-organisms in the Serpentine bathing area is an item of special interest to Londoners.

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* Department of Scientific and Industrial Research Laboratory of the Government Chemist Report of the Government Chemist, 1960 Pp iv+79 (London H M S O , 1961) 5s net

The Laboratory of the Government Chemist is very often involved in legal cases arising out of such illegalities as the dilution of beer, illicit distilling and forgery The present report gives some excellent illustrations on the methods used to detect forgeries

Although mainly concerned with the utilization of existing analytical methods, the Laboratory carries out a considerable amount of research work on the improvement of analytical methods and their adaptation to meet problems peculiar to the types of sample received for analysis The report also indicates that the small but increasing research programme on the development of new methods of analysis is meeting with considerable success The new radiochemical section has begun work for municipal water supplies, and on the analysis of effluents from atomic energy establishments

This report shows admirably that as a means for safeguarding the public interest over a wide field of activity, the Laboratory is second to none This report has been read with considerable interest not only for its scientific content but also for the extreme diversity of its contents The new Government Chemist, Dr D T Lewis, is to be congratulated on the presentation of this, his first report

William I Stephen

AMSTERDAM CONFERENCE ON PARTICLE ACCELERATORS

THE January number of *Nuclear Instruments and Methods** is devoted entirely to the proceedings of the Second Accelerator Conference which was held in Amsterdam during October 4–6, 1960 The Conference, which was sponsored by the High Voltage Engineering Corporation, Burlington, Massachusetts, and High Voltage Engineering (Europa) N V, Amersfoort, The Netherlands, consisted of four sessions, under the chairmanship of Profs H Brinkman (University of Groningen), H H Staub (University of Zurich), K Siegbahn (University of Uppsala) and A Charlesby (Royal Military College of Science), respectively The seven papers in the first session were devoted to nuclear physics, and dealt with resonance reactions, the generation of positrons in a thick target, desirable linear electron accelerator characteristics for nuclear research, Coulomb excitation, experiments using ultra-fast pulse techniques, nuclear widths and life-times, and colliding beam techniques Three of the six papers in the session on particle research discussed tandem accelerators P H Rose of the High Voltage Engineering Corporation described two forms of the three-stage tandem accelerator, one with negative ion injection and the other with neutral beam injection H E Gove of the Atomic Energy of Canada, Ltd, gave an account of the tandem accelerator installation at Chalk River and of two particular fields of investigation during 1960 The Chalk River accelerator began operating in February 1959 and has operated continuously on a two-shift a day, five-day week basis since then The first investigation was concerned with the interaction between beams of heavy ions such as carbon-12, nitrogen-14 and oxygen-16 with targets of nitrogen, oxygen, fluorine, neon, magnesium, aluminium and silicon, and the second with measurements of spins of levels in even-even nuclei lying between oxygen-16 and calcium-40 Recent research work carried out at the Atomic Weapons Research Establishment, Aldermaston, on nuclear reactions using a tandem accelerator and 6-MeV electrostatic generators was described by K W Allen Oxygen ions accelerated in the accelerator to energies of 10–40 MeV were used for Coulomb excitation of energy-levels in both rotational and vibrational nuclei and for the measurement of nuclear life-times by Doppler-shift methods. The final paper in the nuclear physics session was given by J C Nygard and R F Post on recent advances in high-power micro-wave electron accelerators for physics research

The third and final sessions were devoted to physics and radiation research The subjects covered included the use of Van de Graaff accelerators, pulsed neutron sources and ion sources In the industrial applications section, the use of the electron accelerator in solid-state physics, and of high-energy radiation in biochemical and microbiological research were discussed by P Baruch, and S T H Pinner and W T H Davison, respectively The report of the proceedings concludes with a most impressive and long list of accelerators (Van de Graaff, micro-wave linear electron and Cockcroft–Walton) which have been installed in various centres all over the world by the High Voltage Engineering Corporation, and associated companies

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* *Nuclear Instruments and Methods,* 11, No 1 Proceedings of the Second Accelerator Conference, Amsterdam, October 4–6, 1960 Edited by K Siegbahn Pp xii+256 (Amsterdam North-Holland Publishing Company) 90s

CONTINENTAL AND OCEANIC DIFFERENTIATION

DR ROBERT DIETZ'S recent article[1] is a marked contribution to what might be called the oceanographic revolution in geotectonics One gets the impression that with it a stage has been reached like the last but one in fitting together a jigsaw puzzle, with the various parts of the puzzle having been laboriously assembled, piece by piece, at last seeming to look as if they are all part of one single picture

What Dr Dietz has done, effectively, is to combine the notions derived from the recent discovery of oceanographers of mid-oceanic rift ridges and transcurrent faults, with the older pictures, derived from earthquake and gravity measurements, of the pushing up of continental edges along conical thrust faults exposing themselves as mountain ranges, island-arcs and trench systems, according to the ideas of Meinesz and van Bemmelen, and set out specially by Tuzo Wilson The leading new idea, linked with the still hypothetical mantle convexion systems, is one of expanding oceanic floors, split vertically by latitudinal and circumoceanic transcurrent faults, passing under the continents This removes many difficulties and makes it possible to envisage a mantle which can expand under some parts of the crust and contract under others at the same time

Two major difficulties, however, are still left, and it is to these that I wish to direct attention. In the first place, the general acceptance of an asthenosphere ranging from depths of 120–200 km. below the surface of continents and 70–200 km. below that of oceans does not fit with the presence of deep-focus earthquakes ranging down to 800 km. in depth, with a continuity of thrust planes, marked by their epicentres, with those of the shallow-focus earthquakes. This would seem to imply that it is not only the lower crust, the lithosphere, in Dietz's sense, that moves with the continents but also a rigid upper mantle down to about 900 km., below which the heavier material of reduced density 4 and some degree of plasticity seems to predominate.

To be consistent, the expansion of the oceans must occur down to this depth and from it, too, must proceed the material which by successive crack filling provides for the extension of the oceanic floor and for submarine vulcanicity and particularly for extensive basaltic flows and intrusions. This basaltic material may not derive directly from the lower mantle but may be, so to speak, pumped through the cracked crust from the asthenosphere by the buckling of the upper mantle due to the pressure of the convection currents beneath it. This process, as Gaskell has pointed out, is likely to occur catastrophically rather than continuously and its effects disappear in time by subsidence.

The difficulty of reconciling these two sets of facts turns on the rheological properties of the asthenosphere. It is clearly not a liquid or shear waves would not be transmitted at all but, also, evidently it admits easy creep for earthquakes never originate there, in contrast to the rigidity of the layers above and below it. One possibility is that it is crystallographically heterogeneous in which some minor constituent may be melted thus giving it physical properties similar seismically to poorly consolidated sediments. Such a paste sandwich held between rigid rock above and below might well admit both 45° thrust planes and vertical transcurrent faulting as one rigid body. It may also be that the reason why its upper limit lies higher under the oceans than under the continents is because it is actually hotter there owing to upward convection. The apparent equality of flow of heat through continents and ocean beds might well be an almost accidental compensation, the continental masses making up by their greater radioactive content for the heat flow due, under the oceans, to the closer approach of heated material. These speculations might be amenable to test by heat-flow studies on continental margins and oceanic rifts.

The second difficulty is older and more fundamental. It touches the mechanism of the geochemical differentiation between the sima of the ocean beds and the sial of the continents. Apart from an entirely hypothetical solution like the extraction of the Moon from the Pacific basin, it would seem that this is the result of a progressive tangential concentration of sialic material. Continents seem to grow on their active oceanic margins by the pushing in of an undifferentiated crustal material. But how does the mechanism which Dietz invokes, of the sloping under of the ocean floor, transfer its sial content to the base of the crust? Here, I think, more geochemical analysis is necessary. Something might be found by comparative analytical studies of the clearest example of this under-thrust which occurs in Central America where the Pacific type blocks appear to have been pushed right under the Cordillera of the isthmus and come up again in the Antillean arc, limited by the great transcurrent faults of Cuba and Trinidad.

On both these questions of the real depth of the continental blocks and of the concentration of aluminium in these and not in the oceanic block, experimental methods, both chemical and mechanical, as well as observation and analysis should have a part to play.

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[1] *Nature*, **190**, 854 (1961).

REGARDING Prof. J. D. Bernal's communication, I am pleased to learn that he finds the concept of ocean floor spreading, marking the top of mantle convection cells, at least generally palatable.

Regarding the question of deep-focus earthquakes, I am inclined to favour the idea of the lithosphere extending to about 70 km. both under the continents and ocean basins. This is, of course, derived from isostatic evidence. Below this level, in the asthenosphere, permanent stresses seem not to accumulate. Deep-focus earthquakes down to 800 km. apparently indicate that stresses do accumulate for short periods of at least a few years, but this does not prove their accumulation for thousands or millions of years. Hence deep-focus earthquakes do not disprove the existence of an asthenosphere at moderate depths. As Bernal emphasizes, much depends on the rheological properties of the mantle substance, about which little is known. To describe it as a viscous solid, as a quasi-liquid, etc., must all fail as adequate descriptions.

I agree with Bernal that the apparent equality of flow of heat through the continents and ocean basins may well be a matter of accidental compensation. The surprisingly high flow of heat from the ocean floor with poorly radioactive rocks may be explainable by convection, whereas the heat flow from the highly radioactive granite rocks of the continents must depend on diffusion.

Regarding the sialization of the continents, I visualize that the sima, rising from the deep mantle, contains some juvenile sialic material. By spreading, this 'sial-sima' is eventually slid under the continents. On subsidence of the sima beneath the continent, the sialic fraction gets largely squeezed out (gravitationally differentiated) and is plastered to the underside of a continent. Hence the suggested origin of sial is much like the generally accepted origin of juvenile water. But the mantle substance would tend to be dewatered at the convection cell divergence while it would tend to be de-sialized at the convergences which are necessarily under the continents because of continental drift to the convergence zones. At present, the process seems especially operative around the Pacific, where the mantle seems to be slipping under the continental block and not coupled with it, in contrast to the Atlantic. This added new buoyancy would help account for the recent epeirogenic uplift of the Pacific margin. Thus, I support the concept of new sial being added mostly under the continental block rather than merged laterally and annularly as Tuzo Wilson believes.

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WITH most of Dr Dietz's comments on my note I am in agreement In particular, I find his answer to my second objection, namely, the formation of sial by scraping and squeezing out of undifferentiated 'sial-sima' through the sliding under of the oceanic basement most illuminating

However, I must still take issue with him on the nature of the upper mantle as witnessed by the existence of deep-focus earthquakes These cannot, in my opinion, represent merely temporary accumulations of stress in a long-term easy creep material. The location of the conical 45° failure zones must be at least as old as the system of deep sea trenches, island arcs and continental mountain systems they underlie True, they appear to shift discontinuously by distances of the order of 200 km as each phase of the orogenic cycle comes into play However, as Van Bemmelen has demonstrated, the sequence of four parallel thrust planes in the Indonesian arc system goes back at least as far as the base of the Secondary, a period of two hundred millions of years The most natural explanation of this phenomenon may be similar to that of the development of Luders lines in plastically deformed metals, with old zones of failure hardening up and passing the critical stress on periodically to undistorted material It seems to me, in any event, somewhat perverse to attribute events occurring 800 km down in the mantle to causes located in the first 50 km from the surface, the reverse seems much more likely If this is so the downward moving convection current under the continental margins might be pictured not as a flow of liquid against a gravitational field through a rigid and highly compressed rock layer, but rather as a displacement downwards of successive blocks of the upper mantle into the presumably secularly softer material of the lower mantle (Fig. 1) Asthenosphere material could thus be imagined as separating into an upper hardened sialic layer under the continental edge and a lower hardened simatic layer filling in and overlying the depressed former upper mantle surface (Fig 2).

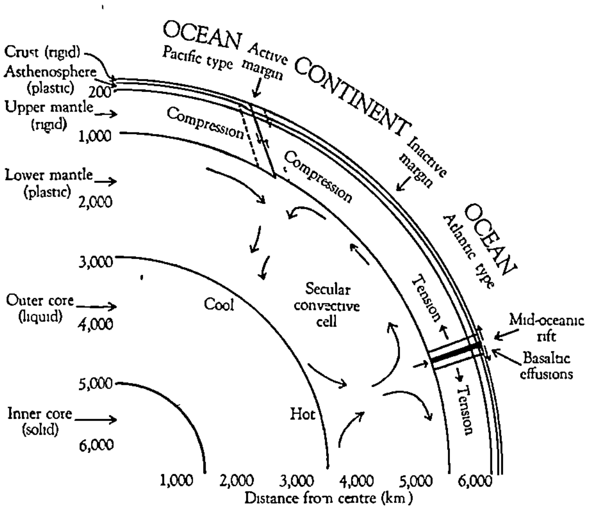

Fig 1 Schematic Earth section showing one secular convection cell in plastic lower mantle and its reactions on the more rigid upper mantle and through the asthenosphere to the crust On the right is shown, corresponding to rising mantle current, a mid-oceanic rift through which magma derived from the lower mantle, directly or indirectly, exudes to the surface through successively opened and consolidated tension cracks On the left is shown, corresponding to a sinking mantle current, an advancing continental edge with trench and mountain building system with the oceanic side of the upper mantle block thrust down into the lower mantle Between is the inactive continental edge marked only by an increased thickness of the crust and the corresponding thinning of the asthenosphere

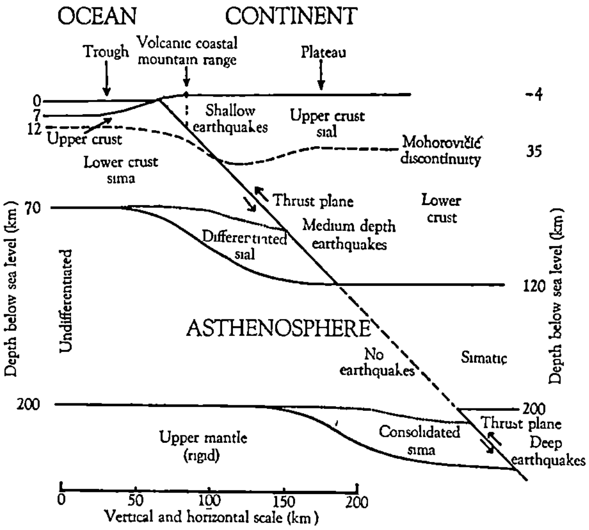

Fig 2 Section of an idealized continental thrust plane system illustrating Dietz's hypothesis of continental sialic differentiation The upper block of asthenospheric sial will in the actual case not be so clearly demarcated from the basal crust as is shown, nor will the corresponding block of sima with the sinking upper mantle The former, as it is differentiated, tends to rise vertically to jam with, and push up, granite batholiths The vulcanicity associated with the mountain building is assumed to arise from the activity of the thrust plane itself

These remain very tentative suggestions but may serve to pull together in one coherent whole the evidence derived from recent geophysical and oceanographic studies J D. BERNAL

IN two recent papers R S Dietz[1] and H H Hess[2] have made similar proposals in which, guided by new discoveries made about palæomagnetism and the ocean floors, they have combined features of several older theories into one which appears to fit many observations J D Bernal has welcomed these views (first communication) and commented on particular points and I should like to do the same

Essentially, Dietz and Hess agree that major convection currents flow in the mantle, as long advocated by Arthur Holmes, F A Vening Meinesz[3] and many others

These currents are held to rise under the active mid-ocean ridges and sink under the belts of active mountains and island arcs which lie along some continental margins The locations of these are well known and so it is possible to sketch on a globe the approximate directions of motion of the upper surfaces of these currents flowing from the mid-ocean ridges towards the active continental mountain systems

Mid-Ocean Ridges

A current rising under the mid-Atlantic ridge provides an explanation for the earlier views of A Wegener[4] and A L Du Toit[5] that the opposite coasts of the ocean have moved apart The presence of this current can also explain the central position of the ridge

The westward current bears the Americas towards and over the limb where the current is supposed to descend beneath the Cordillera and the Andes, while a current flowing eastwards can be assumed to converge towards the Alpine-Himalayan mountain belt from the north-west and the south-west The extension of this concept to the ridge through the Southern Ocean poses a difficulty From the Atlantic the ridge passes south of Africa and Australia to reach the Americas, but a current spreading south from this part of the ridge would flow towards Antarctica from nearly every direction and have no sink into which to descend The only compressional mountains in Antarctica lie along the antarctic peninsula (Grahamland) and these are scarcely adequate To escape the dilemma one must suppose that, instead of a current flowing south from the ridge, the ridge itself is migrating northwards and that the ring which it forms around most of Antarctica is expanding in radius If so, the current flowing northwards on the other side of the ridge is moving at twice the normal rate (taken as of the order of 1 cm /yr by both Dietz and Hess)

This would cause a relatively rapid northward movement of all the southern continents except South America, a fact which is borne out by palæomagnetic observations Blackett, Clegg and Stubbs[6] have recently shown that for the past 2 × 10^8 years Africa, India, and Australia have all been moving north at rates of 2–8 cm /yr

It will be observed that, since the mid-ocean ridge meets the west coast of South America and does not pass south of it, there is not the same reason for South America to have moved north Nevertheless, its fit with Africa suggests such motion It would be useful to have palæomagnetic data for South America

(*Note added in proof* E Irving has directed my attention to palæomagnetic work by K M Creer[16,17] on the Jurassic (Serra Geral) lavas of Uruguay and southern Brazil which suggests that the Brazilian Shield has not changed appreciably in latitude since the Jurassic—a result consistent with the view outlined here)

A similar outward motion may also be taking place in the mid-ocean ridges which in the South Atlantic, Southern and Indian Oceans nearly surround Africa If one is to believe that the African rift valleys are due to spreading, this expansion must be happening

A corollary of this expansion of the ring-shaped ridges around Antarctica and Africa is that, since they cannot expand indefinitely changes must occur occasionally in the location of the currents in the mantle

The ridge across the Pacific, called the East Pacific rise, extends to the Gulf of California[7] Seismicity and topography suggest that two branches reach Juan Fernandez and Galapagos Islands, but these have not been fully investigated If these branches are also upwellings, each ridge will induce a faster flow northwards, for there is no sign of any compressional features between them Following the previous argument there should be no movement of Cape Horn away from Antarctica , but north of Juan Fernandez there should be a motion twice that of the convection-rate, north of Galapagos four times that rate, and off California six times that rate This may provide an explanation of the lack of any shearing or isthmus across the Scotia Sea, the anti-clockwise rotation of the Pacific basin and the strong motion along the San Andreas fault

The current flowing east from the East Pacific rise meets that from the Atlantic under the Andes to produce their present uplift and activity, but Menard's proposal that in the United States and Canada the East Pacific rise now lies along the most active part of the Cordillera, implies that a former downward current has been replaced by an upward one which must have involved some drastic tectonic adjustments One possible consequence of removing the downward current would be to cause North America to move westward relative to South America This could have been a factor in producing the large shear faults in the Caribbean and those recently discovered in the floor of the Pacific off North America of which Menard[7] gives details Unfortunately their age is unknown

The current flowing north-west from the East Pacific rise presumably meets the current flowing south-east from the ridges in the North Atlantic Ocean and Arctic Sea The resulting downflow and compression can be held to have produced the east Asian arcs in much the manner postulated by Scheidegger[8], indeed, no other explanation has yet been offered why failure should take the form of circular arcs with their centres tending to lie on continental margins Thus some features of the contraction theory are preserved This movement of the Pacific basin north-west past the end of the Indonesian-Melanesian belt of mountains and island arcs fits the shearing observed between the Philippines and New Zealand[9]

Hess suggests that the East Pacific rise may have only been formed in mid-Cretaceous time when the location of a rising convection current changed to its present position from an earlier one lying at right angles in a west-north-westerly direction from the Society to the Caroline Islands Before this change, the orogenetic forces would have been quite different from to-day and the Cordillera would have been under strong compression and uplift The change may well have coincided also with the formation of several new island arcs in the Pacific The Kuril, Aleutian and Mariana, Yap and Palau Islands appear to be of about that age

Continental Mountain Systems

The concept that the circum-Pacific and Alpine-Himalayan mountain systems are due to compression over downward flowing limbs of huge convection cells is straightforward except for the complication already mentioned in California and Dietz's enigmatic statement that a rift is opening in the Mediterranean It appears rather to be closing This theory offers an explanation of two formerly puzzling features of older arcs The first is that it was never apparent why island arcs, so symmetrical in youth as for example the Kurils, should lose this with age, for example in the Andes It now appears likely that the Andes formed as arcs off the coast of South America and that these arcs have been overrun and swept up by the westward motion of the continent The contrast between the position of deep-focus earthquakes under the heart of the South American continent[10] and off the margin of east Asia is another indication of this To be thus caught up and overridden appears to the fate of arcs

Another feature, which was hard to explain by earlier theories and which appeared to be contrary to uniformitarianism, is that, whereas the active mountains form one continuous T-shaped pattern, older mountain systems only form short lengths, It is difficult to fit the Hercynian Appalachian mountains together into a single system and quite impossible to do it for Precambrian orogenies.

Relations to Older Orogenetic Theories

Besides admitting of some features of compression in mountains, the new theory admits of continental growth, both by sweeping up sediments on the ocean floor and by the emission of andesitic lavas. This growth along some continental margins implies that coasts separated by rifting need not necessarily fit if later brought together again.

Not only does this marginal growth make it difficult to find the coasts which formerly fitted together, but also old theories of continental drift included the notion that all continents had formerly been united into one or two. This idea, which would have involved untenable distortion of continents, seems unnecessary. The blocks of continents should rather be thought of as sometimes moving together in one place and sometimes splitting and moving apart at another place, always producing scattered patterns.

In one respect the break-up of continents does not seem to be quite random. There appears to be a tendency for them to split along former mountain systems. Thus on the Atlantic coasts of both Africa and South America there seem to be parts of mountain systems formed at the end of the Precambrian or in early Palæozoic time, suggesting that a single system split longitudinally. As pointed out by M. S. Krishnan[11], there is a similar correspondence between the late-Precambrian rocks of East Africa, Madagascar, Ceylon and southern India. The East African rift valleys follow rather closely the boundary between the Mozambique belt and older Precambrian provinces in the interior of Africa. If Menard is correct, the East Pacific rise is starting to split the Cordillera in California.

If convection currents are continually stirring the mantle, they would ensure a uniformity in its composition which would fit the views of Russell and Farquhar on the source of lead ores[12].

A point about possible convection currents which has been generally overlooked concerns the change in viscosity of the mantle with depth. Due to the thermal gradient this is likely to be large. If so, the cross-section of convection cells should be much greater near the surface than near the core of the Earth. Diagrams and discussions of convection in the Earth rarely consider this.

Movements of continents would cause the principal moments of inertia of the Earth to change and would induce polar wandering. This process would be particularly effective where two continents pile up against one another to great elevations as north of India. If such compression proceeds at the rate of a few cm./yr., it would produce hundreds of km. of shortening in ten million years. Since no mountains are compressed more than this, some factor must either change the pattern of convection cells within ten million years after continents come into contact or else a new process must arise by which convection currents begin to shear beneath continents. Three possible cases are worth considering. If some continents during certain periods have had a current flowing beneath them, whereas at other times they have been passively borne along with the current, it may be possible to find some geological phenomena, such, for example, as periods of dike formation, associated with one motion and not with the other. Another alternative is that the convection currents may be stopped or even reversed by the piling up of heat-producing continental material. To test this more heat-flow measurements are needed over mountains. It is tempting to relate the somewhat symmetrical arrangement of currents about the Earth's axis to dynamical control as is the case in the Earth's core. This symmetry was the basis of the tetrahedral hypothesis and has been translated into modern terms of ridges and mountains[13]. If this relation were true then polar wandering would affect the location of convection currents, but the evidence about the rate of polar motion suggests that it is too slow to effect changes in mountain building currents within periods of the order of ten million years. It is also doubtful if the forces are strong enough to make this linkage important. A reason for periodically changing the location of convection cells by large amounts is still needed.

The collision of continents might be expected to produce migrating waves of uplift which could catch klippe of sedimentary rocks on their advancing crests and carry them forward like surfboards on waves, stranding them when the uplift subsided. This offers a possible expanation, heretofore lacking, of some isolated and far-reaching overthrusts.

Persistence of Continental Freeboard

Dietz and Hess both recognize that continents and ocean basins are in isostatic equilibrium and that no large part of a continent can very long remain high because erosion and the rise of its roots will bring it down nearly to sea-level. They also recognize that the constant additions of juvenile water and lava from within the Earth make the problem of raising the level of the continents and of thickening them equally important, but more difficult. They suggest three mechanisms, of which Dietz states that continents "tend to buckle, which accounts for alpine folding, thrust faulting and similar compressional effects", that clastic detritus swept into the deep sea "is carried slowly towards, and then beneath the continents, where it is granitized and added anew to the sialic blocks" and that "new buoyancy is added to continents through the gravitative differentiation from the mantle".

The first two are true under mountains, but not under shields, where the crux of the problem lies. If for the sake of argument we adopt Hess's view that 4×10^9 years ago the continental blocks had half and the oceans a third of their present volumes, and that both have grown at a uniform rate, it can be shown that in Cambrian time, 6×10^8 years ago, the oceans would have been on the average 0.7 km. shallower and the continents 3.3 km. less thick than to-day. Dietz and Hess both maintain that to avoid being drowned beneath the sea, not only mountains but also the crust beneath plains must have been thickened by some such amounts.

The almost flat-lying Palæozoic rocks which cover so much of the interior plains of Canada and United States provide evidence that buckling of the shield has not been the cause of any post-Cambrian thickening of the plains.

To suppose that sediments were carried down along continental margins, metamorphosed and that they rose again is a very satisfactory explanation of the gneissic batholiths in marginal mountains, but what evidence is there that sediments are carried far under continents and that this process operates to uplift plains ? In the western Andes there are many batholiths to which the above explanation might apply, but there is nothing to suggest that batholiths are rising above the foci of the deep earthquakes in the Upper Amazon or Parana-Paraguay valleys These valleys are at low elevations and are not being uplifted Hess's argument about the spreading of mountain roots fails for the same reason

The third concept, that of differentiation of acid rocks in the mantle and their rise, is one which I have supported as an explanation of andesitic volcanism in the continental mountain systems , but what evidence is there that any such process has added about 3 km to the thickness of the interior plains of North America since the Cambrian? The only evidences of igneous activity there are a few nepheline basalt volcanoes of Cretaceous age, forming the Monteregian hills near Montreal, small carbonatite intrusions like the ten between Montreal and Lake Superior[14], very small pipes of ultra-basic rocks as in Kansas, some crypto-volcanic structures and a few other igneous intrusions, all small, isolated and rarely siliceous Most of these rocks are not acid differentiates and most of them can only have come from the mantle If these intrusions could find their way to the surface, and if 3 km of acid rocks had been added to the base of the crust during the same period, surely there would be more signs of the acid rocks All this evidence suggests that continents grow at their margins Although it is often stated that continental shields thicken, there is a lack of evidence that this has happened, at least since early Precambrian time.

The impasse is avoided if no new material is being added from the interior of the Earth, but this is contrary to several sound arguments Another possibility is that the Earth is slowly expanding A rate of a few mm /yr would suffice to maintain continents above sea-level This would not interfere with and indeed might assist the other orogenetic processes described As Dicke[15] has pointed out, this is a matter of fundamental importance in physics and cosmology and one which it is very hard to prove or disprove

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CARBONYL CONTENT OF THE VITRAIN COMPONENT OF BITUMINOUS COALS*

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IT is generally recognized that oxygen occupies a position in the structure of coal comparable with that of carbon Consequently, much work has been carried out in order to determine the percentage of oxygen contained in the various functional groups in coals of a wide range of carbon content A summary of the methods used and the results obtained has been given by Blom *et al* [1]

Of the methods used previously for determining the carbonyl fraction, that which has found widest application involves the use of excess phenylhydrazine[1,2] In this method the finely divided coal is treated with phenylhydrazine at 100° C in an aqueous medium, followed by volumetric determination of the excess reagent Although fairly consistent results have been obtained the method suffers from several disadvantages which make the results subject to error For example, both coals and humic acids react with compounds containing the NH_2 group (for example, aqueous ammonia at 100° C [3], ethylenediamine[4], etc), forming compounds which are not hydrolysable

Other errors result from the fact that many quinones are reduced by phenylhydrazine so that they do not yield any hydrazone In addition, the presence of aromatic rings inhibits the complete formation of the hydrazone[5]

In view of these difficulties, a method based on the reaction of carbonyl compounds with hydroxylamine was evolved[1] Coal of known nitrogen content is treated with hydroxylamine in pyridine at 90° C for 20 hr The nitrogen content of the coal remaining is then determined before and after hydrolysis with sulphuric acid The hydrolysed oxime is determined as ammonia In every instance it was noted that more hydroxylamine was taken up than could be split off by hydrolysis The anomalous results were attributed to reactions of hydroxylamine with non-carbonyl substances and to the fact that the oximes of some carbonyl compounds are not readily hydrolysable

As neither of the above techniques was completely reliable, it was decided to investigate the possible application of the specific reagent, trimethylacetoaminohydrazide hydrochloride (Girard's reagent-*T*)[6], to the determination of the carbonyl content of vitrains. Girard's reagent is a white, crystalline

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* Based on a paper presented at the fourth International Conference on Coal Science, Le Touquet (May 30–June 2) See p 116 of this issue

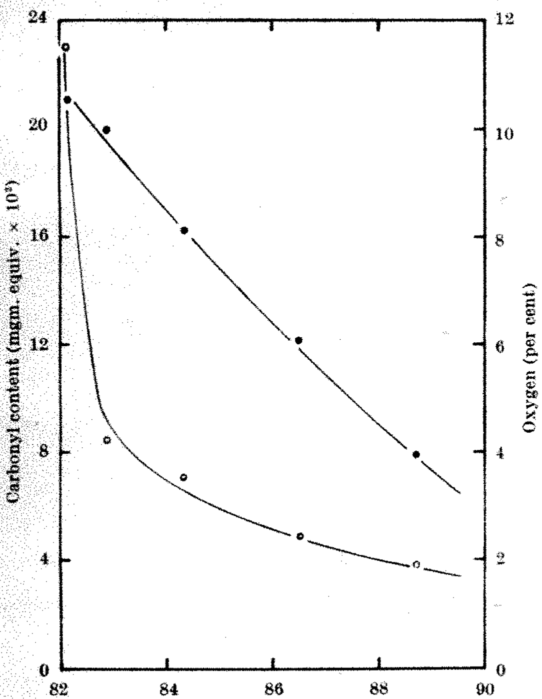
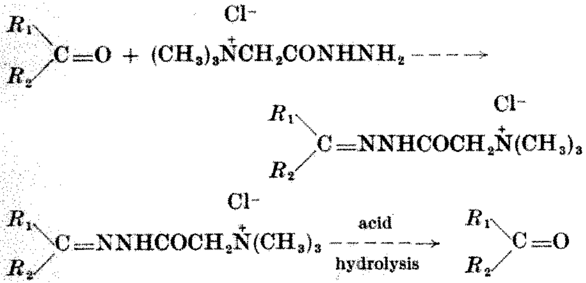


Fig. 1. Relationship between carbonyl content, oxygen content and coal rank. ○, carbonyl; ●, oxygen

solid which is very soluble in water and organic solvents containing a hydroxyl group. The reagent has a great affinity for ketonic carbonyl compounds and reacts with them quickly and quantitatively even when they are present in very small amounts[7-10].

The function of the reagent is to produce a water-soluble derivative by a condensation reaction with the carbonyl compound which can then be separated from the remaining inactive material. The carbonyl fraction thus removed can be regenerated by acid hydrolysis and afterwards estimated by conventional methods. The reactions involved are illustrated in the following reactions:



$$R_1R_2C{=}O + (CH_3)_3\overset{+}{N}CH_2CONHNH_2\,Cl^- \dashrightarrow R_1R_2C{=}NNHCOCH_2\overset{+}{N}(CH_3)_3\,Cl^-$$

$$R_1R_2C{=}NNHCOCH_2\overset{+}{N}(CH_3)_3\,Cl^- \xrightarrow[\text{hydrolysis}]{\text{acid}} R_1R_2C{=}O$$

Thus the obvious advantages of Girard's reagent are: (1) it reacts exclusively with carbonyl compounds; (2) it extracts the carbonyl compounds as water-soluble derivatives which are easy to manipulate.

The analytical data of the vitrains used are summarized in Table 1 and the experimental procedure is as follows: About 2·0 gm. dry vitrain (– 200 mesh B.S. sieve) were weighed accurately and then transferred to a 500-ml. flask to which were added 100 ml. absolute ethyl alcohol, 10 ml. glacial acetic acid and 5 gm. Girard's reagent-*T*. After boiling under reflux for 24 hr., the solution was cooled by the addition of 300 ml. distilled water containing sufficient caustic soda to neutralize 90 per cent of the acetic acid present. After allowing the insoluble material to settle, the solution was filtered through a weighed sintered glass crucible (porosity *G*4); the residue was washed and dried at 110° C. until of constant weight.

Table 1. Analytical Data of the Vitrains

					Analysis (Parr basis)				
No. of coal	N.C.B. code No.	Water per cent	Ash per cent	S (d.a.f.) per cent	C per cent	H per cent	N per cent	O (diff.) per cent	V.M. (d.a.f.) per cent
1	301	6·40	0·97	0·68	88·67	5·38	2·04	3·91	23·53
2	501	1·66	0·06	1·14	86·49	5·44	1·98	6·09	34·50
3	602	6·83	1·22	1·26	84·34	5·05	1·92	8·11	37·59
4	701	5·09	5·43	0·97	82·88	5·36	1·78	9·98	33·41
5	802	6·99	1·78	1·18	82·16	5·37	1·90	10·57	40·43

In order to hydrolyse the water-soluble derivative, 10 ml. concentrated sulphuric acid were added to the combined filtrate and washings and the resulting solution was warmed on a water-bath at 70° C. to complete the hydrolysis. The solution, after cooling, was added from a burette into an excess of 2,4-dinitrophenylhydrazine. The mixture was allowed to stand for 1 hr. to ensure complete precipitation of the hydrazone. The resulting hydrazone was filtered through a weighed sintered glass crucible (porosity *G*4), washed and dried at 110° C. until of constant weight.

The results obtained by determining the carbonyl content by reaction with Girard's reagent-*T* followed by hydrolysis and estimation with 2,4-dinitrophenylhydrazine are given in Table 2. The results are quoted in terms of mgm. 2,4-dinitrophenylhydrazone (column 3) and have been converted to mgm. equivalents of C=O in column 4.

Table 2. Carbonyl Contents of the Vitrains

Coal	Carbon (per cent)	mgm. dinitrophenyl-hydrazone/gm.	mgm. equiv.* C=O/gm.
301	88·67	6·0	0·03
501	86·49	10·0	0·05
602	84·34	15·0	0·07
701	82·88	17·5	0·08
802	82·16	48·0	0·23

* Maximum values.

The vitrains which were investigated ranged from 82 per cent carbon (10·6 per cent oxygen) to 89 per cent carbon (4 per cent oxygen). The expected decrease in carbonyl content with increasing coal rank, that is, decreasing oxygen content, is clearly demonstrated in Fig. 1. The sensitivity of the reagent in detecting the differences in carbonyl contents of coals lying in such a narrow range of carbon content is of particular interest and is indicative of the specificity of the reagent. Other investigators suggest that this trend is correct although this has not been demonstrated previously.

The use of Girard's reagent in conjunction with 2,4-dinitrophenylhydrazine has been extended to give an indication of the weight average molecular weights of the ketones involved. Full details of this are to be published elsewhere.

We wish to thank Prof. M. W. Thring for his permission to submit this article.

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FORMATION OF CRACKS IN ICE PLATES BY THERMAL SHOCK

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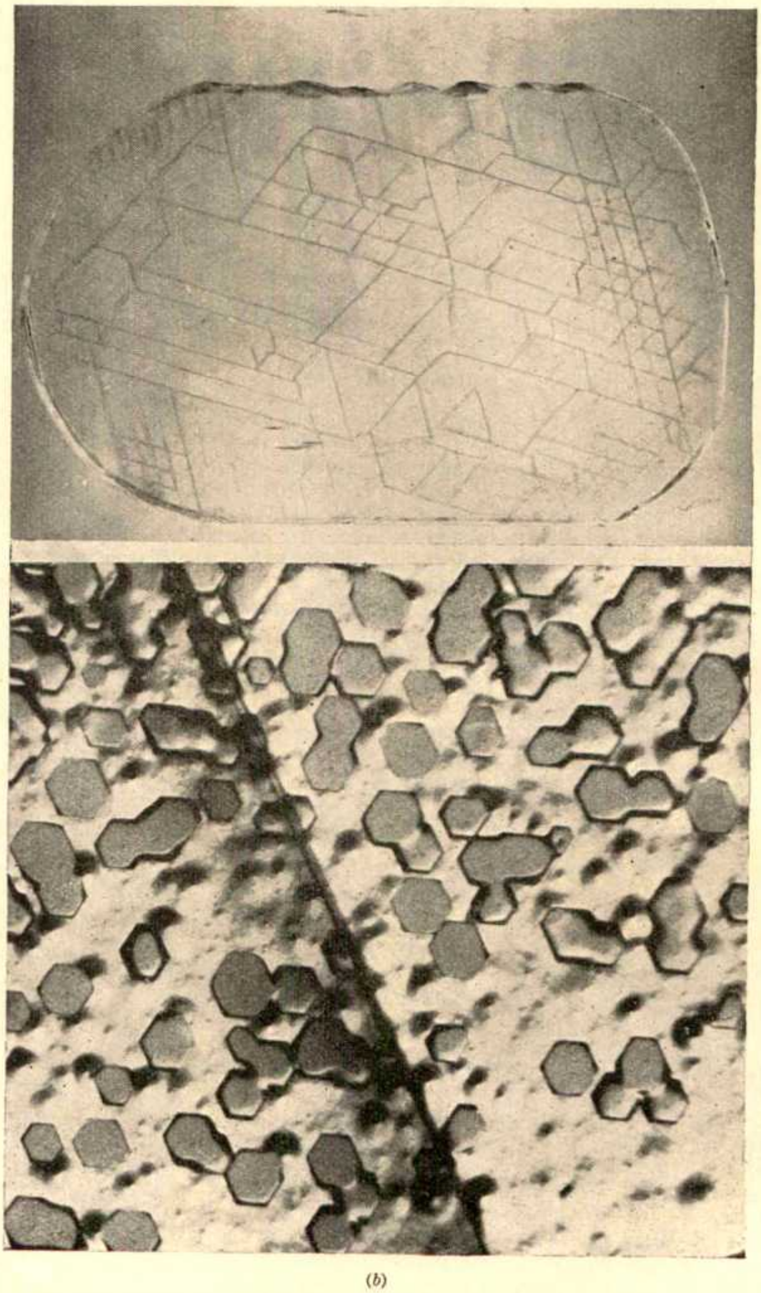
IN work on crack formation in multi-grained ice during creep under constant compressive load[1], the question arose as to whether the direction of the formation of a crack depended on the crystallographic orientation of the grains in which it formed. Experiments were undertaken to investigate this possible dependence. In one set of experiments a thermal shock was applied to the flat surface of an ice plate in such a way that the resulting thermal stress exceeded the cohesive strength of the ice almost instantaneously.

The first observations were made on irregularly shaped ice plates prepared from single crystals grown by a technique described by Landauer[2]. The plates were about 10 cm. in length and width and about 2 cm. thick. They were prepared with a milling machine using a helical cutter about 15 cm. long. Some plates were machined so that the axis of hexagonal symmetry, or '*c*' axis, of the ice crystal was normal to the surface and some so that the '*c*' axis was parallel to the surface. After machining, the plates were stored in kerosene at − 10° C. for at least 24 hr. Earlier work on the elastic and strength properties of ice had shown that immersion of the ice in kerosene for periods of up to 30 days or more did not appear to affect these properties.

Thermal shock was applied by bringing the smooth surface of an ice plate, at − 10° C., in contact with the smooth flat surface of a brass plate cooled to below −45° C. with dry ice. To ensure good thermal contact, the brass plate was immersed in kerosene just deeply enough to ensure that a very thin film of the liquid lay over the surface to which the ice plate was applied.

When the ice plates were brought into contact with the brass plate, cracks formed very quickly, certainly within 1 sec. For this stress condition, outside the region influenced by the edge the principal stresses are equal and the shear stresses are zero on any plane parallel to the ice surface. Therefore very little, if any, creep could have occurred in such planes or perpendicular to them in the short time that it took for the cracks to form.

(*a*)



(*b*)

Fig. 1. (*a*) Crack pattern for axis of hexagonal symmetry perpendicular to surface of plate; (*b*) orientation of cracks with respect to hexagonal etch pits. Crack is line running from upper left to lower right. (× 50)

The crack pattern which developed was found to depend almost entirely on the crystallographic orientation of the ice with respect to the shocked

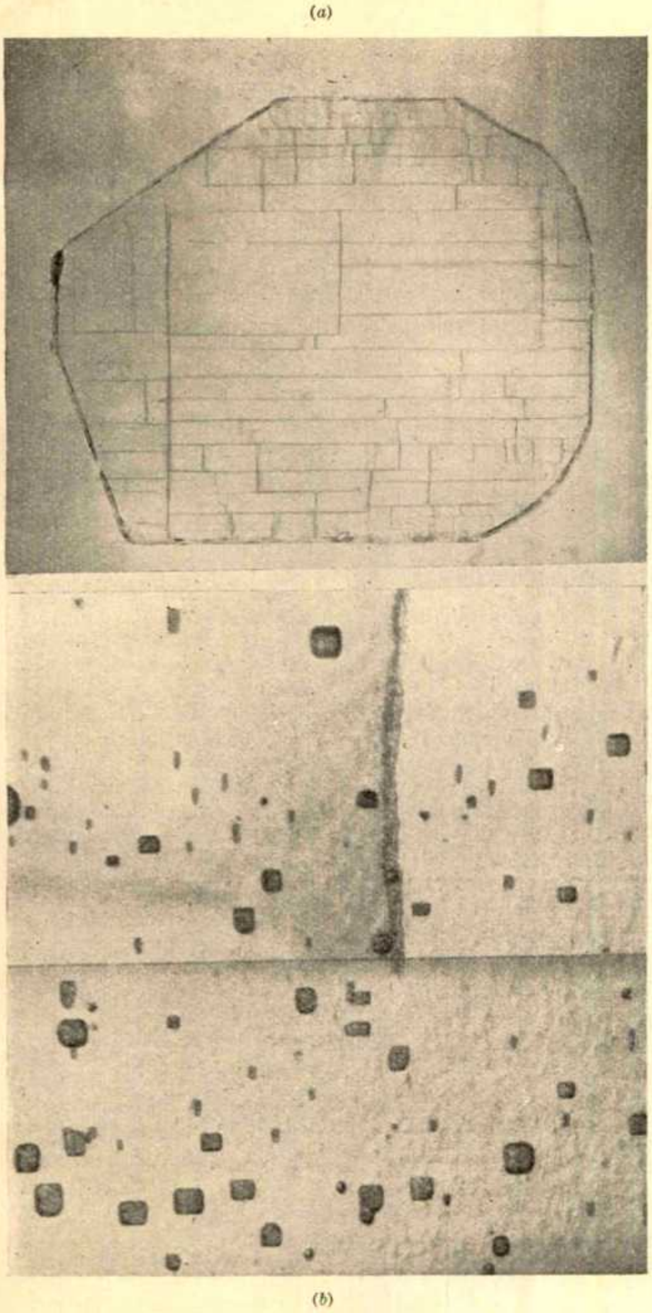


Fig. 2. (a) Crack pattern for axis of hexagonal symmetry parallel to surface of plate; (b) orientation of cracks with respect to rectangular etch pits. Basal plane of ice perpendicular to surface and parallel to the bottom edge of the photograph. (× 40)

surface. An example is shown in Fig. 1*a* for the case where the axis of hexagonal symmetry is perpendicular to the plate surface. The crack pattern is a system of equilateral triangles. As shown in Fig. 1*b*, the trace of the cracks on the surface was found to be parallel to the sides of hexagonal etch pits obtained by a technique described by Higuchi[3]. This shows, accepting Higuchi's conclusions on the orientation of the pits with respect to the crystallographic orientation, that with the symmetry axis perpendicular to the surface, there is a preference for the trace of the cracks at the surface to be parallel to the crystallographic '*a*' directions.

The rectangular crack pattern which develops when the symmetry axis lies in the plane of the plate surface is shown in Fig. 2*a*. Fig. 2*b* shows that the edges of the cracked segments are parallel to the sides of the rectangular etch pits that form on an ice surface with this orientation. The long dimension of the cracked segment was usually perpendicular to the crystallographic '*c*' direction, that is, this edge was normally in the basal plane.

The edges of each plate were cut in an irregular shape to see if this would have any influence on the crack pattern. It was observed, as can be seen in Figs. 1*a* and 2*a*, that only very close to the edge did the boundary conditions have an influence.

Figs. 1*b* and 2*b* show that there is a preference for the trace of the crack at the plate surface to be in the planes containing the '*a*' and '*c*' crystallographic directions. Etch pits formed on surfaces machined perpendicular to the plane of the crack and to the plate surface showed that near the plate surface the cracks tend to be parallel to the planes containing the '*a*' and '*c*' directions, but often curve out of these planes farther into the interior of the ice. This is considered to be due to the tendency for the cracked segments to curl under the tensile stresses parallel to the plate surface. This curving of the crack may be aided by the fact that the maximum tensile stress at the root of a crack is not perpendicular to the plane of the crack but at an angle to this plane[4]. The shaded area parallel to some of the cracks is due to the curving of the crack below the surface. If the thermal shock is severe and long enough, some of the crack segments will separate from the plate in a way similar to the spalling of concrete.

The experiments have now been extended to multigrained ice of average grain size as small as 2 mm. and with the crystallographic orientation of the grains at various angles to the surface. The preference for the trace of the cracks at the surface to be parallel to the planes containing the '*a*' and '*c*' directions is maintained for all grain sizes observed so far, a crack often changing direction abruptly as it passes from one grain to the next.

Some refinements have been made in the experimental technique. By using ice instead of brass for the cold plate and making both plates quite thick the change in temperature at the surface can be calculated and readily controlled and expressions obtained for the dependence on time of the temperature in the ice plates and the associated thermal stress. Using these expressions, a cohesive strength of 40–50 kgm./cm.² has been obtained for ice for the particular stress condition applied. The experiments and their analysis are continuing and will be reported in full when completed.

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PHOTOCHEMICAL PROPERTIES OF WATER-SOLUBLE CHLOROPHYLL

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THE water-soluble chlorophyll, potassium chlorophyllin, obtained by saponification of chlorophyll with alkali, has been known for more than fifty years[1], yet the photochemistry of this interesting pigment has not been investigated. Since chlorophyllin is soluble in water, its photochemical properties can be compared directly with other water-soluble dyes the photosensitizing action of which for oxidations and reductions have been studied in detail[2]. Water is a more natural *milieu* than are the organic liquids which are required to dissolve chlorophyll. Water-soluble dyes will bind to high-polymer substrates[3] with dramatic changes in photo-chemical properties[4,5] and chlorophyllin would be expected to behave similarly. Furthermore, since chlorophyll is in the bound state in the living plant, the photochemical properties of bound chlorophyllin may have some immediate relevance to the photochemical steps in photosynthesis. Another reason for studying the photochemistry of chlorophyllin is that it has some structural differences from chlorophyll and hence theories as to the reactive groups can be tested.

Chlorophyll *a* was obtained chromatographically from spinach extract[6] and the dried pigment was dissolved in benzene and diluted 1 : 14 with pentane. This solution was shaken with 7 per cent methanolic potassium hydroxide to saponify the chlorophyll. The methanolic solution of potassium chlorophyllin *a* was placed under vacuum in order to remove the solvent and the material was stored dry. The photochemical investigations were carried out with chlorophyllin in 0·6 *M* phosphate buffer at *p*H 7·0.

Treatment with methanolic potassium hydroxide causes the material to be soluble in water. The phytol and methyl esters are saponified. Furthermore, the cyclopentanone ring (ring V in the chlorophyll structure) is broken so that three carboxylate groups result. The magnesium is retained at neutral and at basic *p*H values.

The infra-red spectra of chlorophyllin (potassium bromide pellets and measured in a Perkin-Elmer model 21 spectrophotometer) do not have the 1611 cm.$^{-1}$ band associated with porphyrins containing a cyclopentanone ring[7] and ascribed to the C=C bond of this ring in its enolic form[8]. The two principal absorption bands in the visible spectrum of chlorophyllin (Fig. 1), measured in a Cary spectrophotometer, are at shorter wave-lengths than chlorophyll *a*, and the satellite on the short wave-length side of the Soret band is absent. Similar changes in the visible spectrum are observed in one of the fractions of allomerized chlorophyll where the cyclopentanone ring is believed to have been transformed[9]. Other losses in detail in the absorption spectrum further indicate that the molecule resulting from cleavage of ring V is less rigid. The strong red fluorescence of chlorophyllin has its maximum at 646 mμ while chlorophyll *a* has its maximum fluorescence at about 20 mμ farther into the red part of the spectrum.

Chlorophyllin in the bound state is a good photosensitizer ; free in solution, it is much less effective. Chlorophyllin, like other anionic dyes[3], binds to polyvinyl pyrrolidone (PVP) with a spectral shift of the long-wave peak to 660 mμ. The bound chlorophyllin sensitizes the photoautoxidation of *para*- and *ortho*-aromatic amines just as do triphenyl methane dyes if they are in the bound state[10]. The well-known chlorophyll-sensitized photoreduction of azo dyes in non-aqueous media[11] can be duplicated in water by bound chlorophyllin[12]. Although ascorbic acid and phenylhydrazine are effective reducing agents for this reaction, chelating agents which can serve as electron donors in photoreductions of synthetic dyes[13] are inactive for chlorophyllin.

Chlorophyllin undergoes the Krasnovsky reaction[14], that is, reversible photoreduction in the presence of ascorbic acid. With chlorophyll *a* this reaction is carried out in pyridine with some water present[15] to give an intermediate with a maximum at 523 mμ ; with chlorophyllin *a* the major solvent constituent is water (with 10 per cent pyridine) and the maximum of the pink solution is at 550 mμ. Evidently the cyclopentanone ring is not required for this reaction[16]. Bound chlorophyllin, on the other hand, is photoreduced rapidly and irreversibly to a colourless species under the conditions of the Krasnovsky reaction.

Chlorophyllin, on standing in aqueous solution, is slowly transformed into a colloidal species, and this

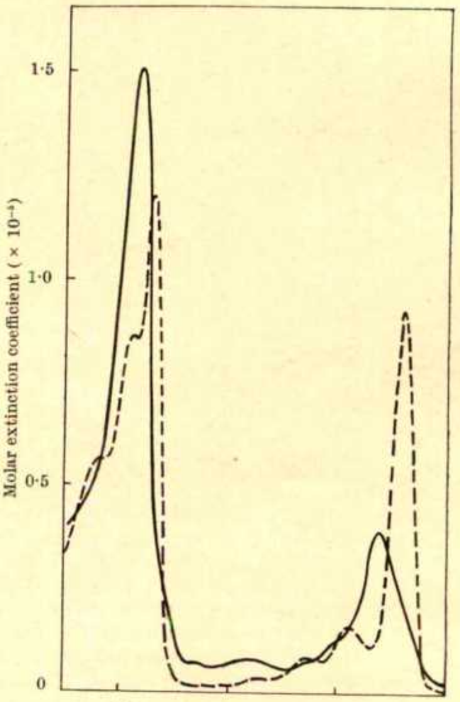

Fig. 1. Spectra of chlorophyllin *a* (continuous line) and chlorophyll *a* (dotted line)

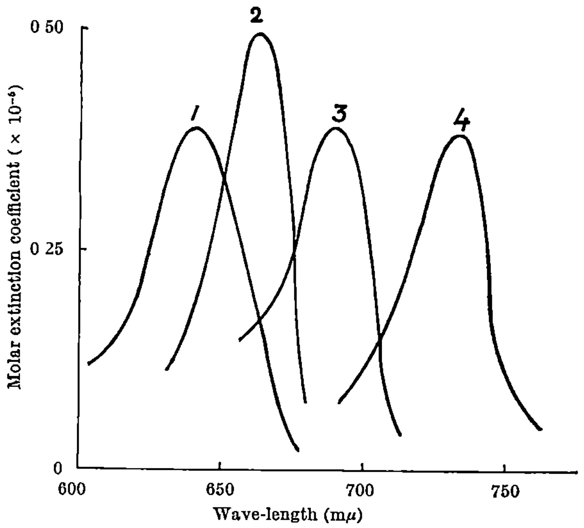

Fig 2 Changes in long wave-length peak of chlorophyllin 1, freshly prepared in water, 2, addition of PVP to No 1, 3, after illumination of No 2, 4, long standing of No 1 (reverts to No 3 on addition of PVP and to No 1 on addition of alkali)

transformation is accelerated by light or heat The system is non-fluorescent and absorbs at 735 mμ (Fig 2) as is also the case for suspensions of chlorophyll microcrystals[17] This suggests that the state of aggregation, rather than the difference in details of structure, determines the position of this peak Addition of PVP or of water-miscible organic solvents disperses the colloid to produce a strongly fluorescent solution with an absorption maximum at 688 mμ. This latter species can be obtained directly by binding chlorophyllin to PVP and exposing to light (Fig 2) Both the colloidal suspension and the solution absorbing at 688 mμ revert to the original chlorophyllin solution when the system is rendered alkaline The species which forms colloidal suspensions in water has an infra-red band at 1,730 cm $^{-1}$ as compared with the analogous band of chlorophyllin itself at 1,680 cm $^{-1}$ These facts suggest that the water-insoluble species results from a dimerization of the carboxylate groups in chlorophyllin The existence of a dimer of chlorophyll *a* absorbing at about 682 mμ has been deduced from difference spectra of chlorophyll solutions[18]

The photochemical reactions of chlorophyllin described here supplement the extensive work already carried out on chlorophyll[19], but new features are apparent Our results show that (*a*) photochemical activity of a chlorophyll derivative does occur in predominantly aqueous media, (*b*) the spectra of aggregates of chlorophyllin are the same as those of chlorophyll although there are differences in molecular structure, (*c*) certain spectral features and photochemical reactions occur for bound pigment but not for the molecules free in solution, and (*d*) the cyclopentanone ring is not required for photochemical activity We are now carrying out quantitative work to determine the mechanism of the photochemical reactions of chlorophyllin

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A QUANTITATIVE THEORY OF THE CHARACTER OF CHEMICAL BONDING IN BINARY COMPOUNDS

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IN view of the great difficulties encountered in the case of a strict quantum-mechanical treatment of many chemical problems, especially in the field of inorganic chemistry, many attempts were aimed at a more qualitative approach to the problems involved by other, much simpler methods Of such, the most widely known are Pauling's theory of electronegativity, the theory of oxygen attraction, first put forward by van Arkel and de Boer, Fajans' theory of ionic polarization and his quanticule theory and Weyl's theory of screening It appears to me that, accepting as a first approximation a purely electrostatic character of chemical bonding, it is possible to establish a quantitative theory of binary chemical compounds

The proposed theory is based on the following assumptions:

(1) Every kind of chemical compound (salts, metals, molecular compounds) is made of cations, or positive cores equivalent to cations, and a number of valency (or bonding) electrons, which form various configurations of electron clouds

(2) Homonuclear compounds (pure elements) are built of one kind of cation or positive core only Differences in their chemical characteristics are due to the different degrees of polarization of electron

clouds by the specific cations or cores. If there is no perceptible effect of polarization a purely metallic bonding results. A growing effect of polarization results in a more and more atomic (covalent) character of binding.

(3) The ionic character of bonding in binary heteronuclear compounds is due to the asymmetry of the attraction of the valency electrons by the two different kinds of cations or positive cores present, one of which is pulling the electrons more strongly than the other. In a purely ionic binary compound the strength of the field of one cation is completely negligible in comparison with the other which, in consequence, forms a positive core of an anion.

The proposed system may be presented in the form of a diagram with the following co-ordinates:

(*a*) The values of the ionic potential φ, which, according to Cartledge (1926), is a measure of the polarizing ability of cations, are taken as abscissæ. The abscissa is the axis of homonuclear compounds, along which the ionic potential φ increases from zero (the origin of the co-ordinate system) corresponding to a purely metallic bond up to the value of 87.5 for F^{7+}, corresponding to an extremely atomic (covalent) character of bonding in F_2.

(*b*) As the ordinate, the relative values of the differences between the ionic potentials of cations or positive cores, expressed in percentage of the ionic potential $\varphi_1 (\varphi_1 > \varphi_2)$, may be accepted $\frac{\Delta\varphi}{\varphi_1} \times 100$.

The value of this parameter is zero for a purely metallic bond (taken as the origin of the system) and for purely ionic bonds it reaches the value of 100.

The diagram for the proposed system of bond character of binary compounds may be constructed in the form of a right-angled triangle with apexes representing purely metallic, ionic and most nearly atomic (covalent) character of bonding (Fig. 1).

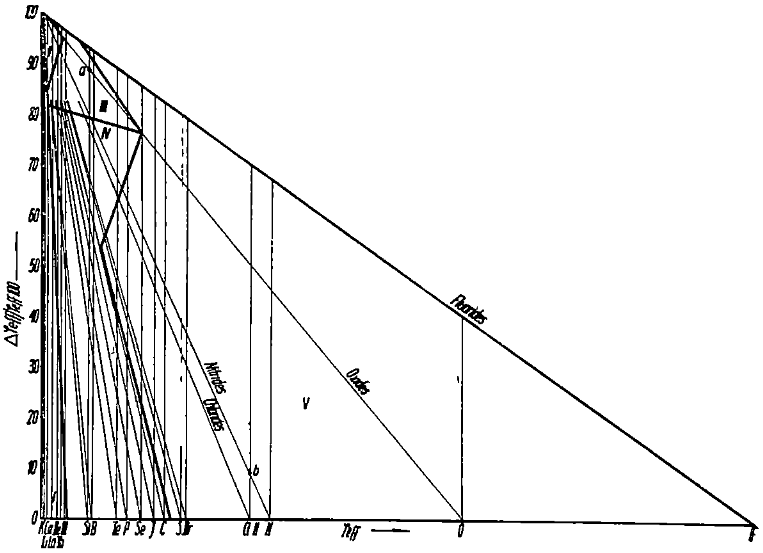

Fig. 1

The values of ionic radii given by Ahrens[1] were accepted in computations of ionic potentials throughout this article.

It is, however, more correct to use instead of 'formal' ionic potentials, as formerly proposed by Cartledge, the effective (actual) values of ionic potentials. The effective ionic potential φ_{eff} is related to the electronegativity as proposed by Pauling (log φ_{eff} is a linear function of Pauling's electronegativity). It constitutes also a measure of the acidity (or basicity) of the cation[2].

$$\frac{\varphi_{eff}}{\varphi} = x$$

For noble gas-type cations with the same charge, the coefficient x has the same constant value. As a measure of the actual field strength of cations the values of the so-called field function $F = I^p/r$ are proposed here[1], where I^p is the pth ionization potential with charge p of the chemical element forming a given cation) and r is the ionic radius. As may be demonstrated, the relation $F = I^p/r = x \times \varphi - a$ is strictly valid for all inert gas-type cations of the same charge. Putting $I^p/r + a = \varphi_{eff}$ we can find the value of the coefficient x (Table 1). Only inert gas-type cations are considered here, the more complex problems of the elements of the sub-groups of the periodic system will be treated in a separate paper.

The character of bonding of a binary compound is represented by a point on the diagram (Fig. 1), lying at the intersection of two lines, one of them parallel to the axis of the ordinate ($M - I$) and cutting the axis of the abscissa at φ_{eff} of the weaker cation or core (that is, one which has the smaller value of φ_{eff}) and the second connecting the value φ_{eff} of the stronger cation or core (that is, one which has the greater value of φ_{eff}) with the ionic apex of the triangle. For example, point *a* represents the character of the bond Si—O, and point *b* that of N—Cl.

As Fig. 1 shows, all points representing fluorides lie on the same line connecting the φ_{eff} value for

Table 1. VALUES OF THE COEFFICIENTS x* OF CATIONS OR POSITIVE CORES OF INERT GAS-TYPE WITH THE CHARGE z

z	x
1	0.65
2	1.08
3	1.50
4	1.90
5	2.30
6	2.70
7	3.10
8	3.50

* The coefficient $x = \varphi_{eff}/\varphi$ may be regarded as a relative electrostatic electronegativity value of the cations or positive cores.

Table 2

Field* (Figs. 1 and 2)	Type of binary compound	Character of electrostatic bonding	State of aggregation †
I	Metals and intermetallic compounds	Metallic	Solid
II	Salts (alkaline oxides included)	Ionic	Solid
III	Polymers	Ionoidic (sub-ionic)	Solid (glass-forming)
IV	Sub-metals (semi-conductors and semimetals)	Sub-metallic (metalloidic)	Solid
V	Molecular compounds	Atomic (covalent)	Solid, liquid or gas
VI	Inert gases	None	Gas (free atoms)

* Many of the properties of the binary compounds, resulting from the different nature of their chemical bonds, are, of course, changing more gradually than is suggested by the sharp boundary lines shown on Figs. 1 and 2.

† At room temperature and pressure.

F^{7+} with the apex *I*, and also points for all other compounds with common anion, namely, oxides, sulphides, chlorides, nitrides, etc , lie on their respective lines

Various physical and chemical properties of binary compounds (as, for example, their structures) are reasonably ordered by the proposed system It enables a classification of binary chemical compounds (Table 2)

Some irregularities noticeable among the halides are due to the fact that the proposed diagram (Fig 1) is a projection of a space diagram with *n* as the third axis of co-ordinates (*n* being the period of the Mendeleyevian system to which the given element belongs) The diagram log *n* – log φ_{eff} ordering the homonuclear compounds (pure elements) will be presented in a later communication

It is to be noted that for the elements of the main groups of the periodic system log- φ_{eff} is a linear function of log *z*, *z* being the group of the periodic system It is also to be mentioned that the expression $\Delta\varphi_{\mathrm{eff}}/\varphi_{\mathrm{eff}1}$, where $\varphi_{\mathrm{eff}1} > \varphi_{\mathrm{eff}2}$, may be considered as a measure of the chemical affinity of the elements forming a heteronuclear compound It was used by me to systematize the binary systems of equilibria for compounds with common anions[3]

The projection points on the diagram given by Fig 1 are very unevenly distributed To obtain a more uniform dispersion of points we must resort to a logarithmic scale This may be done by accepting the value φ_{eff} for Fr^+ as unity ($\varphi'_{\mathrm{eff}}Fr = 1{,}000$) This choice is justified by the fact that francium, as a homonuclear compound, must have the most nearly pure metallic character of bonding among all known elements As the others (atomic or covalent) we take F^{7+}, because fluorine in the F_2 molecule forms the most nearly atomic bonds known. Putting log φ'_{eff} value for Fr (that is, zero) at the origin of the system of co-ordinates (point *M*) and accepting the log φ'_{eff} value for F (2 876) as 100 (point *A*), we obtain a relative logarithmic scale on which the atomic character of bonding in homonuclear binary compounds may be expressed in percentages of that found in the F_2 molecules The diagram *I* – *M* – *A* may now be presented in the convenient form of an equilateral triangle (Fig 2) The ionic character of bonding is here also expressed in percentages $\Delta \log \varphi'_{\mathrm{eff}}/\log \varphi'_{\mathrm{eff}1} \times 100$ The ionic character of bonds in Fr—F has on the absolute scale (Fig 1) the value 99 87 per cent in comparison with 100 per cent on the relative logarithmic scale (Fig 2) The bond Si—O in SiO_2 is 35 8 per cent ionic on this scale (point *a*, Fig 2) The vertical line on this diagram is only an auxiliary scale

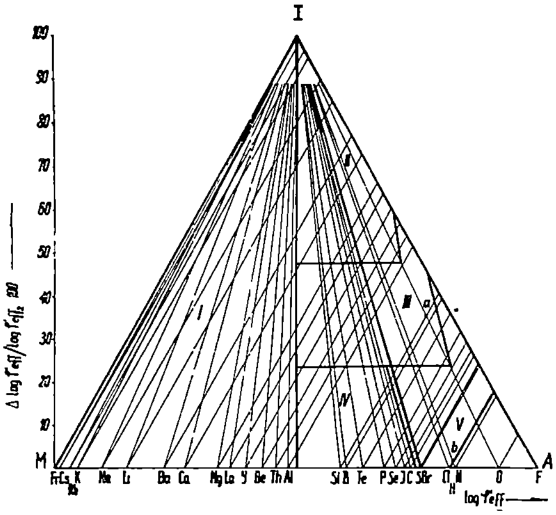

Fig 2

All points for fluorides, oxides and so on lie on the respective lines connecting the values for F, O and so on, lying on the *M–A* axis with the *I*—apex of the triangle—as on the diagram Fig 1 The fields bear the same denominations as on the previous diagram The proposed systems are valid for room temperature and atmospheric pressure. Possible defects in solids and the dispersion energy share in bonding are not taken into account here, nor are the magnetic forces

Sincere thanks are due to Prof A Bielański for valuable discussions

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CHITINASE: AN ADDITION TO THE LIST OF HYDROLASES IN THE DIGESTIVE TRACT OF VERTEBRATES

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IT was thought for a long time that, in animals, chitinases were present in snails only The distribution of these enzymes among invertebrates has appeared to be much more extensive They have been found by Tracey in protozoa[1], in nematodes[2], and in earthworms[3], and by me in arthropods[4–6], cœlenterates[7], polychæte worms[7], etc The presence of chitinolytic enzymes among vertebrates has, however, never been suspected The enzymic lysis of chitin is known to be actually performed by two different enzymes, acting consecutively[8–9] a chitinase (mucopolysaccharidase) and a chitobiase (β-acetylglucosaminidase) This 'chitinolytic system' and these two enzymes have been searched for in the digestive juices and in aqueous extracts of digestive glands and digestive epithelia of some fishes, lizards, birds and mammals

In order to detect the chitinolytic system (chitinase + chitobiase), the enzymic extracts were incubated with a chitin suspension (from *Sepia* pen) at 37° C and *p*H 5 2 for 3 hr , the acetylglucosamine liberated was measured by a method described by Reissig *et al* [10] Controls without enzyme or without substrate were run simultaneously

In order to detect chitinase in enzymic extracts containing little or no chitobiase, the activity of these extracts on chitin has also been investigated after addition of an excess of chitobiase in the enzy-

Table 1 CHITINOLYTIC ENZYMES IN DIGESTIVE JUICES OF SOME VERTEBRATES

Species	Digestive juices or contents	Activity of chitinolytic system acetylglucosamine liberated, μgm /hr /ml
Carassius auratus L	Anterior intestine	113
	Posterior intestine	814
Gasterosteus aculeatus L	Whole digestive contents	872
Lacerta viridis Laur	Gastric juice	1,200
Testudo hermanni J F. Gmelin	Gastric juice	0
	Intestinal contents	0
Passer domesticus L	Gastric juice	180
Turdus merula L	Gastric juice	1,550
Oryctolagus cunniculus L.	Gastric contents	0
	Intestinal contents	2 5*
	Cæcal contents	0

* Non-significant result

Table 2 DISTRIBUTION AND LOCALIZATION OF CHITINASE AND CHITOBIASE IN DIGESTIVE GLANDS AND TISSUES OF SOME VERTEBRATES

		Activity = acetylglucosamine liberated, μgm /hr /gm fresh tissues		
Species	Organs	Chitinolytic system*	Chitinase†	Chitobiase‡
Carassius auratus L	Mucosa anterior intestine	43–88	490	440
	Mucosa posterior intestine	288	—	72
	Pancreas (+ Liver)	—	67	62
Lacerta viridis Laur	Gastric mucosa	1437–1570	8,064	250–740
	Intestine	0–3 3§	—	45
	Pancreas	810–1350	13,120	0–160
Testudo hermanni J M Gmelin	Gastric mucosa	0	0	45
	Intestinal mucosa	0	0	57
Turdus merula L	Mucosa of glandular stomach	340	3,114	89
	Intestine (first third)	26	144	56
	Pancreas	13§	13§	0
Rhinolophus ferrum-equinum Schreb	Gastric mucosa	347	5,280	71
	Intestine	0	12§	23
	Pancreas	0	0	29
Oryctolagus cunniculus L	Gastric mucosa	0	0	0
	Intestine	0	0	70
	Pancreas	0	7 8§	72

* Liberation of acetylglucosamine from chitin, by the enzymic extract

† Liberation of acetylglucosamine from chitin, by the enzymic extract after addition of an excess of chitobiase

‡ Liberation of acetylglucosamine from 'depolymerized chitin', a preparation containing chitobiose and other small polymers of acetylglucosamine

§ Non-significant results

mic test A commercial preparation of β-glucosidase (Nutritional Biochemicals Corporation) provided a convenient source of chitobiase, free from chitinase In these cases, the presence of chitinase was also confirmed by the nephelometric method[11], which does not depend on the presence or absence of chitobiase[9] In order to measure the chitobiase, the liberation of acetylglucosamine has been followed, using a preparation of 'depolymerized chitin' as substrate[12] (a preparation which contains chitobiose and other small polymers of acetylglucosamine)

The results are summarized in Tables 1 and 2. Chitinolytic enzymes have been found in the digestive tract of six species of vertebrates, among the eight species so far investigated, the digestive juices and tissues of turtle and rabbit being entirely devoid of chitinases In all these eight species, muscles, kidney and liver extracts were found to be free from chitinases, the presence of chitobiase only being evident in the livers of *Lacerta* and *Testudo*

The following tentative conclusions may be drawn from these results ·

(1) The chitinolytic enzymes have been found not only in the digestive contents, but also in glands and in carefully washed gastric or intestinal mucosa. Such localization does not support the hypothesis of a bacterial origin

(2) All the species where chitinase has been found are insectivorous, or eat chitin-covered preys, at least occasionally In contrast, the turtle and the rabbit, which do not possess any trace of chitinase secretion, are typical phytophagous animals This observation, if confirmed for other species, would represent the only clear-cut case, with that of lactase, of a correlation between diet and digestive enzymes among vertebrates

(3) The site of chitinase secretion seems to show an evolution towards a higher specialization of tissues: in the goldfish, the secretion of chitinase is a property of the whole epithelium and of pancreatic islets This faculty is restricted to the gastric mucosa and the pancreas in *Lacerta* in bats, the gastric mucosa alone has this property

In contrast, the secretion of chitobiase, although often very weak, occurs in the majority of tissues, even in the turtle and in the rabbit

(4) The cases of the pancreas of *Lacerta* and of the gastric mucosa of *Turdus* and of *Rhinolophus* are the first known examples of tissues being able to synthesize large amounts of chitinase, but secreting only very small amounts of chitobiase

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A PRECIPITATING ANTIBODY TO INSULIN

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ANTIBODIES to insulin preparations have been detected by many of the conventional immunological methods in the sera of insulin-treated diabetic and mental patients, and of animals immunized with insulin, but convincing demonstrations of antibodies to insulin itself are few[1] Furthermore, Lapresle and Grabar[2] have directed attention to the presence of precipitating antibodies in the sera of some diabetics, to antigens other than insulin These antigens were found in the mother liquor which remains after the initial crystallization of insulin. Precipitating antibodies to insulin were found by Moloney and Aprile[3], in a horse hyperimmunized with crystalline insulin, and both insulin and the antibody were recovered from the specific precipitate They were, however, unable to find precipitating antibodies in several other species, including guinea pigs, which were immunized in the

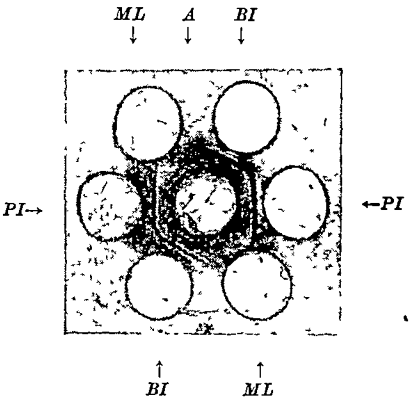

Fig 1 Precipitation of guinea pig anti-bovine insulin serum *A* (centre of figure) and some insulin preparations in agar gel, Boots bovine insulin, *BI*, Wellcome pig insulin, *PI*, and mother liquor, *ML*

same manner Berson and Yalow[4] have attributed their failure to find precipitating antibodies in the sera of insulin-treated diabetics to the univalence of insulin Nevertheless, we have found that guinea pigs readily produce precipitating antibodies when immunized with large doses of crystalline insulin preparations, and we present here evidence that these are antibodies to insulin

Bovine insulin, crystallized six times (Boots, Ltd), was dissolved in 0 002 *N* hydrochloric acid and carefully emulsified with Freund's 'Complete Adjuvant' (Difco) to give a final insulin concentration of 1 5 mgm /ml Adult guinea pigs each received 1 0 ml , injected beneath the skin and distributed between three or four sites After 4 weeks a further 1 0 ml was similarly injected and one month later precipitating antibodies to the insulin preparation could be demonstrated in the serum Thirty immunized guinea pigs all produced this type of antibody

Precipitation of the antigen–antibody complex was first detected by the gel diffusion technique of Ouchterlony[5] (Fig 1) Solutions containing 200 μgm / ml of several-times crystallized bovine and pig insulins were placed in the outer wells as was a solution containing 200 μgm per ml of mother liquor solids from an initial crystallization (kindly provided by Dr Lapresle, Paris) This last material contained only 16–18 per cent of the concentration of insulin present in the other wells The guinea pig antiserum in the centre well, although forming a single identical precipitation band with the crystalline insulins, gave no visible reaction with the mother liquor

Specific precipitates of antigen and antibody were measured quantitatively[6], using 1 0 ml amounts of two pools of guinea pig antiserum (*A* and *B*) and varying amounts of two different bovine insulin preparations, crystallized six times (Boots, Ltd , 22 2 units/mgm and Burroughs Wellcome and Co , 25 units/mgm) Mixtures of antiserum and antigen were incubated for 4 hr at 37° C and for a further two days at 4° C The precipitates were washed three times in 2 ml of saline and then dissolved in 2 5 ml of 0 1 *N* sodium hydroxide Total protein was determined from the E_{280}, measured by means of a 'Uvispek' spectrophotometer (Hilger and Watts) with a 1-cm quartz cell A factor of 0 715, estimated for guinea pig gamma globulin in 0 1 *N* sodium hydroxide, was used to convert the E_{280} to mgm protein/ml (Fig 2) At the point of maximum precipitation, pool *B* antiserum gave 1 36 mgm. of specific precipitate with 75 μgm of Boots insulin; pool *A* gave 1 00 mgm with 50 μgm of this insulin preparation but only 0 82 mgm with the Wellcome preparation Assuming that all the antigen was precipitated at the point of maximum precipitation, the ratio of antibody to antigen at this point varies between 15 5 and 19 A single curve using pool *A* and once-crystallized pig insulin (Wellcome) is also shown (Fig 2) The smaller quantity of specific precipitate formed with this heterologous insulin preparation suggests a species specificity which is interesting in view of the finding of Berson and Yalow[7] that bovine insulin is more firmly bound to human 'antibovine-pig insulin' serum than is pig insulin, and also accords with the difference in chemical composition of pig and bovine insulins[8]

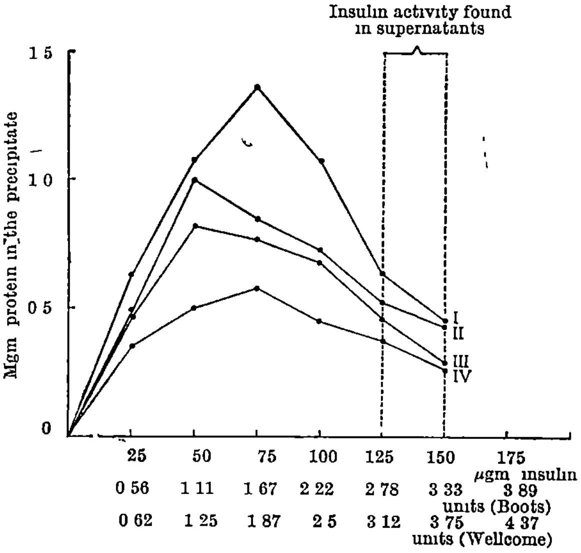

Fig 2. Quantitative precipitation curves using 1 0 ml of antiserum Curve I, pool *B* + bovine insulin, and curve II, pool *A* + bovine insulin (Boots, 22 2 units/mgm), curve III, pool *A* + bovine insulin, and curve IV, pool *A* + pig insulin (Wellcome, 25 units/mgm)

The supernatants obtained after removing the specific precipitates were tested for free antigen and antibody. In relatively large antigen excess, insulin activity was detected by the mouse convulsion test (Fig 2) and both antigen and antibody were detectable in great antigen and antibody excess respectively by precipitation in agar gel Both these methods, however, are relatively insensitive and incapable of detecting amounts smaller than 0·1 unit (4·5 μgm) of insulin in each supernatant

Precipitation, at the point of maximum precipitation, was probably not complete, since, in several experiments with longer incubation periods, somewhat larger specific precipitates were obtained For example, with an incubation period of 7 hr at 37° C , 1 0 ml of pool *B* and 75 μgm of insulin (Boots) gave 1 72 mgm of specific precipitate, corresponding to an antibody–antigen ratio of 22 Attempts were made to check the completeness of precipitation using iodine-131 labelled insulin with a specific activity of less than 1 mc /mgm. (kindly prepared by Dr Samols) Larger amounts of the labelled than of unlabelled antigen were needed to produce maximum precipitation, suggesting that some denaturation of the insulin had occurred Because of this, results were not entirely conclusive; however, 47 per cent of the total radioactivity added was recovered in precipitates which contained only 66 per cent of the total precipitable antigen–antibody protein found when unlabelled insulin was used

An attempt was made, using gel diffusion methods devised by Allison and Humphrey[9], to estimate the size of the antigen molecules which initiate this precipitation The diffusion coefficient was estimated to be 9 6, assuming that the diffusion coefficient of guinea pig antibody is 3 8 The 'molecular sieve' effect of gelatin gels was also used and the antigen diffused through 38 per cent gelatin Both methods indicate that the molecular weight is less than 24,000

These preliminary results suggest that the precipitating guinea pig antibody is indeed antibody to insulin and not to an impurity in the preparations If this is so, it can be concluded from the results of the experiments designed to estimate molecular size of the antigen, that the insulin antigen is mostly in the aggregated form.

We are grateful to Dr E Samols, Royal Free Hospital, London, for preparing the labelled insulin This work is supported by grants from the British Diabetic Association and the Joint Research Committee of King's College Hospital and Medical School

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ANTIBIOTIC PROPERTIES OF MYCELIUM AND METABOLITES OF *MYROTHECIUM RORIDUM* TODE

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THE fungus *Myrothecium roridum*, belonging to the Fungi Imperfecti, group Moniliales[1], is known as a parasite of leaves of *Gardenia*, tomatoes, violets, kidney beans and some other plants[2,3] It has been also found in soil[4] and in the dead plant tissues A detailed morphological analysis of species of *Myrothecium roridum* and related *Myrothecium verrucaria* and *Myrothecium inundatum* was undertaken by White and Downing[5] on the basis of observations of several strains They confirmed the great morphological similarity between *Myrothecium roridum* and *Myrothecium verrucaria*, and also found that these two species differed only in their manner of decomposition of cellulose, and their occurrence They also demonstrated the complete identity of *Myrothecium roridum* with a species known as *Metarrhizium glutinosum* Pope, which produced, according to Brian and McGowan[6], a new metabolite, glutinosine

The scarcity of data on metabolite production by *Myrothecium roridum*[7,8] directed our attention to this problem, and now we present some observations on the activity of the mycelium and the metabolites of *Myrothecium roridum* towards some fungi pathogenic for cultivated plants

The strain of *Myrothecium roridum* isolated by one of us[9], and used for further experiments, has been characterized by formation on glucose-potato-agar of a dense, white mycelium with concentric black sporodochia The smoky, light olive-green conidia (8–10μ long, 2–4μ thick) form black, slime masses, consisting of cylindrical or spindle-shaped cells containing oil drops The fungus grows and sporulates very easily both on malt-agar and on malt liquid (20 gm malto-extract produced by Spółdzielnia Pracy 'Warta' Śrem (Poland) in one litre distilled water), in the latter case the concentration of sporodochia not being easily visible

For the biological tests of fungicidal activity of *Myrothecium roridum* the following species of fungi were used *Penicillium notatum*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium equiseti*, *Fusarium anguioides*, *Fusarium* sp, *Mucor* sp, *Alternaria*

Table 1 INHIBITING EFFECT OF THE MYCELIUM OF *Myrothecium roridum*, AND ITS METABOLITES ON THE GROWTH OF SOME FUNGI

	Mycelium		Metabolic solution						Metabolites			
Species	malt-agar	potato-agar	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	control	myrothecin	minor metabolite of m p 204–205°C	mother liquor	control
Penicillium notatum	++	++	++	++	+	+	++	+	++	++	+++	—
Aspergillus fumigatus	+	++	++	+	+	+	++	+	+	+	++	—
Aspergillus niger	++	++	++	+	+	+	+	+	—	+	++	—
Fusarium equiseti	+	+	+	+	+	+	+	+	—	—	+	—
Fusarium sp	+	+	+++	+	+	+	++	+	—	+	++	—
Fusarium anguioides	++	++	+++	+++	+	+	+	+	—	—	++	—
Verticillium albo atrum	+++	+++	+++	+++	+	+	+++	+	+	++	+++	—
Phoma betae	+++	+++	+++	++	+	+	++	+	+	++	+++	—
Botrytis cinerea	+++	+++	+++	+++	+	+	+++	+	+	++	+++	—
Mucor sp	—	—	+*	+	+	+	+	+	—	—	+	—
Rhizopus nigricans	—	—	+*	+	+	+	+	+	—	—	—	—
Trichothecium roseum	+	+	++	++	+	+	+	+	—	+	++	—
Alternaria tenuis	++	++	++	+	+	+	++	+	—	+	++	—
Hormodendrum sp	+	+	++	++	+	+	++	+	+	+	++	—
Cephalosporium roseum	++	+−	++	++	+	+	++	+	—	—	++	—

+++, Zone of inhibition more than 10 mm or insignificant growth of mycelium for exp 2 *a b c d e*
++, „ „ „ between 5 and 10 mm or average „ „ „ „ „ „
+, „ „ „ between 0 and 10 mm or normal „ „ „ „ „ „
—, No inhibition
* Only weak sporulation observed

tenuis, *Verticillium albo-atrum*, *Botrytis cinerea*, *Phoma betae*, *Cephalosporium roseum*, *Trichothecium roseum*, *Hormodendrum* sp., *Rhizopus nigricans*.

Analysis of the metabolic solution of *Myrothecium roridum* in the usual way, that is, extraction with chloroform, chromatography on activated alumina and crystallization, yielded one major and two minor crystalline metabolites. The major metabolite, which we called myrothecin, has a molecular weight of 334, and on this basis and on its elemental analysis it was assigned the formula $C_{18}H_{22}O_6$. The substance does not melt at temperatures up to 350° C and has $[\alpha]^{20}_D = +174°$ (in chloroform).

The second metabolite has a melting point of 204–205°, the third one 115–120°. It must be stated that these compounds could be isolated only when *Myrothecium roridum* was grown on malt liquid, otherwise an entirely different production of metabolites occurred.

The biological tests were carried out in the following manner:

(1) The mould *Myrothecium roridum* was grown in Petri dishes on potato- or malt-agar, and simultaneously, or after 4 days, the species investigated were inoculated at distances of 4 cm. The zones of inhibition were measured in millimetres. (2) The crude filtrates of the metabolic solution of 4-week-old cultures of *Myrothecium roridum* were investigated for their fungicidal activity in the following way: filtrates were used for the preparation of the 5 following media, on which the test fungi were grown:

(*a*) 1 litre filtrate + 20 gm. agar; (*b*) 0.5 litre filtrate + 0.5 litre malt liquid + 20 gm. agar; (*c*) 1 litre filtrate passed through active charcoal + 20 gm. agar; (*d*) the same filtrate + 0.5 litre malt liquid + 20 gm. agar; (*e*) dried mycelium from 4-week-old cultures of *Myrothecium roridum* suspended in 1 litre distilled water + 20 gm. agar, and (*f*) control medium prepared from malt liquid + 20 gm. agar.

(3) The chloroform extracts of the metabolic solution of *Myrothecium roridum* were investigated by means of the paper-disk plate method. Approximately 10 mgm. dried chloroform extract (or crystals) were dissolved in chloroform, and 20 filter paper disks were saturated with this solution. After drying, each disk was moistened with water and put on a malt-agar medium, into which the suspension of spores of the test fungus was inoculated. The zones of inhibition were measured in millimetres.

The results are presented in Table 1; they show that:

(1) The growing mycelium of *Myrothecium roridum* inhibits strongly the growth of the majority of test fungi except the quickly developing species. (2) The metabolic solution of *Myrothecium roridum* also has a strong inhibiting effect, when not treated with charcoal, which adsorbs the fungicidal metabolites. The suspension of dried mycelium is not very active. (3) The major metabolite (myrothecin) is not very active, whereas the first minor metabolite (melting point 205° C.) and the non-crystalline mother liquors are highly active. (4) *Myrothecium roridum* does not produce glutinosine, but a number of crystalline compounds (at least three) and possesses high fungicidal activity.

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EFFECT OF WHOLE-BODY IRRADIATION AND CORTISONE ON THE DEVELOPMENT OF *OSTERTAGIA* SPP. IN SHEEP

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Effect of Whole-Body Irradiation on Antibody Response of Sheep

In the course of experimental work on the life-cycle of sheep helminths it became necessary to utilize experimental methods of suppressing the sheep's antibody response. One method of accomplishing this is by whole-body irradiation; in view of the lack of available data on response of the animal to whole-body irradiation the following is presented to indicate the results that I have obtained.

Information available (*Handbook of Biological Data*, W. B. Saunders Co.) indicated that the LD_{50} (30 day) for goats, the species nearest sheep for which data were available, was 350 rads. It also appeared that the dose necessary to suppress antibody response is about 80 per cent of the LD_{50}. Hence the initial test dose was approximately 300 rads. The irradiation source used was 310 c. cobalt-60, to which the sheep was exposed at a distance of 160 cm. Half the exposure was delivered to each side of the sheep. Total exposure was 4.5 hr. to deliver 300 rads. The test antigen used was a formalin-killed suspension of *Salmonella typhimurium* injected 36 hr. and 5 days following irradiation. This had been shown to induce marked agglutinin production in normal sheep. In a sheep exposed to 300 rads only a slight reduction in antibody response was noted although the leucocyte count dropped to a minimum (48 hr. post-irradiation) of 960 per mm.^{3} with only 2 per cent lymphocytes in the differential count.

Following this initial test two sheep were each exposed to 440 rads (6 hr. exposure, 160 cm. from 310 c. cobalt-60) and subjected to similar antigenic stimulus. No antibody was detected by orthodox agglutination techniques up to 19 days following irradiation in the first sheep. The second sheep died suddenly 14 days after irradiation without having developed any detectable antibody to the *Salmonella* antigen. At autopsy there was evidence of septicæmia and a Gram-negative bacillus was cultured from heart blood, but not identified.

These results indicated that 440 rads, applied equally to both sides of the sheep, suppressed antibody production as assessed by the response to a formalin-killed *Salmonella typhimurium* antigen

Following these preliminary experiments, four sheep have been irradiated at the above dose In all cases the hæmatological picture indicated a very severe depletion of body lymphocytes with total white cells dropping in all cases to less than 20 per cent of the pre-irradiation figure, the minimum being reached 48 or 72 hr following irradiation The lymphocytes were the cells most affected initially, dropping to less than 2 per cent in all cases within 72 hr and then rising again during the next two weeks The granulocytes reached a minimum 7–10 days after irradiation so that at this stage the total count was sub-normal, of which about 80–90 per cent were lymphocytes From the experimental results obtained in this work on the role of immunity to developing helminths it did appear that the host immunological response had been depressed The nature of the experiment prevented any attempt to confirm this serologically

In all the sheep a febrile reaction was noted which became severe (temperature exceeding 106°F) in two animals The experiment did not allow antibiotic therapy so that these sheep were killed and autopsied 13 and 14 days after irradiation The remaining two sheep survived the full 15-day experimental period with little evidence of harm The two sheep severely affected both showed septicæmia with severe gastro-enteritis, but again the Gram-negative bacillus isolated did not fit a readily identifiable bacterial group Splenic and lymphoid tissue in all sheep was markedly atrophied and bone marrow smears showed severe agranulocytosis The hæmatological picture had showed that the early lymphocytopenia was later replaced by a relative lymphocytosis as the total granulocyte count became depressed (7–10 days following irradiation) In two sheep that survived irradiation and experimentation the hæmatological picture took 12 weeks to return to normal

It appears that 440 rads whole-body irradiation will largely suppress the antibody response of sheep, but the sheep is likely to succumb to bacterial infection unless protected by antibiotics

Effect of Cortisone and Whole-Body Irradiation on Development of *Ostertagia* spp. in Sheep

It has been shown previously[1] that the development of a large percentage of the parasites recovered from a sheep following a single large dose of *Ostertagia* spp larvæ is arrested, and that 14 days following infestation the larvæ are still only in the early 4th stage of development In an attempt to determine whether this arrested development is due to an immunological response by the host, an experiment was carried out with the object of destroying or reducing the host immunological response by the use of cortisone and by high-level, whole-body irradiation

In the experiment, 4 groups were used each of 3 sheep which had been raised free of strongyle parasites and were approximately 6 months old One animal in each group received larvæ only and acted as an untreated control, one was irradiated and one was treated with cortisone Irradiation at the rate of 440 rads per animal delivered in two equal parts to each side has been found to be effective in largely eliminating agglutinin response to an injected formalized *Salmonella typhimurium* antigen (see previous article) This dose was given 24 hr prior to larval administration Hydrocortisone alcohol was injected intramuscularly at a dose of 4 mgm /kgm body-weight daily commencing 5 days prior to administration of larvæ 100,000 *Ostertagia* spp larvæ were injected directly into the rumen, each member of each group receiving larvæ from the same batch

The sheep were killed 14 days after administration of larvæ and the parasites were collected after thorough washing of the abomasum and peptic digestion of the abomasal wall The parasites were counted and classified as 'normal' or 'arrested' forms (Table 1) Those recorded as 'arrested' were still in the early 4th stage of development At least in the untreated control group the unarrested larvæ should properly be classified as 'retarded', these larvæ not having attained the size that can normally by expected of *Ostertagia* spp if given at a low level of infestation Thus a population of *Ostertagia* spp might include some 'arrested' forms (that is, development temporarily ceased in the very early 4th stage), some 'retarded' (the final morphological stages of development have been reached but the size attained is sub-normal) and normal adults of the genus

Table 1

	Group No	1	2	3	4	Means
Cortisone treated	Sheep No	57	54	64	72	
	Total recovered	53,600	31,400	29,150	15 540	32,420
	Number 'arrested'	6,930	1,200	200	2,740	2,770
	Percentage 'arrested'	12 9	3 8	0 7	17 6	8 5
Irradiated	Sheep No	61	63	55	59	
	Total recovered	43,670	27,520	13,200	43,200	31,900
	Number 'arrested'	13,540	2,860	2,600	4,860	5,960
	Percentage 'arrested'	31 0	10 4	19 7	11 3	18 7
Control	Sheep No	68	53	56	62	
	Total recovered	50 130	26,000	20,450	24,000	30,140
	Number 'arrested'	16,130	7,340	14,600	14,060	13,030
	Percentage 'arrested'	32 2	28 2	71 4	58 5	43 2

Larval arrest was very much less in cortisone-treated (8 5 per cent) and in the irradiated sheep (18 7 per cent) than in the controls

Although both cortisone and irradiation cause a variety of effects on the physiological status of sheep, an alteration common to both treatments is a reduction in the capacity of the animal to respond immunologically to a parasitic invader It is suggested that reduced immunological responsiveness by the treated sheep may have been the factor which permitted the greater percentage of unarrested development of *Ostertagia* spp as compared with that in the control animals It is postulated that in normal sheep which have had no contact with *Ostertagia* spp or related parasites, a rapid, probably local, immunity reaction leads to the formation and release of factors causing a specific arresting of development of many of the parasites Although the larvæ were introduced into the rumen by a single injection it is probable that entry to the abomasum will extend over a period of possibly up to 48 hr It may be that the first larvæ which reach the abomasum cause a local immunity reaction leading to the arrested development of later arrivals

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HEART DIPOLE REGIONS AND THE MEASUREMENT OF DIPOLE MOMENT

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Measurement of Specific Dipole Regions within the Heart

MOST electrocardiograph leads used to-day do not differentiate between dipoles from various parts of the heart, but instead give a mixture of all dipoles in unknown proportions Fischmann and Barber[1] have shown that this applies to so-called 'unipolar' chest leads no less than to standard lead I If it is assumed that there are N dipole regions in the heart, each of which can be represented by a single dipole, then it is possible to make an electrolytic tank analogue of homogeneous conductivity enclosing a heart model of somewhat higher conductivity The N dipoles can be placed in the appropriate positions on the heart model If N electrodes are now distributed over the body surface it is possible to measure N sets of N potentials as each of the dipoles $a,b,c,$, etc , is excited to unit strength in turn

Alternatively, if all the N dipoles are excited to different strengths simultaneously and N voltages are measured in the same positions on the body surface, then it is possible to calculate the strengths of each of the N individual dipoles by solving N linear simultaneous equations The coefficients in the equations are obtained from the $N \times N$ set of potentials described above The dipole strengths will appear in the form

$$\mu_a = a_1 V_1 + a_2 V_2 + a_3 V_3 + \qquad + a_n V_n$$

where V_1, V_2, V_3, etc , are the body surface potentials

In practice it is convenient to use the formulæ to design resistor networks which perform automatically the additions of element voltages to give μ_a, μ_b, μ_c, etc By switching to a particular network it is then possible to display the effects of a chosen dipole on a two-terminal electrocardiograph The effects of all other dipoles will be eliminated

Work applying this method to the human torso is in progress and will be published Accuracy will depend obviously on how closely the analogue represents the electrical properties of the human body under investigation

Clinical Method for the Quantitative Measurement of the Heart's Total Dipole Moment

THE electrical phenomena associated with the human heart may be attributed to a group of dipoles arbitrarily orientated within a homogeneous medium and producing a set of potentials V_1, V_2, V_3, V_n at n points on the body surface An application of Green's reciprocity theorem[2,3] makes it possible to measure the collective dipole strength[4] by applying currents at each of the n points and measuring the fields at the dipole positions

For example, if the dipoles of strengths μ_1, μ_2, μ_3, , μ_n establish potentials V_1, V_2, V_3, . V_n on the surface, then field strengths E_1, E_2, E_3, E_n, will exist at the dipole positions when equal currents of magnitude I are applied to each of the n points, where

$$I(V_1 + V_2 + V_3 + \ldots V_n) = \mu_1 E_1 + \mu_2 E_2 + \mu_3 E_3 + \ldots \mu_n E_n$$

If the field in the region of the dipoles is uniform and Z directed (E_Z) then the resultant Z component of all the dipoles may be written

$$\sum_1^n \mu_z = \frac{I \Sigma V}{E_Z} \tag{1}$$

Since the dipoles are likely to be orientated randomly it is desirable to establish three mutually perpendicular fields and thereby measure the three resultant dipole components The main problem is to measure the electric field since the interior of the body is generally inaccessible This can be resolved, however, by arranging that the flow of current from the electrode is uniform and calculable throughout the body

If a homogeneous three-dimensional body is box-shaped, it is obviously possible to establish a uniform field within it by applying a uniform current density over one pair of opposite faces If the conducting medium is replaced by a rectangular three-dimensional fine-mesh resistor network then a similar result will be obtained if equal currents are applied to all leads emerging from one pair of opposite faces If an irregular-shaped body is now removed from within the box then the uniform field within the body can be preserved if the original currents are maintained at the newly exposed boundaries, that is, equal currents in one direction but zero on the other two

The situation described can be approximated on the human torso if electrodes are uniformly spaced on a flat platform placed on the chest Similar electrode clusters can be used for sides and back although for the back electrode it is usually sufficient to use electrodes mounted on a flexible sheet[5] The electrodes protruding from the platforms can be sprung so that they press firmly on to the body contours

The approximately uniform field within the body between a pair of such electrodes is simply

$$E_z = \rho\, i$$

where ρ is the conductivity of the body, assumed uniform, and i is the average current per unit area projected on to the platform

The expression may also be written as

$$E_z = \frac{\rho I}{A_{xy}} \tag{2}$$

where I is the current applied to each of the elements of the cluster and A_{xy} is the small rectangular area enclosing one and extending half-way in all directions to the adjacent element Combining equations (1) and (2) we obtain

$$\sum_{1}^{n} \mu_z = \frac{\Sigma V}{\rho} \quad A_{xy}$$

as the resultant z component of the dipoles distributed throughout the chest region

The field produced by the electrode will obviously not be exactly uniform if the electrode elements are spaced at finite intervals and cover a limited area However, with a frontal cluster having 30 elements and covering an area 10 in × 14 in it has been found from model experiments that the fields in the heart region are calculable to within 10 per cent assuming the body to be perfectly homogeneous

A paper describing, in detail, an orthogonal electrocardiograph lead system, which employs platform-mounted multiple electrodes, will be published[6] Using a new non-paste electrode material developed by Fischmann *et al* [7], consisting of balsa-wood impregnated under vacuum with lithium chloride, leads *X*, *Y* and *Z* take as much time to record as three standard electrocardiograph leads

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CATION-EXCHANGE CAPACITIES OF TISSUES OF HIGHER AND LOWER PLANTS AND THEIR RELATED URONIC ACID CONTENTS

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SINCE the early work of Devaux[1], which showed that plant tissues possess cation-exchange properties, many workers have measured the cation-exchange capacity (C E C) of the roots of higher plants[2] Their findings are that dicotyledons have roughly twice the C E C of monocotyledons and that the C E C 's of the common agricultural crops lie in the range 10–60 m equiv /100 gm dry matter Much of the recent interest in this topic stems from the possible effect of C E C on the mineral composition of the plant In particular, if Donnan distribution principles hold, the higher the root C E C the higher would be the sorption of divalent over monovalent cations (valence effect) The characteristic differences in calcium and potassium contents of monocotyledons and dicotyledons—the former low in calcium and high in potassium, the latter high in calcium and low in potassium—are in keeping with the measured differences in their root C E C

The majority of measurements of C E C of roots have been made on agriculturally important[3] or ornamental plants[4] grown in nutrient culture (nutrient solution, sand or gravel culture) for ease of recovery of roots These solutions are generally too concentrated to allow the valence effect to be reflected in the plant composition Under these conditions it has been found that the C E C of a particular species can be altered to some extent by change in nitrogen[5], phosphorus[6] or heavy metal supply[7]

The indiffusible anions in the plant roots to which the C E C could be attributed were demonstrated to be pectic substances by Mattson[8], using staining tests Decarboxylation methods of estimating uronic acids, supported by chromatographic identification of extracted uronic acids, were used by Keller and Deuel[9] for plant roots and by Knight *et al* [10] for disks of plant storage tissue It was shown that a large part of the C E C could be accounted for as pectic substances

Brief reports on the C E C of other organs (leaves and stems) of higher plants[11], and on the C E C – uronic acid relationship[12] have been published recently. The exchange properties of lower plants have been noted (marine algae[13], yeasts[14], bacteria[15], fungi[16], mosses[17]) but not in relation to uronic acid content

An adequate test has never been published of the influence of soil factors on the C E C of plants Earlier work had shown that it was possible to recover plant roots from soil[18], and it was therefore decided to examine the effect of soil-type on the C E C and uronic acid content of natural vegetation Such plants, since they grow under very poor nutritional conditions, also seemed more likely to offer experimental material which might show the relationship between root C E C and mineral composition

Plants were therefore collected from sites varying widely in drainage, *p*H and calcium supply The sites are described more fully in Table 1 In all, about 80 species were collected, but only 12 (5 monocotyledons and 7 dicotyledons) were common to all 4 soil types Tops were separated into leaves and stems (where appropriate), dried overnight at 80° C and major element cations determined in the ash After washing, root systems were divided into tap roots, rhizomes and fine roots (where appropriate), dried overnight at 80° C and milled to pass a 0 7 mm sieve The C E C was measured using an acid-washing technique followed by titration to *p*H 7 using 0 01 *N* potassium hydroxide[7] Uronic acids were estimated by a micro-decarboxylation method[19] and the results are expressed in m equiv /100 gm assuming all carbon dioxide was derived from carboxyl groups

Fig 1 shows the relationship found between the uronic acid content and the C E C of the roots The diagonal line represents the theoretical 1 1 rela-

Table 1 DESCRIPTION OF SITES

Soil type	Site	*p*H	% Loss on ignition	Exchangeable cations m equiv /100 gm oven-dry soil Ca	K
Sand	Newburgh, Aberdeenshire	4 8–6 8	—*	—*	—*
Peat	Gardrum Moss, near Falkirk	4 0	72	12 9	0 75
Acid soil	Craigiebuckler, Aberdeenshire	6 5	6	7 5	0 39
Calc soil	Limehillock, Banffshire	8 0	12	55 0†	0 38

Additional sites sand, Maryculter and Glendye, Aberdeenshire, peat, Mossmaud and Lyne of Skene, Aberdeenshire , acid soil, Bridge of Alford, Aberdeenshire , calcareous soil, Blackhillock, Banffshire
* Not determined , † This soil yielded 15 3 per cent CO_2, equivalent to 34 8 per cent $CaCO_3$

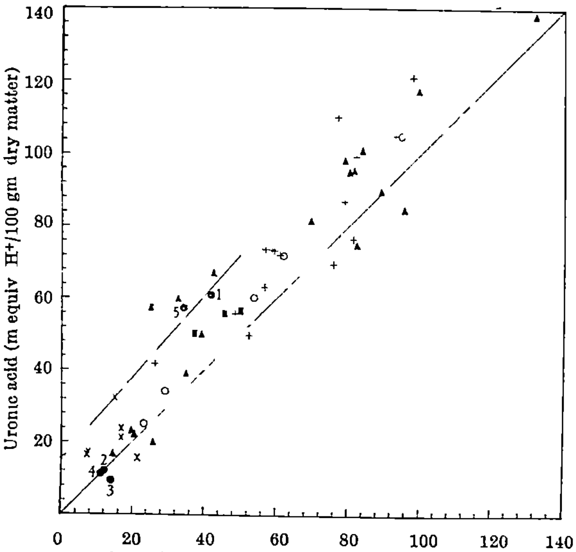

Fig 1 Relationship between cation-exchange capacity (C E C) and uronic acid content (in m equiv /100 gm) for the roots of higher plants Lower plants are indicated thus ferns (4, ■), mosses (15, +), marine algae (18, ▲), and fungi (6, ×) Numbered points are 1, liverwort, 2, lichen, 3, bacteria, 4, yeast, 5 *Equisetum* Open circles are Keller and Deuel's results

tionship The short solid line is derived by the method of least squares from the results of 62 samples, embracing the 12 angiosperm species ranging in C E C from 6 to 50 m equiv /100 gm dry roots, which were common to all 4 soil types The total of 62 samples is explained by the fact that there were several sampling sites for each type of soil Table 2 gives the mean values for these 12 plants Statistical examination shows that there are no significant differences between the individual regression lines calculated from the data for each of the 4 soil types

The slope and intercept of the line in Fig 1 show that C E C is, in general, accounted for by uronic acid content, but there is included in the ordinate a proportion of esterified carboxyl groups which yield carbon dioxide, but do not take part in cation exchange The effect of interfering substances on the decarboxylation results has been discussed[10,19]

It was found that the C E C–uronic acid relation also held for different parts of the same plant For example, for *Heracleum* sp the values (in m equiv /100 gm dry matter) for C E C and uronic acid are, respectively roots, 25 8, 49 5; tap root, 26 0, 52 0, stem, 26 7, 52 5, leaves, 26 3, 70 7 It would appear that leaves contain a higher proportion of substances which yield carbon dioxide but do not take part in cation exchange

To extend the data to include gymnosperms, the roots of 23 species of one-year-old conifer seedlings growing on the acid soil were also examined and the C E C. (17 9–30 8, mean 25 3) and uronic acid values were found to fall on the same line as the Angiosperms, but for clarity these results are not plotted For comparison with the results of other workers, values obtained by Keller and Deuel[9], using somewhat different techniques of plant culture and analysis, have been plotted. These are shown by open circles and are for the roots of 4 crop plants and one ornamental plant, larkspur[20], which gives the highest value

Table 2 AVERAGE VALUES (IN M EQUIV /100 GM) OF C E C AND URONIC ACID (U A) CONTENT OF PLANT ROOTS GROWN IN DIFFERENT SOIL TYPES

Growth medium	Sand		Peat		Acid soil		Calcareous soil	
	C E C	U A	C E C	U A	C E C	U A	C E C	U A
Monocots (5 spp)	13 2	28 1	12 3	27 2	12 6	24 8	13 3	28 5
Dicots (7 spp)	25 0	45 7	32 8	49 1	27 2	48 3	30 8	48 0

Having established the line for the higher plants, it was decided to perform tests on a few species from the other major groups in the plant kingdom These results have been plotted on Fig 1 and are made up of. ferns (4, ■), mosses (15, +), marine algae (18, ▲), fungi (6, ×) Single samples of liverwort, lichen, soil bacteria, yeast, and *Equisetum* sp are indicated by solid circles numbered 1–5, respectively These determinations were carried out on whole plants except in the case of ferns and *Equisetum* where roots were used All samples were oven-dried, then milled as for the higher plant roots

It would appear that the C E C and its related uronic acid value is a characteristic of the roots of angiosperms and is independent of a wide range of growth conditions The C E C–uronic acid relationship is also shown by other organs of the plant For lower plants there also seems to be a general relationship between C E C and the decarboxylation value of the dry matter Different substances may be involved in the production of carbon dioxide for the different plant groups, and 'uronic acid' is used only as a convenient overall expression It is reasonable to suppose that the uronic acid in the roots of angiosperms is mainly polymerized galacturonic acid (in other words, pectic substances) but little is known of the occurrence of these compounds in the lower plants In brown algae, the uronic acid is probably alginic (that is, a polymer of mannuronic acid) From the results obtained here, it is noteworthy that among the marine algae the C E C values fell into two clear groups, those of the brown algae being 70 and above and those of the red and green algae 56 m equiv /100 gm dry matter and below The presence of ethereal sulphate in seaweeds which would contribute to C E C but not to the decarboxylation value would obviously affect the relationship

We thank R, Boggie, E Birse, Miss M E K Henderson and Dr H G M Hardie for their assistance, J E Shackleton, of Ben Reid's Nursery, Aberdeen, for supplying the conifer seedlings, and E Booth, of the Institute of Seaweed Research, Inveresk, for samples of marine algae

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IMPORTANCE OF OSMOTIC POTENTIAL AS A COMPONENT OF THE TOTAL POTENTIAL OF THE SOIL WATER ON THE MOVEMENT OF NEMATODES

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THE force with which water is held in a soil can be specified in terms of a total water potential (ψ), identified as the free energy of the soil water, and is conveniently expressed as $pF = \log_{10}(-\psi)$ This total potential is the sum of a matric potential or suction (h), caused by hydrostatic pressure deficiencies existing in the soil water, and an osmotic potential (π), caused by dissolved salts

Horizontal migration of the nematodes, *Heterodera schachtii* Schmidt and *Ditylenchus dipsaci* (Kuhn) Filipjev, through sands wetted with distilled water is affected by the matric potential applied to the sands[1] In these conditions the total potential equals the matric potential, that is, the suction applied, and there is no information on the effects of osmotic potential I therefore compared the movement of *D dipsaci* larvæ in a sand of particle size 250–500μ with and without an osmotic potential In one lot of experiments the sand was saturated with distilled water and suctions of 0–100 cm water applied The larvæ that moved horizontally more than 3 cm in 5 hr through the sand column were counted and expressed as a percentage of the number of larvæ introduced In the other experiment the sand was saturated with a 0 1 M solution of urea and suctions of 0–100 cm water applied

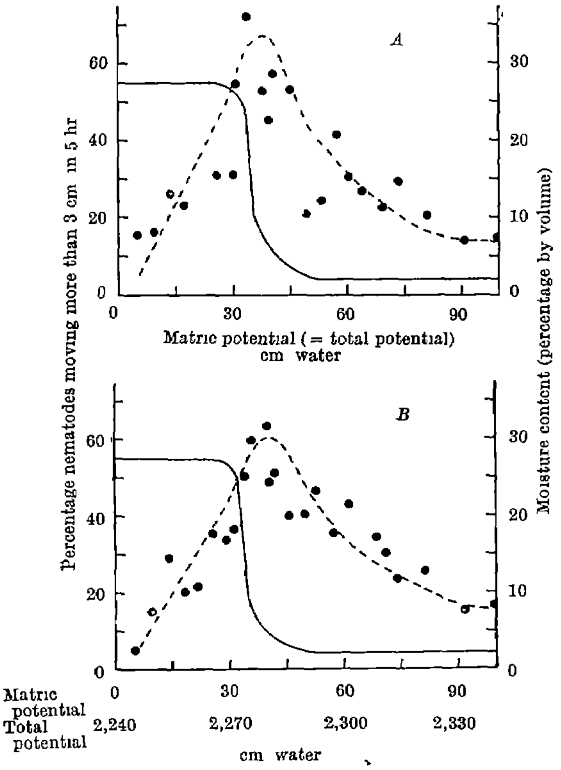

Fig 1 Horizontal migration of *D dipsaci* through sand at different total potentials Each point is the mean of two replicates In *A* the sand is wetted by distilled water (osmotic potential = 0) and in *B* by 0 1 M urea solution (osmotic potential = 2,240 cm water) The moisture characteristic of the sand is shown as a solid curve

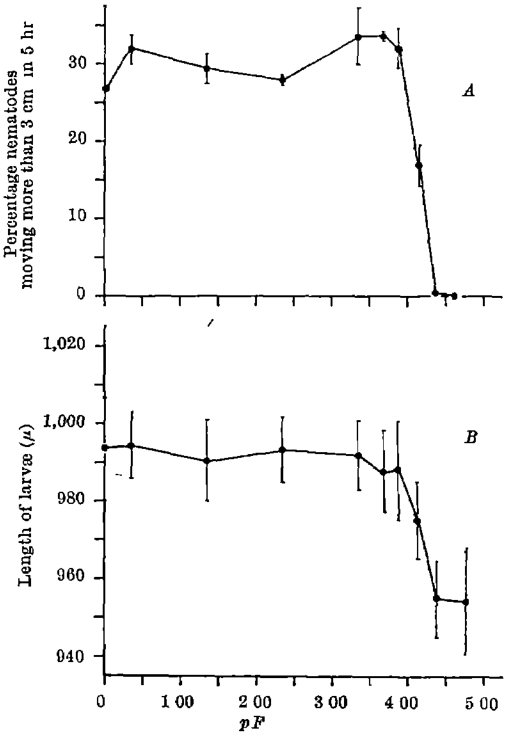

Fig 2 *A*, Horizontal migration of *D dipsaci* through sand wetted with urea solutions of different concentrations Each point is the mean of five replicates and the standard error of each mean is shown by a solid line through the point *B*, Length of *D dipsaci* larvæ measured after immersion for 36 hr in urea solutions of different concentrations Each point is the mean of fifty measurements and the standard error of each mean is shown by a solid line through the point

Fig 1 shows that the larvæ moved similarly whether the sand was wetted with distilled water or urea solution, movement in both was related to the moisture characteristic and was fastest as the pores of the sand began to drain, showing that movement depends only on the moisture content and is independent of the osmotic potential The extrusion of the mass of larvæ of *Anguina agrostis* (Steinbuch) Filipjev from flower galls was also sensitive to suction but unaffected by changes in osmotic potential within the range of 0–300 cm water[2]

The mobility of *D dipsaci* larvæ was also measured in 250–500μ sand saturated with solutions of 0·0001 M–2 M urea and drained by a suction of 32 cm water The percentage of larvæ that moved more than 3 cm in 5 hr was not influenced by the osmotic potential of the sand solution until the concentration exceeded

Table 1 EXPECTED CHANGES IN THE TOTAL POTENTIAL OF A TYPICAL SOIL, MOISTURE CONTENT 20 PER CENT W/W, AFTER ADDITION OF DEXTROSE AT SIX LEVELS/ACRE TO A DEPTH OF 8 IN

Rate of application of dextrose to soil		Matric potential of soil (cm water) (h)	Osmotic potential of soil before dextrose added (cm water) (π_1)	Total potential of soil before dextrose added (cm water) ($\psi_1 = h + \pi_1$)	Molar concentration of dextrose in soil water	Osmotic potential of dissolved dextrose (cm water) (π_2)	Total potential after dextrose added ($\psi_2 = \psi_1 + \pi_2$)	
Per cent w/w	Tons/acre						cm water	$pF = \log_{10}(-\psi_2)$
0 1 (1,000 p p m)	1	75	400	475	0 028	627 2	1102 2	3 04
1	10	75	400	475	0 28	6272	6747	3 83
2	20	75	400	475	0 56	12544	13019	4 11
3	30	75	400	475	0 84	18816	19291	4 29
4	40	75	400	475	1 12	25088	25563	4 41
5	50	75	400	475	1 40	31360	31835	4 50

0 3 *M* (*pF* = 3 87), when it decreased and movement ceased at 1 *M* (*pF* = 4 35) (Fig 2*A*)

The length of the fourth-stage larvæ of *D dipsaci* suspended in urea solutions decreased as the osmotic potential of the solutions increased

Shrinkage was greatest in the range of 0 001–1 *M* concentration and the larvæ reached their minimum lengths at *pF* approximately 4 1 (Fig 2*B*) Larvæ in the 1 *M* and 2 *M* urea solutions were killed, but those transferred to distilled water after 36 hr in solutions of 0 001 *M*–0 3 *M* urea regained their activity and former length

Dextrose and three salt solutions ranging from 0 2 to 1 *M* depressed hatching of eggs of *H rostochiensis* Woll and *Meloidogyne arenaria* (Treub) Chitwood, but in all experiments hatching was resumed when the eggs were transferred to water[3] Emergence of larvæ from cysts of *H schachtii* similarly decreased rapidly as the solute concentration exceeded 0 01 *M* regardless of the solute used[4] The speed of movement of *D dipsaci* declined when the solute concentration exceeded 0 1 *M* and the movement of *D dipsaci* and the emergence of *H schachtii* both ceased at about 1 *M* (*pF* = 4 35) This potential approximates to the permanent wilting point of plants

Matric potential affects nematode migration by varying the thickness of the water films surrounding the soil particles[5] Figs 1 and 2 show that the rate of movement of *D dipsaci* in sand depends only on the matric potential and is independent of the osmotic potential until the solute concentration is such as to cause incipient plasmolysis of the nematode In most fertile soils the salt concentration of the soil water does not exceed 0 05 *M* (*pF* = 3 05) and so nematode movement through a soil is normally governed not by the total potential but by the matric potential of the soil system

Sucrose or dextrose applied to soil at the rate of 1–5 per cent by weight of soil has been claimed to be nematicidal and proposed as a method for controlling plant parasitic nematodes in field-crops[6] Assuming the dextrose was thoroughly mixed in a soil of moisture content 20 per cent w/w to a depth of 8 in , that the osmotic potential of the soil water before treatment was equivalent to 400 cm water and that the matric potential of the soil was 75 cm water, it is of interest to calculate the changes in *pF* of the soil after adding dextrose at the rates of 1–5 per cent by weight suggested (Table 1)

The growth of most plants becomes slower as the total potential exceeds *pF* = 3 30 and stops at *pF* = 4 00 (ref 7) With 0 1 per cent dextrose, the resultant *pF* of 3 83 may retard plant growth, but from Fig 1 such a total potential would probably not slow nematode movement The *pF* at which nematodes are killed by ex-osmosis approximates to the *pF* at which plants permanently wilt Unless sugar were applied only during a period of bare fallow, it is difficult to see practical uses for the treatment if it were economic Even during bare fallow, such large quantities of pure carbohydrate could be expected to render nitrogen temporarily unavailable and induce profound changes in the soil microbial community

This work was done while I held the Sir Benjamin Fuller Trust scholarship and was on leave from the Department of Agriculture, Sydney, Australia

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CHROMOSOMAL ABERRATIONS INDUCED BY AN ANIMAL VIRUS

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SEVERAL agents known to cause mutation of mammalian cells have also been shown to induce chromosomal aberrations[1–4] and it is generally accepted that such aberrations represent one stage of the mutagenic process induced by these agents No similar effect on chromosomes has been shown in the case of animal viruses, although there is evidence implicating viruses as possible mutagens which can produce heritable changes in one or more cell characteristics[5–8] This article describes the effect of herpes simplex virus on the cytology of chromosomes in a line of Chinese hamster (*Cricetulus griseus*) cells in tissue culture In this instance, infection produced cytological changes similar to those previously described as the result of treating mammalian cells with X-irradiation[1], alkylating agents[2,3], and bromodeoxyuridine[4]

The cell line used (designated *MCH*) appeared spontaneously in a bottle seeded with a normal 23-chromosome line of Chinese hamster cells received from Dr G Yerganian, and designated by him as 44–*FAF*–10 [9] No cellular proliferation had been evident for 8 days after seeding Since healthy-appearing cells were still present, the culture was trypsinized and passaged to several T-flasks Slow proliferation of the passaged cells was noticed during

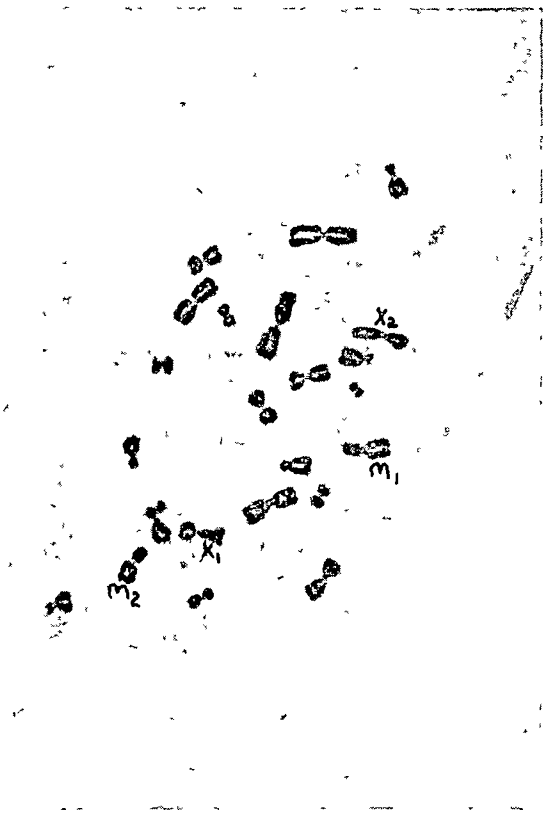

Fig. 1. Chromosomes of an uninfected *MCH* cell. M_1 and M_2 indicate marker chromosomes

several days, and these cells have now been maintained for 30 passages. Their generation time is at present similar to that of normal Chinese hamster cells (approximately 14 hr.). The *MCH* line is characterized as hyperdiploid, containing 23–28 chromosomes, and approximately 5 per cent of the cells are in the polyploid region (29 or more chromosomes). In comparison with chromosomes of normal Chinese hamster cells which have been examined in considerable detail[10-12], the unique characteristic of the *MCH* line is the presence of a marker chromosome not previously seen (Yerganian, G., personal communication). Fig. 1 shows the chromosomes of an uninfected *MCH* cell, in this instance containing two marker chromosomes differing slightly in their length. The sex chromosomes in the illustration are essentially normal, but slight variations in the X_1 and X_2 chromosomes have been observed. The marker chromosome is found in all cells regardless of chromosome number.

Cells were grown in a mixture consisting of Puck's medium (36 per cent), Hanks' balanced salt solution (37 per cent), N.C.T.C.-109 (6 per cent), lactalbumin hydrolysate (0.5 per cent), horse serum (10 per cent) and fœtal calf serum (10 per cent). The medium also contained penicillin (50 units/ml.) and streptomycin (50 μgm./ml.). All experiments were run in a humidified atmosphere containing 10 per cent carbon dioxide. For karyotype analysis, 2.5×10^5 cells were inoculated into 50 mm. Petri dishes containing 2 coverslips. Colchicine (1 μgm./ml.) was added after appropriate intervals and the cultures were incubated for an additional period, generally 6 hr. Carnoy's solution and acetocarmine were used for fixing and staining. Squash preparations were made according to the method described by Yerganian (in the press). In those experiments involving viral infection, the inoculum was added to the cells when the cultures were initiated. Mitotic indices and plating efficiency studies were carried out in parallel with chromosome analysis.

The strain of herpes simplex virus was isolated in 1952 on the chicken chorioallantoic membrane[13] and had been stored at − 70° C. until 1960, when it was adapted to growth in HeLa cells. The 49th and 50th tissue culture passages were used.

We have confirmed Yerganian and Gresser's finding (Yerganian, G., and Gresser, I., personal communication) that normal Chinese hamster cells are not susceptible to infection by herpes simplex virus. When the *MCH* line is inoculated with this agent, no cytopathic effect is observed nor do characteristic intranuclear inclusions develop, but chromosomal aberrations are seen during the first cell division following infection. Virus can be recovered from the supernatant culture fluids during two cell passages following infection—even though the cells are repeatedly washed free of released virus—as well as from frozen-thawed cells after three subsequent passages. Viral infection at the time of plating does not alter the cloning efficiency, which is approximately 80 per cent. Clones formed in the presence

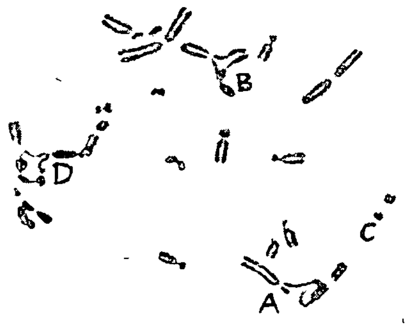

Fig. 2. Chromosomes of an infected *MCH* cell. *A*, chromatid break on chromosome 1 which has translocated to a medium-sized metacentric; *B*, chromatid exchange involving chromosome 2 and the X_1 chromosome; *C*, centromeric fusion of two telocentric chromosomes; *D*, chromatid separation and translocation involving the X_2 chromosome

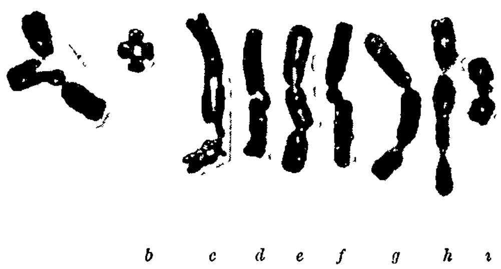

Fig. 3. Examples of chromosomal aberrations in infected *MCH* cells. *a*, chromatid break at the centromere of chromosome 2 showing terminal association with the short arm of the X_2 chromosome; *b*, reciprocal (chromatid) translocation involving two small metacentric chromosomes at their centromeric regions; *c*, chromatid deletion of chromosome 1 with segmental interchange; *d*, chromatid break at centromeric region of chromosome 1; *e*, *f*, chromatid break along chromosome 1; *g*, *h*, chromosome dicentric or exaggeration of secondary constrictions on chromosome 1; *i*, centromeric fusion of two telocentric chromosomes

of virus consist of moderately ballooned cells in contrast to the characteristically long and narrow fibroblastic cells seen in uninfected clones These results warrant the assumption that a type of carrier culture has been produced

Figs 2 and 3 illustrate the types of chromosomal changes produced by virus infection (We are indebted to Dr G Yerganian for his assistance in interpreting the karyotypes) Most aberrations occur at the centromeric and secondary constriction regions of chromosomes 1 and 2 as shown in Figs 2 and 3*a*, *c*, *d*, *e* and *f*, as well as at the centromeric regions of subtelocentric chromosomes, with the resultant formation of acentric fragments Where definitive breaks do not occur, exaggeration or elongation of centromeric and secondary constriction areas are evident (Figs 3*g* and *h*) Fusion at the centromeric regions of two chromosomes (Fig 3*i*) is seen infrequently

Table 1 presents a compilation of the chromosomal aberrations that result when cultures are infected at a multiplicity of one Chromatid breaks are common in infected cultures whereas uninfected controls and cultures inoculated with heat-inactivated virus show only chromosome breaks Translocations are recorded as to the number of cells, and these cells are not included in other categories Aberrations are not restricted to any particular karyotype, and are observed in cells bearing varied chromosome numbers Heat-inactivated virus shows no effect, as indicated in Table 1 Aberrations seem to be restricted to the metaphase of the first cell division following infection, even though virus is present during several subsequent passages The mitotic indices of infected and uninfected cultures show no significant variation, both reaching a maximum in approximately 40 hr after initiation of the cultures

Table 1 CHROMOSOMAL ABERRATIONS IN NORMAL AND VIRUS-INFECTED *MCH* CELLS

Age (hr) when colchicine added*	No of cells scored	Chromatid and chromosome breaks per cell	Accentuated secondary constriction or centromeric regions per cell	Percentage of cells with translocations
Infected				
2	102	0.18	0.07	3.9
14	200	0.23	0.08	9.5
40	196	0.21	0.12	4.1
72	218	0.09	0.007	0.0
Uninfected or inoculated with heat-inactivated virus				
2–72†	400	0.075	0.002	0.75

* Colchicine treatment lasted 6 hr except in the case of 2-hr -old cultures which were exposed to colchicine for 18 hr

† No variation with time observed in these cultures

The chromosomes of cells no longer releasing virus are now being examined in detail Preliminary work indicates that if chromosome variations are present after infection, they are not qualitative but rather quantitative or cryptostructural[14] The only variations noted thus far between infected and uninfected cells are based on slight morphological alterations which cannot in themselves be considered conclusive proof of induced mutation The fact, however, that virus infection does not result in any recognizable cell destruction and that a short-term carrier state can be established in the *MCH* line, indicates that the aberrations seen are non-lethal It is therefore possible that some permanent alteration in cellular characteristics has occurred

This work was supported by a research contract [*Nonr* 266(63)] between the Office of Naval Research and Columbia University, and by a grant from the Bristol-Myers Company One of us (B H) is a post-doctoral Research Fellow of the National Institute of Dental Research, National Institutes of Health

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INFLUENCE OF THE AGE OF POLLEN AND STIGMAS ON SEX DETERMINATION IN HEMP

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PREVIOUS investigations on the influence of the age of pollen on sex determination in hemp have given conflicting results[1,2] The possibility of regulating the number of the male and female individuals in a population would have great practical significance For this reason investigations were carried out during 1955–60 concerning the influence of the time of storing of pollen as well as the age of stigmas on sex determination in hemp derived from Poland, a type intermediate between that of central Europe and central U S S R

Female plants of hemp, with different or similar ages of stigmas, were pollinated with pollen of determined age in the same doses Plants which were pollinated naturally were used as control series In the next generation the relations of sex were analysed in all the combinations In 1955 the age of stigmas was not taken into consideration The female flowers in full blossom, that is, with stigmas of different ages, were pollinated with pollen in three stages of ripeness (1) gathered from flowers immediately after dehiscence , (2) stored in uncovered Petri plates in free air for 6 hr , (3) stored in the same way for 12 hr In the experiment in 1956 both the ages of pollen and that of stigmas were known Female plants with stigmas 7–12 days old were pollinated with pollen (1) fresh, gathered immediately after opening of male flowers, (2) stored in uncovered Petri dishes in the laboratory for 24 hr The experiments were carried out in a greenhouse with artificial or space isolation The results are summarized in Table 1

The plants begin to blossom gradually on different nodes, for this reason the age of stigmas may be very different in the female flowers flowering at the same

Table 1

Stigmas	Pollen	Year	Number of plants in 1st generation			Per cent plants in 1st generation		Number of plants in 2nd generation			Per cent plants in 2nd generation	
			Total	*M*	*F*	*M*	*F*	Total	*M*	*F*	*M*	*F*
All ages	fresh	1955/56	535	224	311	41 9	58 1	—	—	—	—	—
7–12 days old	,,	1956/57	736	256	480	34 8	65 2	—	—	—	—	—
1–3 days old	,,	1958/59	242	90	152	37 3	62 7	284	130	159	45 8	54 2
12 days old	,,	1958/59	36	14	22	38 9	61 1	47	12	35	25 5	74 5
All ages	kept for 6 hr	1955/56	663	262	401	39 5	60 5	—	—	—	—	—
,,	kept for 12 hr	1955/56	648	290	358	44 7	55 3	—	—	—	—	—
7–12 days old	kept for 24 hr	1956/57	696	307	389	44 1	55 9	—	—	—	—	—
1–3 days old	kept for 14–17 days	1958/59	158	45	113	28 4	71 6	212	93	119	43 9	56 1
12 days old	kept for 14–17 days	1958/59	22	7	15	31 8	68 2	—	—	—	—	—
control		1955/56	550	242	258	44 0	56 0	—	—	—	—	—
,,		1956/57	700	315	385	45 0	55 0	—	—	—	—	—
,,		1958/59	138	67	71	48 5	51 5	166	83	83	50 0	50 0

time It was observed that the hemps investigated began to bloom at the 7th or 8th leaf node (counting the cotyledon node as the first) On the basis of this observation, in the experiments of 1958 only the flowers at the 7th and 8th nodes were pollinated, that is, those which had stigmas 1–3 and 12 days old Pollination was carried out with fresh, and with 14–17-days-old, pollen In this experiment the mother plants were sown on the ground and covered with double sacks of dense fabric, while pollen was kept in a desiccator with calcium chloride Plants which were pollinated naturally were used as controls, but seeds were taken from the 7th and 8th node only Both in controls and in most of the combinations with fresh pollen the seeds were set normally , only in combinations with stigmas 12 days old and fresh pollen, and stigmas 12 days old with pollen 14–17 days old (Table 1), was the number of seeds considerably less, and consequently the number of plants in these series was relatively small

Nevertheless the results of these two series were also taken into account when the conclusions were drawn, since similar sex-ratios were observed in previous years

From Table 1 one may conclude that (1) the age of the stigma does not influence the direction of sex-determination in hemp, (2) depending on the period of storage, pollen has a certain influence on the numerical relations of male and female individuals of the given population

In the first and second generations a predominance of female plants occurred when pollination was carried out with fresh pollen (immediately after the beginning of pollen emission by the flowers and up to 6 hr afterwards) and when pollen was 14–17 days old In crosses which were pollinated with fresh pollen the number of female plants was about 2–11 per cent higher in the first generation and 13 per cent higher in the second than in the control series When pollination was performed with pollen 14–17 days old the majority of female plants in relation to the control series amounted to 20 per cent in the first generation and 5 per cent in the second Pollen 12 and 24 hr old did not cause any changes in the sex ratio, the results being identical with those obtained in the control series

The results of investigations suggest that (1) The rhythm of development of pollen with female tendency (x) and of that with male tendency (y) is different (2) It is possible that in pollen populations with male tendency a certain percentage of grains ripens later than those of the populations with female tendency. In consequence, a shift of direction of sex ratio towards the female side may occur in the populations of plants derived from pollination with young pollen (3) The preponderance of female plants after the pollination with pollen 14–17 days old may be evidence either of the frequent occurrence of longer duration of vitality of pollen with female tendency, or (and it means the same in the case of phenotypic observations) of shorter duration of vitality of some part of pollen with male tendency (4) The ratio of male plants to female plants near to 1 1 obtained in the populations derived from pollination with pollen 12 and 24 hr old may be due to equal quantities of pollen of both tendencies at this age and, in this case, to the random process of pollination

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SIMULATED WAVE-RIDING DOLPHINS

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THE explanation of the mechanism by which dolphins are able to ride bow waves of ships, or natural surf and wind-generated waves, has stimulated much discussion and controversy Since an excellent summary of previous research on the wave-riding problem has been given by Fejer and Backus[1], only a few remarks by way of review will be given here

Although dolphins had been observed riding on bow waves of ships many times over the years, Woodcock[2,3] published the first account which included considerations of the forces estimated to be required Shortly thereafter, Woodcock and McBride[4] undertook to explain in more detail the nature of these forces They found that a 200-lb dolphin submerged in sea water would have, at most, a net

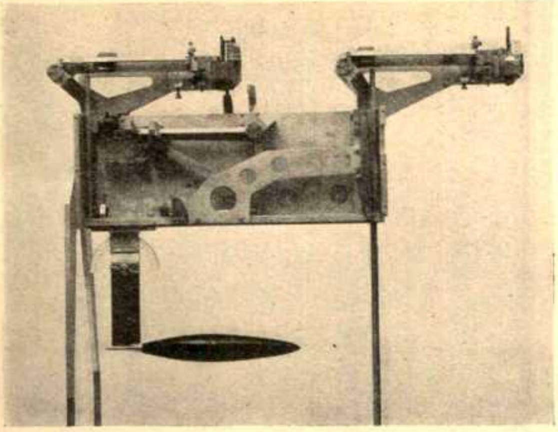

Fig. 1. The 'dolphin-like body' mounted on the 3-component force balance used in these experiments

weight of 9·2 lb. The component of this weight in the direction of the local flow was thus shown to be too small to overcome the expected viscous drag, unless the dolphin was in some manner able to maintain a laminar flow over its body-surface. The Reynolds number for these observations was $8 \cdot 4 \times 10^6$ based on body-length. For rigid bodies tested under many conditions it is known that at such a Reynolds number the boundary layer is almost certainly turbulent; hence, the conclusion would follow that the dolphin had achieved the surprising feat of maintaining a laminar boundary layer (and hence, very low drag) at a very high Reynolds number.

It was Hayes[5] who pointed out that it was the component of the total weight rather than the net weight in the direction of the local flow that provided the thrust. With this force available, there is no need to suppose the dolphin has a viscous drag appreciably lower than any other streamlined body moving at the same Reynolds number.

In spite of the lucid arguments set forth by Hayes, some workers remain unconvinced. Scholander's experiments in the open sea[6] convinced him that the Hayes explanation was not satisfactory. Scholander and Hayes[7,8] thereupon arrived at an impasse, with Scholander suggesting that further experiments be conducted under better-controlled conditions.

In discussing these considerations with Prof. Scholander, it occurred to us that the free-surface water tunnel in the Hydrodynamics Laboratory at this Institute could be used to help resolve this controversy.

Apparatus and Experimental Procedure

At tunnel operating speeds approximating 7 ft./sec., it is possible to establish a stable, standing wave in the working section of the free-surface water tunnel[9]. Such a wave was formed, and the simulated dolphin model (consisting of a streamlined body of revolution 2 in. in diameter and approximately 1 ft. long) was immersed in the wave and the hydrodynamic reactions were measured. The test body, strut support, and force balance assembly are shown in Fig. 1. Extensive force measurements had already been made on this body in previous investigations[10] using wind tunnel, water tunnel and drop tank facilities, and were therefore available for comparison. The configuration of this body, however, had been slightly modified for the present experiments by replacing the original tail and boom assembly with a shorter one. Also, an adjustable streamlined strut had been attached to this boom, thereby permitting the attitude of the test body to be determined within an angle of ½ degree.

Prior to starting the tunnel, tare forces were measured with the test body in air, at each of the angles of the test body used in the experiment. By means of an elevating mechanism, the balance and body assembly were lowered until the centreline of the body was at a depth of about 0·2 ft. The buoyant force acting on the body in still water was then measured. The tunnel was started and the standing wave established. Force readings at the two tunnel locations were made for angles of 0° and ±8° at various submergences.

Position 1 (Fig. 2) was located on the upstream slope of the wave, considerably ahead of the crest. It had been hoped that the body could be located somewhat farther downstream; but this could not be done easily because of interference with a tunnel structural member. Position 2 (Fig. 3) was in the trough of the wave. The water velocity in these tests was of necessity near the critical channel velocity, but varied locally with position along the wave. A miniature *HRS* current meter about ½ in. in diameter was used for measuring these velocities at the same longitudinal position and depth as the midpoint of the test body. These results are summarized in Table 1 as well as are some other conditions not described.

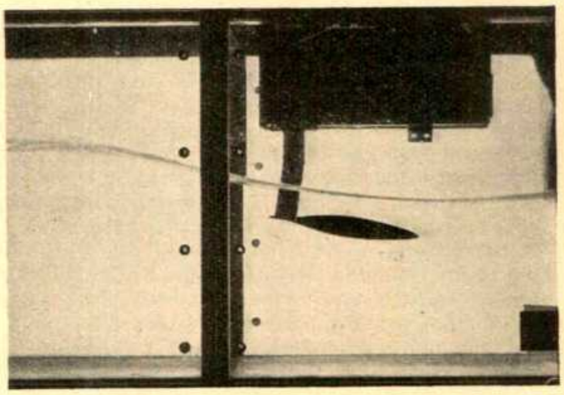

Fig. 2. 'Dolphin-body' located at tunnel position No. 1 on the forward slope of a standing wave. Net forward thrust = 0·007 lb. at a velocity of 7·02 ft./sec.

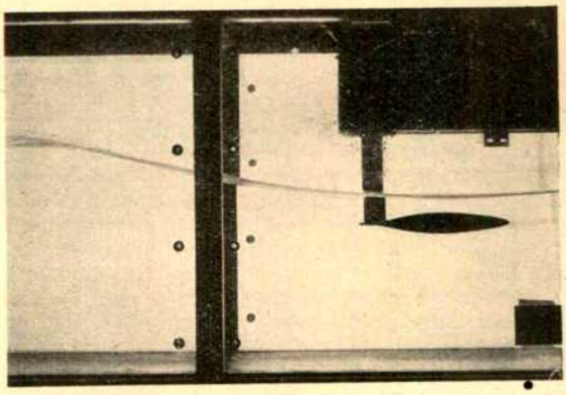

Fig. 3. 'Dolphin-body' located at tunnel position No. 2 in the trough of the standing wave. Net drag = 0·071 lb. at 7·24 ft./sec. Note the dye streak ahead of the model near the model centre-line

Table 1

Run No.	Tunnel position	Angle	Depth	Drag*	Lift	Water velocity	Remarks
1	1	−10 deg.	0·25 ft.	0·000 lb.	0·774 lb.	0·00 ft./sec.	Stationary
2	1	0	0·1	0·000	0·776	0·00	
3	1	0	0·2	0·000	0·777	0·00	
4	1	− 8	0·25	0·000	0·776	0·00	
5	1	+ 8	0·15	0·000	0·778	0·00	
6	1	0	0·2	0·031	0·889	2·78	Flat free surface (no standing wave)
7	1	0	0·2	0·032	0·775	3·54	
8	2	0	0·2	0·071	0·842	7·24	In trough of wave
9	2	+ 8	0·25	0·106	0·900	7·22	
10	2	− 8	0·05	0·070	0·829	7·26	
11	1	+ 8	0·2	0·059	1·022	7·08	Forward slope of wave
12	1	0	0·2	0·001	0·870	7·12	
13	1	− 8	0·1	0·008	0·641	7·02	
14	1	− 8	0·15	−0·011	0·748	7·08	
15	1	− 8	0·2	−0·007	0·793	7·02	
16	1	− 8	0·25	−0·009	0·800	7·04	
17	1	− 8	0·35	−0·001	0·821	7·02	
18	1	− 8	0·45	0·006	0·824	7·00	

* Negative values imply thrust.

Observations and Analysis of Results

When the test body was aligned with the slope of the water surface and submerged beneath the upstream slope of the wave in position 1, it was immediately apparent that a small, forward thrust between 0·007 and 0·011 lb. was measured (see runs 14–17). However, when the body was located in the trough of the wave at a similar submergence (runs 8, 9, 10) the body experienced a drag force ranging from 0·07 to 0·106 lb. These figures can be compared with the still-water buoyant force of 0·78 lb. to establish scale. Although no attempt was made to find the optimum location of the body with respect to the wave in so far as thrust is concerned, it is clear that a net thrust can be achieved with a rigid streamline body under even the gentle slope of the wave produced in the tunnel.

The observed thrust varies with depth and changes to a drag both when the body broaches and when the submergence exceeds about three diameters of the body. It should be remembered that the drag of the strut is included in these results so that the present results do not reflect the body drag alone. The submergence for least drag observed herein—about one body diameter—is roughly the same as that reported for dolphins in bow waves.

Further insight into the mechanics of the force system acting on the body is obtained by resolving the forces along axes parallel to and perpendicular to the local flow angle[5]. For example, for run 14 of Table 1 the resistance parallel to the local flow is 0·054 lb. and the force normal to the flow (or free surface) is 0·745 lb., with the estimated local flow angle of 5°. This latter force, perpendicular to the wave surface, can be regarded as an inclined buoyant force as mentioned by Hayes. These forces, it must be recalled, are hydrodynamic in origin and do not include the body-weight. Let it be supposed now that the self-propelling capability of a body, the weight of which is equal to that of the displaced fluid, is to be determined. This weight of 0·777 lb. resolved along the direction of flow must exceed the flow resistance of 0·054 lb. For the present example, this force is not only exceeded but there is also, in fact, a net propelling force of 0·013 lb. available. The forces are not precisely in equilibrium for this example; the body, if free, would tend to sink to a slightly greater depth, where the vertical force is seen to be larger.

The flow resistance of 0·054 lb. resolved along the local flow-angle is roughly comparable with the drag measured in the trough of the wave (run 8) and both are typical of viscous drag measurements in a turbulent flow[9,11]. Laminar skin friction is therefore seen not to be a necessary condition for bow wave-riding as was originally contained in the suggestion by Woodcock.

It is also interesting to observe that the vertical forces, both in the slope of the wave and in the trough, differ noticeably from the buoyant force in still water. This is thought to be due to a combination of two effects: the well-known repulsion experienced by a submerged body moving under an otherwise flat, free surface, and more important, the curvature of the local streamlines. Summary calculations have shown the contributions of the latter term to the lift force to be of the correct magnitude.

Conclusion

The mere existence of the measured forward-acting thrust on the test body in a wave demonstrates that a rigid body with turbulent flow can perform the wave-riding trick. Analysis of the measurements shows further that the force system is equivalent to a dynamic buoyant force perpendicular to the inclined wave surface and a resistance parallel to this surface. The dynamic buoyancy is approximately equal to the weight of the displaced liquid, while the viscous resistance is substantially equal to that measured on the body when not in the wave.

In spite of these incomplete experimental results, it appears inescapable that Hayes's arguments are substantially correct.

This work was supported, in part, by the Department of the Navy, under Contract No. 123 (60530) 24917*A*.

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LETTERS TO THE EDITORS

SPACE SCIENCE

Errors in Orbital Predictions for Artificial Satellites of Earth

SEVERAL workers[1-3] have carefully investigated variations in the accelerations of artificial satellites of Earth, but no one seems to have published a systematic study of the errors in orbital predictions. The results of such work[4] are presented in this communication. A stochastic model was constructed which takes into account fluctuations in atmospheric density, observational errors, and the correlations introduced by smoothing. Errors in actual predictions issued by the Vanguard Computing Center, National Aeronautics and Space Administration Computing Center, Smithsonian Astrophysical Observatory, and Naval Weapons Laboratory were also computed, for comparison with the theoretical model.

Table 1. ERRORS IN PREDICTION NEAR PEAK OF SUNSPOT CYCLE

Satellite	Dates	No. of predictions	$-dP/dn$ (min./rev.)	N (rev.)	E_0 (min.)	E Actual (min.)	E Theoretical (min.)
Explorer IV	1958	8	$2{\cdot}15 \times 10^{-3}$	165	0·024	3·2	3·3
Sputnik III	1958	7	$1{\cdot}32 \times 10^{-3}$	220	0·01	3·3	1·9
Vanguard I	Autumn 1958	20	$5{\cdot}5 \times 10^{-5}$	154	0·056	0·25	0·22
Vanguard I	Summer 1959	11	$2{\cdot}1 \times 10^{-5}$	154	0·056	0·13	0·097
Vanguard I	Winter 1959–1960	7	$6{\cdot}5 \times 10^{-6}$	154	0·056	0·062	0·061
Atlas-Score	Dec. 1958–Jan. 1959	1*	$2{\cdot}2 \times 10^{-2}$	271	0·3	67	74

* A single observation has no statistical significance. This case is included merely to show how large the error can be when the rate of change of period is large.

Table 2. ERRORS IN PREDICTION HALF-WAY BETWEEN SUNSPOT MAXIMUM AND MINIMUM

Satellite	Dates	No. of predictions	$-dP/dn$ (min./rev.)	N (rev.)	E_0 (min.)	E Actual (min.)	E Theoretical (min.)
Tiros II	Dec. 1960–May 1961	12	$3{\cdot}7 \times 10^{-6}$	250	0·08	0·12	0·08
Vanguard I	Oct. 1960–May 1961	12	$7{\cdot}4 \times 10^{-6}$	150	0·06	0·12	0·06
Transit III-B	Feb.–Mar. 1961	10	$1{\cdot}05 \times 10^{-2}$	22	0·04	0·74	0·50
Echo I	Oct–Dec. 1960	6	$6{\cdot}8 \times 10^{-4}$	145	0·04	4·4	3·3

The actual and theoretical errors in orbital predictions are given in Tables 1 and 2 and Fig. 1. Table 1 contains the errors in 1–2-week predictions made near the peak of the sunspot cycle. Fig. 1 presents the same data graphically. Table 2 shows the errors in predictions half-way between sunspot maximum and sunspot minimum. In Tables 1 and 2, N is the number of revolutions predicted, beginning at the centre of the smoothing interval. The smoothed rate of change of period is dP/dn (min./revolution). The root-mean-square prediction error, E (min.), includes the contributions of observational errors and drag fluctuations. The theoretical prediction error caused by observational errors alone is designated by E_0.

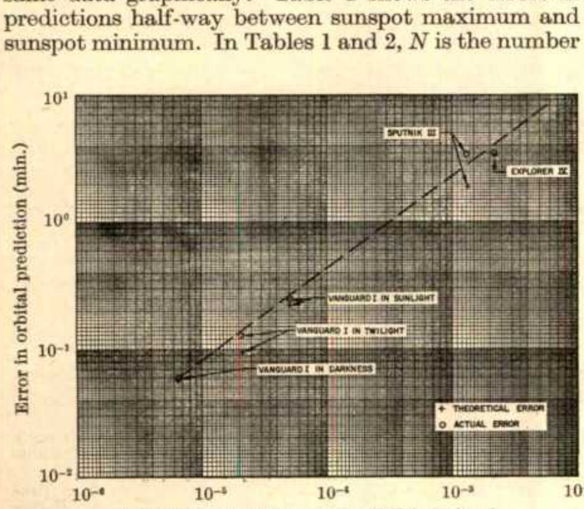

Fig. 1. Errors in orbital predictions running for 1–2 weeks

It is interesting to note that observational errors were the principal cause of errors in orbital predictions for only one of the cases shown in Fig. 1, that of *Vanguard I* with its perigee in darkness (winter 1959–1960). In all the cases shown in Fig. 1, the prediction errors attributable to observational errors were smaller than the total error for *Vanguard I* in darkness. If the errors in the predictions had been caused only by observational errors, then the prediction errors would have been independent of the smoothed rate of change of period. A detailed discussion of the theory and the method of calculation is given in ref. 4.

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ASTROPHYSICS

Secular Variations of Short-lived Sunspots

LARGE secular variations have been found in short-lived sunspots as recorded in "Sunspots seen on one Day Only" of the Greenwich Photo-Heliographic Results, 1879–1957. The 11-yr. period often appears like a subsidiary perturbation.

A pertinent example is seen in Fig. 1, which shows the ratio K between the number of one-day spots at less than 65° from the central meridian, and the number of spots seen throughout two days or more. In Fig. 2 is plotted the number of one-day spots without umbra as a percentage of the total number.

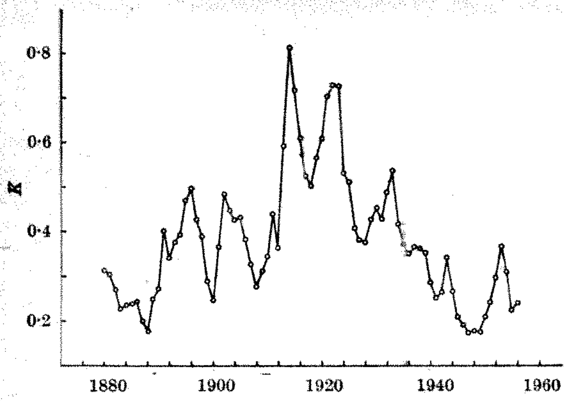

Fig. 1. Sliding 3-yr. mean for the period 1880–1956 of the ratio between the number of one-day spots and the number of spots observed throughout two or more days

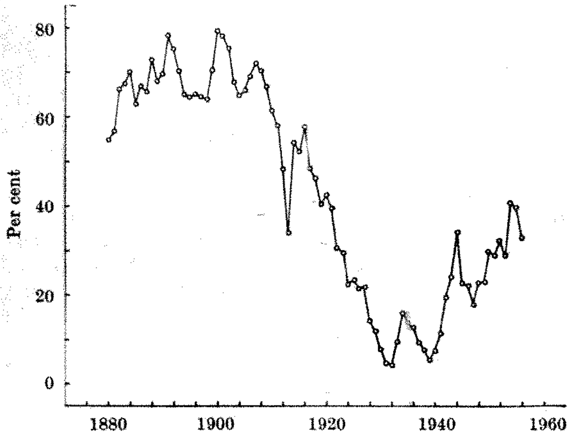

Fig. 2. Sliding 3-yr. mean for the period 1880–1956 of the number of one-day spots without umbra as a percentage of total spot number

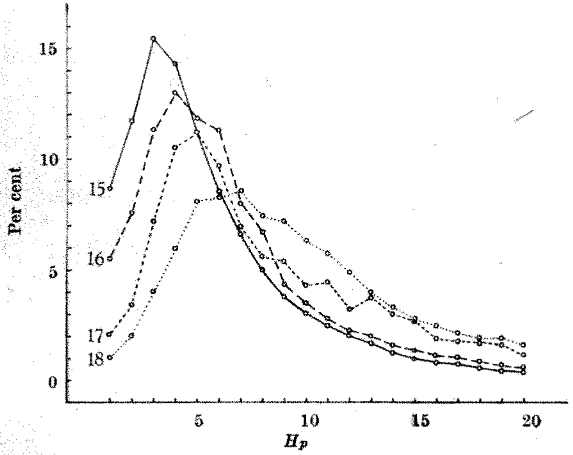

Fig. 3. Sliding mean of the number of one-day spots with determined total area as a percentage of total numbers for cycle 15 (1914–24), cycle 16 (1922–34), cycle 17 (1932–45) and cycle 18 (1942–54)

The frequency distribution with respect to area (both total area and area of the umbra) varies from one cycle to the next, and in such a way that the frequency maximum decreases with increasing area. This is shown in Fig. 3, which gives the smoothed frequency distribution with respect to total area for the solar cycles 15–18.

In an earlier paper[1] secular variations were found in the relation between magnetic field-strength H and total area A_p of sunspots, $H = a . \log A_p + b$, where a and b are coefficients that vary systematically from one cycle to the next. A connexion was predicted between the secular variations and variations in number and distribution of area of short-lived spots. This is borne out by the present investigation, which shows a marked correlation between the coefficients a and b and the mean values of K and A_p for one-day spots in the various cycles.

The research is supported in part by the Geophysics Research Directorate, Office of Aerospace Research, United States Air Force.

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RADIOPHYSICS

Coincidence of the Explosive Phase of Solar Flares with 10·7-cm. Solar Noise Bursts

The observational evidence of an explosive phase in the development of the solar flare, as reported by Athay and Moreton[1], is of great interest. This phase appears after the commencement of the flare, is well defined, and thus provides a stage in the evolution of certain flares which may be of significance for examination of flare-related phenomena, such as solar noise bursts, etc. The Athay and Moreton paper lists eleven selected flares with 'explosive' phases. Two of these flares occurred outside the normal observing period at Ottawa, but the remaining nine can be compared with 10·7-cm. solar noise bursts observed at Ottawa. These cases, while few in number, sharpen the already well-established close relationship between flares and 10·7-cm. radio bursts which have been derived from other work done on the Ottawa data[2].

The relationships between the starting times of the flare, the explosive phase (as given in Table 1, of ref. 1) and the starting time of the 10·7-cm. burst (as given in the "Tabulation of the 10·7-cm. Outstanding Occurrences" published in the *CRPL-F*, Part *B*. *Solar-Geophysical Data*) are shown in Fig. 1. In order to provide identification, each radio event has been assigned the same letter as the associated flare event. The upper histogram shows the distribution of the difference in time of start of the flare and of the explosive phase; the lower histogram shows the dis-

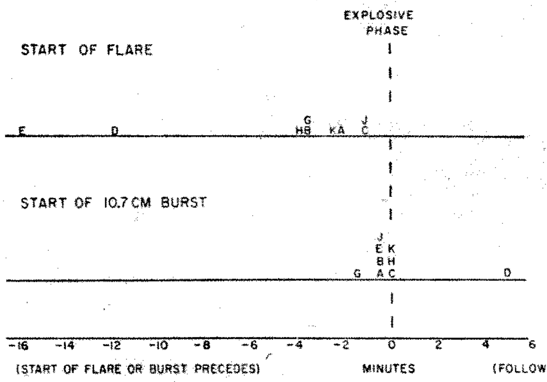

Fig. 1. Upper, distribution of the difference in time of start of the flare and of the explosive phase; lower, distribution of the difference in time of start of the 10·7-cm. burst and the explosive phase of the flare

tribution of the difference in time of start of the 10 7-cm burst and of the explosive phase of the flare. Comparison of the two histograms shows that, on the average, the start of the 10 7-cm burst precedes the explosive phase of the flare by approximately 1/2 min (excluding event *D*) whereas the start of the burst follows the commencement of the flare by approximately 2 min (excluding events *E* and *D*).

The coincidence of the time of start of the burst and the time of the explosive phase of the flare is not altered significantly when the criterion used to determine the start of the burst is examined. Burst commencement is that time when the rising flux has departed from the steady background-level by an amount equal to the residual receiver noise. In each of the nine cases examined here, the bursts are of the impulsive type, with a well-defined and rapid increase of emission from the steady background to a peak. Variations in interpretation and timing errors could introduce an uncertainty in the time of the start of the bursts of no more than ± 1/2 min.

Examination of the time profile of the 10 7-cm burst shows that there are two basic types of bursts: the impulsive burst, and the long-enduring burst (gradual rise and fall)[3]. As mentioned above, the bursts which are here associated with the explosive phase of the flare are all of the impulsive type. They are all, also, of more than average intensity. It may be of some significance that, in every case but one, the impulsive bursts are superimposed on long-enduring enhancements of radio emission of lesser intensity, and that in the one exception the occurrence of sunset precludes any observation.

The occurrence of an impulsive burst on a long-enduring enhancement is regarded as a single composite event from the frequency with which the association appears. In the large number of cases which have been observed, the separation of the commencements of the impulsive burst and the long-enduring enhancement range from a few minutes to several hours. In the extreme, when the two bursts presumably start at the same time, two types of profiles have been observed in the resultant composite event, and have been classified differently in the published 10 7-cm. burst data. When the maximum of the long-enduring burst appears after the occurrence of the impulsive burst, the composite event is classified as a long-enduring burst with the impulsive burst superimposed on it. However, when the impulsive burst is followed by a long-enduring enhancement which shows only a steady decline, the event is classified as a single impulsive burst with a post-burst increase.

In the case of the nine radio events associated with the flares with explosive phases, both of these types of composite events have appeared. In cases *D* and *K*, the impulsive bursts are superimposed on long-enduring bursts which commence 23 min before the impulsive event, and continue for approximately 1 hr after the end of the impulsive burst. Burst *D* is the only impulsive feature in this composite event, but burst *K* is the third and most intense, by a factor of 3, of a group of three impulsive features in this composite event. Radio event *H* is a period of irregular activity in which a great number of impulsive events of various intensities appear superimposed on an elevated background. The most intense of these impulsive events has an intensity which is 17 times greater than the next largest of the superimposed features, and it is this intense impulsive feature which corresponds in time with the explosive stage of the flare. The period of irregular activity starts 6 min. before and ends approximately 30 min after this impulsive burst. In case *J*, sunset coincides with the final decline of the impulsive burst, and, while no long-enduring enhancement of emission was in evidence just prior to the burst, it is impossible to know whether any enhancement followed it. In all the other cases, *A*, *B*, *C*, *E*, and *G*, the impulsive bursts are followed by relatively long-enduring enhancements of emission of much less intensity (post-burst increase).

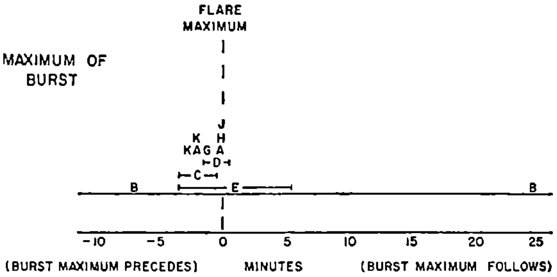

Fig 2. Distribution of the difference in time of 10 7-cm burst maximum and the time of flare maximum

The comparison of the time of burst maximum with the time of flare maximum is shown in Fig. 2. In the case of complex bursts, the times of both maxima are indicated by the letter identifying the event. In the cases where the burst produced off-scale readings and the maxima were not recorded, the off-scale period is indicated by a line. Although this histogram shows more scatter than that of the start of the bursts in relation to the explosive phase, it indicates that burst maximum is reached shortly before flare maximum in general.

The preceding detailed analysis involving a group of flares selected for the explosive phase in their evolution shows that the time of start of the 10-cm burst coincides with the explosive phase rather than the commencement of the flare, and these bursts, without exception, are of the impulsive type. A second type of 10-cm burst, the long-enduring burst (gradual rise and fall), cannot be directly related to this feature. This suggests that it is related to some other feature in the evolution of the flares.

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OCEANOGRAPHY

Sea Surface Temperature in Coastal Waters of the British Isles

MR. A. J. LEE has recently commented[1] on my paper dealing with variations of sea surface temperature in coastal waters of the British Isles. He points out that I made no mention of the part played by currents in determining the winter pattern of sea surface temperature. I was, however, primarily concerned with the *shape* of the isotherm patterns. The presence of the relatively shallow water of the Dogger Bank area will, in itself, ensure that the pattern will consist of two tongues, although the level of temperature of these tongues will be largely determined by the strength of the currents mentioned by Mr. Lee.

Mr. Lee also criticizes the statement that the English Channel is isothermal or very nearly so throughout the year. Taking this statement in isolation, the criticism is fully justified, but it was intended to be taken as a comment on Fig. 1(*b*), which shows the mean temperature profiles at a position (49° 49′ N., 02° 33′ W.) towards the middle of the English Channel. Perhaps this should have been made clearer in the text.

I did not discuss the accuracy of the observations because the paper was written as an aid to local weather forecasting, and for this purpose an accuracy of ± 0.5° C. is adequate. Most of the sea temperatures used were obtained by the bucket method, and it is generally recognized that the above-mentioned standard of accuracy can be obtained by this method, if the readings are taken with reasonable care. Measurements of sea temperature made recently on Ocean Weather Ship *Monitor* by a thermistor, supplied by the Fisheries Laboratory, Lowestoft, and floated astern of the ship, steaming slowly at about 2 knots or when stopped 'on station', at a distance of 10 m., show that the differences from the bucket measurements rarely exceed 0.5° C. (only 1 occasion in 34 over a period of four days). There is always a danger of much larger errors of reading, for example, 5°, but as shown in my paper these can be readily distinguished if the sea temperature isotherms are drawn intelligently.

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PHYSICS

X-Ray and Neutron Diffraction Examination of *p*-Diphenylbenzene

The crystal structure of *p*-diphenylbenzene was first determined by Pickett[1], using projection X-ray diffraction techniques. Because of the considerable theoretical interest in the bond-lengths of conjugated carbon-carbon bonds[2,3] a refinement of the structure has been commenced, using full three-dimensional X-ray and two-dimensional neutron diffraction techniques.

The refinement of the data on *h*0*l* neutron diffraction projection has given some interesting results, which are discussed here. The neutron intensity data were collected on the single crystal spectrometer installed at the *Hifar* reactor at the Australian Atomic Energy Commission Establishment. The intensity of the neutron beam from the (1,1,1) plane of a copper monochromating crystal was 140,000 counts/sec./cm.^{2}, when set to reflect neutrons with a wave-length of 1.0015 ± 0.0005 Å. The intensities at all the *h*0*l* reflexions out to sin θ/λ = 0.8 Å^{-1} were measured, and of the 248 possible reflexions, 139 had intensities sufficiently greater than the incoherent scattering background. By comparison, in the *h*0*l* projection in the X-ray case, 107 of the 140 possible reflexions out to the copper $K\alpha$ limit were obtained.

The *h*0*l* projections of both the neutron and the X-ray structure have been refined simultaneously. This has enabled a comparison to be made of the relative power of the two methods. The X-ray structure determinations showed that the thermal motions of a number of the atoms in the molecule were markedly anisotropic. Significant peaks were found in the difference synthesis at positions corresponding to the hydrogen atoms, when these were not included in the structure factor calculation. From this synthesis approximate positions of the hydrogen atoms could be determined. The resolution obtained was poor, however, there being considerable overlapping of the features due to thermal anisotropy with those caused by hydrogen atom contribution. Consequently, an accurate evaluation of the thermal parameters and the positions of the hydrogen atoms was not possible. The corresponding neutron diffraction projection showed much better resolution and confirmed the X-ray observations. The atomic peaks in the Fourier synthesis[4] (Fig. 1), based on the neutron diffraction data, are well resolved. The form of the peaks gives clear indications of the extent of the anisotropy in the thermal motion of the atoms. The negative peaks corresponding to the hydrogen atoms are well resolved. This has led to an accurate determination of their positional parameters.

Certain interesting features of the structure are obvious from an examination of the neutron diffraction Fourier synthesis. First, there are significant deviations of some of the atoms from their normal positions so that the carbon skeleton of the molecule is slightly distorted, presumably for packing considerations. Each of the benzene rings has its normal configuration, but the two end rings are out of parallel with the central ring by approximately 1° 30′ (Fig. 2). Secondly, several of the hydrogen atoms are distorted from their normal positions due to steric hindrance within the molecule. Thus the hydrogen atoms attached to the C_1 and C_5 atoms (Fig. 2) are displaced in directions which increase the distance between them. This is in accordance with expectations, for if they occupied their normal positions they would be only 1.8 Å apart, whereas the

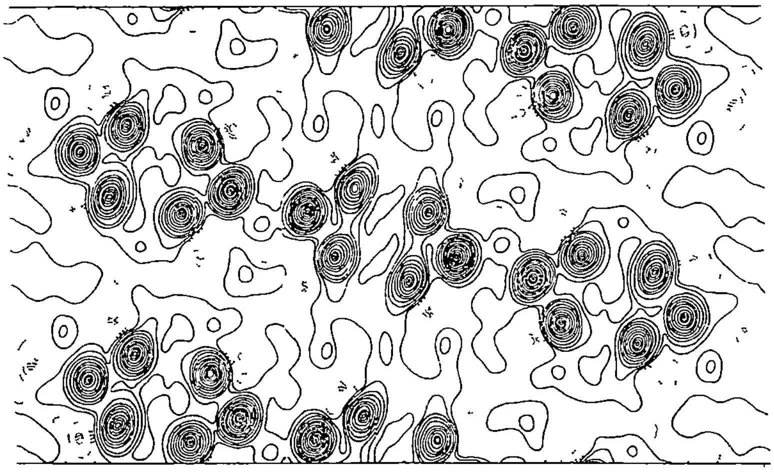

Fig. 1. A Fourier projection on the (010) plane of *p*-diphenylbenzene at room temperature based on neutron diffraction data. Contour intervals are in arbitrary units. Full lines are positive, broken lines are negative contours

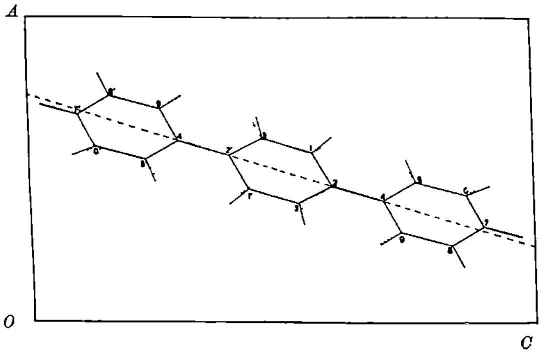

Fig 2 Atomic positions in the Fourier projection of *p*-diphenylbenzene Full lines are the undistorted C—H bonds

sum of their Van der Waals radii is 2 4 Å The equivalent pair of hydrogens attached to the C_3 and C_7 atoms is similarly displaced

It is also possible from work on the thermal parameters to deduce in projection the predominant modes of thermal vibration within the molecule The C_2 and C_4 atoms have thermal motions considerably less in magnitude than those of the remaining carbon atoms The motions of all the atoms are markedly anisotropic, the direction of maximum vibration being perpendicular to the long axis of the molecule It may be shown from this that two modes of vibration predominate The first appears in projection as an oscillation about the centre of gravity of the molecule, and the second is an oscillation about the long axis of the molecule, that is, the line joining the C_7 and $C_{7'}$ carbon atoms The presence of the former mode confirms the recent suggestions of Bacon and Curry[5] that the outer benzene rings in mòlecules of this type have considerably more freedom of movement than the inner ones

The X-ray and neutron diffraction work is continuing, and a full report will be published elsewhere

The first three authors are indebted to the Australian Institute of Nuclear Science and Engineering for the generous financial assistance that has made the neutron work possible The co-operation and assistance of the *Hifar* reactor staff is gratefully acknowledged

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Energy-Level Model for High-Resistivity Gallium Arsenide

ALTHOUGH gallium arsenide is usually prepared with low resistivities (< 1 ohm-cm), it has also been prepared for several years with very high resistivities (> 10^6 ohm-cm) This latter material can be produced by zone refining[1], by the addition of impurities such as oxygen[1], or under growth conditions where the main shallow donor, silicon, is reduced below roughly one part per million[1] To explain the occurrence of high-resistivity gallium arsenide, Welker[2] suggested that the donors and acceptors are built into the lattice in pairs More recently, Allen[3] has termed this material 'semi-insulating', and claims that the existence of *n*-type high-resistivity material requires that there be a very high degree of compensation between the shallow donor and a deep acceptor He also states that because of the frequency of occurrence of high resistivities, some mechanism of automatic compensation must be operative Later, to explain gradual transitions in resistivity along floating-zone refined ingots, Gooch *et al* [4] have shown that a wide range of resistivities can still occur even on assuming a high degree of automatic compensation However, according to their model, the degree of automatic compensation must vary along the ingot, yet cannot vary too much otherwise *p*-type material is predicted, which is not observed

A three-energy-level model is proposed here which explains the existence of high-resistivity material without requiring that there be a high degree of compensation, and therefore no need for invoking the occurrence of automatic compensation Similar to the above model[4], a wide range of resistivities will be shown to be possible The energy-level scheme is shown in Fig 1, where N_D is the density of shallow donors, N_{DD} the density of deep donors, and N_A is the density of acceptors It is easily seen that high-resistivity material will result when $N_A > N_D$ and $N_{DD} > (N_A - N_D)$ These conditions are not very restrictive, and explain why high-resistivity gallium arsenide can occur so frequently For example, for a given concentration of N_{DD}, high resistivity will result when N_D is removed at a faster rate than N_A, as possible in zone refining, or else that the concentration of N_A is increased, which might occur when oxygen is added Of course, when N_D is somewhat greater than N_A, the material is low-resistivity *n*-type, and the N_{DD}-levels are neutral It is also seen that so long as $N_{DD} > N_A$, *p*-type material will not occur

The model of Fig 1 can be treated simply mathematically assuming that the activation energy of the deep donor is E_d, and that of N_D and N_A is zero, however, relaxing these latter restrictions does not detract qualitatively from the results Then the electron concentration, n, is given by

$$n = \frac{N_c \, N_{DD} \exp \left(-E_d/kT\right)}{2n + N_c \exp \left(-E_d/kT\right)} + \frac{n_i^{\,2}}{n} - (N_A - N_D) \quad (1)$$

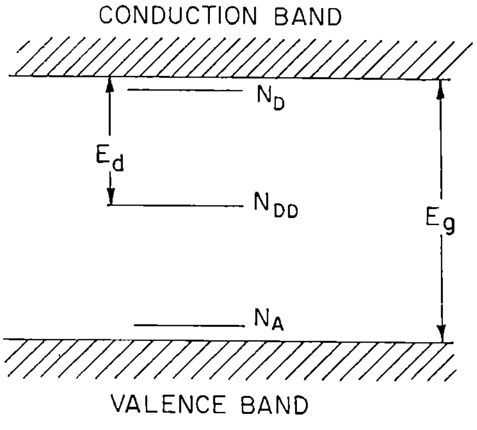

Fig 1 Energy-level diagram for the model

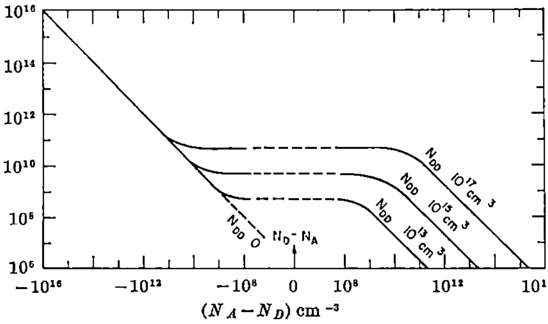

Fig 2 Variation of electron concentration, n, with $(N_A - N_D)$ at room temperature for various values of N_{DD}, with $E_d = 0\ 76$ eV

where n_i is the intrinsic electron concentration, and N_c is the effective density of states in the conduction band

A value for E_d of 0 76 eV has been deduced by Gooch *et al* [4] Using this value, the quantity N_c exp $(-E_d/kT)$ in the denominator of equation (1) can be neglected for n-type samples and then equation (1) can be easily solved for n The variation at room temperature of n with $(N_A - N_D)$ is shown in Fig 2 for $N_{DD} = 10^{13}$, 10^{15}, and 10^{17} cm^{-3}, using $N_c = 6\ 3 \times 10^{17}$ cm^{-3} and $n_i^2 = 1\ 1 \times 10^{13}$ cm^{-3} The results of this model are quite similar to those of Gooch *et al* [4] in that a wide range of resistivities can occur, and further three conductivity-ranges are predicted At high electron concentrations, the Hall coefficient should be independent of temperature, at very low electron concentrations, the activation energy deduced from Hall effect measurements should give E_d, however, only in the case of extremely close compensation where $N_D \approx N_A$ should the activation energy be $E_d/2$, since in this range the deep donor is effectively uncompensated

A donor located at the centre of the energy band gap of gallium arsenide has been detected by thermally stimulated current measurement[1,5], and has been found, where experimentally feasible, in every crystal to date at densities of 10^{16} cm^{-3} or greater From Fig 2, it can be seen that the electron concentrations will be between 10^{6} and 10^{8} cm^{-3} for values of $(N_A - N_D)$ in the range from 2×10^{15} to 2×10^{13} cm^{-3}, which can easily occur as the shallow donor concentration is reduced This would also explain why there is almost no p-type gallium arsenide produced in undoped growth of gallium arsenide, but instead why usually either very high or very low resistivity n-type material is found Actually, thermally stimulated current measurements[1,5] have also shown the presence of other deep donors with shallower levels than 0 76 eV In this case, the three-level model is an over-simplification, and one would expect that the variation of Hall coefficient and conductivity with temperature would exhibit intermediate energy-levels, especially below room temperature, and this has already been observed[5]

Conductivity versus temperature slopes have been measured in n-type gallium arsenide with activation energies greater than half the band gap, that is, as large as 0 8 and 0 9 eV (C Hilsum, private communication, R H Bube, private communication) This behaviour is explicable on the present model In equation (1), it can be shown that at room temperature and above, for $N_{DD} > 10^{11}$ cm^{-3} and for $E_d \gg 0\ 8$ eV, both n_i^2 and the term N_c exp $(-E_d/kT)$ can be neglected Then so long as $(N_A - N_D)^2 \gg 2\, N_C\, N_{DD}$ exp $(-E_d/kT)$, n will vary as

$$n = \frac{N_C N_{DD}}{2\,(N_A - N_D)} \exp\,(-E_d/kT) \qquad (2)$$

The above condition is quite easily met Typical values could be $N_{DD} = 5 \times 10^{16}$ cm^{-3}, $N_D = 10^{12}$ cm^{-3}, and $N_A = 10^{13}$ cm^{-3}, and the sample will still be n-type It is also interesting that, depending on the magnitudes of $(N_A - N_D)$ and N_{DD}, a sample of gallium arsenide might never become intrinsic, and can exhibit a conductivity or Hall constant versus temperature slope greater than the intrinsic slope up to the melting point

Finally, it should be noted that the distinction drawn by Gooch *et al* [4] between 'semi-insulating' gallium arsenide, and 'high-resistivity semiconducting' gallium arsenide prepared by diffusion of copper into low-resistivity material[6], is non-existent in principle on the present model, since the concept of automatic compensation has not been invoked

We are indebted to Dr R H Bube for helpful discussions, and to Drs Gooch, Hilsum and Holeman for placing their paper at our disposal prior to publication This work was sponsored by the Electronics Research Directorate of the U S Air Force Office of Aerospace Research under contract

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GEOLOGY

Natural Glass from beneath Red Clay on the Floor of the Atlantic

During April and May 1961 on a cruise of the R V *Chain*, a programme of coring deep-sea sediments from the Atlantic for geochemical investigations was conducted At 19° 23′ N, 40° 53′ W a modified Kullenberg corer was lowered through 4,430 m of water to the summit of a rise on the ocean floor The topography of the sea bed in this region is irregular, but between the hills and rises the average depth is approximately 4,900 m At the point where the corer struck the sea bed the surface sediment is a red clay, but no sediment core was obtained since at a very short distance below the surface the red clay is underlain by solid material The corer struck and broke the surface of this solid material, a fragment of which was recovered in the pilot corer, together with the overlying red clay From the position of the solid fragment in the pilot corer the thickness of the clay can be estimated to be not more than 38 cm The solid fragment is a very dark brown, almost black natural glass of irregular shape, but with approximate dimensions 3 cm × 2 5 cm × 1 3 cm Two faces of the fragment were freshly broken surfaces On two other faces a thin (1 mm) zone of devitrification occurs with small areas of manganese deposition on the outer surface The two other faces appear to be natural fractures Internally the fragment contains small irregular cavities about which slight devitrification has also occurred When collected the fragment showed a marked tendency to disintegrate into smaller fragments, a consequence, no doubt, of the impact of the corer on the brittle glass Red clay containing broken shards of the same glass was found adhering to the piston of the main corer, and it is likely, therefore, that the glass formation is too extensive to be interpreted as a boulder contained within the red clay It is believed to represent the solid surface on which the overlying sediments rest So far as is known this is the first sample collected of solid rock indisputably from below the sediments of the deep Atlantic

The glass fragment when collected weighed approximately 18 gm Part of it was crushed and natural glass fragments hand-picked from devitrified material for chemical analysis Examined optically these glass fragments appear homogeneous without crystallites, microlites or any other crystallization phenomena The chemical composition of this glass is quite remarkable, namely (percentages), SiO_2 25 81, Al_2O_3 19 35, TiO_2 2 02, Fe_2O_3 15 05, FeO 0 63, MnO 0 15, MgO 1 29, CaO 1 25, Na_2O 1 68, K_2O 0 89, H_2O 31 02, P_2O_5 0 08, Cl_2 0 97, uncorrected total 100 19 per cent, less 0 for Cl 0 22 per cent, corrected total 99 97 per cent It is quite different from compositions of natural igneous glasses from the continental regions of the world The high chlorine content suggests contamination with sea salts However, the fragments analysed were hand-picked from the interior of the larger fragment collected, and such a high chlorine content, equivalent to 1 6 per cent sodium chloride, cannot be attributed to surface contamination by sea water during collection The high water content also suggests contamination with sea water Attempts to determine the distribution of the water between loosely bound positions (H_2O-) and tightly bound positions (H_2O+) did not meet with success The loss in weight on heating the powdered glass for 20 hr at 108° C corresponded to 22 18 per cent of the total powder, but this figure slowly rises with continued heating Loss of water appears to be accompanied by crystallization of the glass so that any H_2O- figure determined does not correspond to the original material Thus the total H_2O figure has not been broken down into H_2O+ and H_2O- The very high Fe^{+++}/Fe^{++} ratio suggests that the material was hydrated while still in a molten condition, supporting the view that the water content of this glass is original, despite being so much greater than the water content of silicate glasses produced experimentally in the laboratory

A more extensive account of the properties of this glass and its possible origin will be published elsewhere Meanwhile we urge oceanographers to make further attempts to collect samples from the subsediment surface of the Atlantic floor in order to demonstrate whether this first sample is truly representative of that surface or merely an unusual type of limited distribution

Grateful acknowledgment is made to all aboard the R V *Chain* for help in the coring operations and particularly to R G Leahy, P L Sachs and G L Erlanger This work was supported by the U S Office of Naval Research under a contract with the Woods Hole Oceanographic Institution

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Ultrabasic Liquids

FOR a number of years we have been investigating the problem of ultrabasic rocks with particular reference to evidence for and against the existence of ultrabasic liquids[1,2] We believe that our published results have largely established their existence and this has been further substantiated by Wyllie[3], and by Clark and Fyfe[4] Although Clark and Fyfe chiefly confine the application of their experimental results to what may be broadly termed the serpentinite problem, their conclusions have a very restricted validity if it is implied that serpentinites are the only type of ultrabasic rock which could have been mainly or wholly liquid In the ultrabasic rocks investigated by us most of the olivine is not serpentinized

The results of our recent investigation of picritic and peridotitic rocks add further support to the existence of ultrabasic liquids, but at the same time have revealed certain complicating factors the significance of which has yet to be evaluated The principal purpose of this communication is briefly to record and discuss some of these results

In West Greenland and the Scottish Hebrides we have made an extensive and detailed examination of marginal phenomena in what is by far the best developed series of ultrabasic (picrite and peridotite) minor intrusions within our knowledge As recorded in ref 2*a*, the degree of contact metamorphism is very variable and has no apparent relationship with the size of the intrusion To the evidence of high temperature on Soay[3] we can now add similar evidence from the Island of Scalpay in Skye and from Ubekendt Ejland in West Greenland On Scalpay, a picritic intrusion 3 ft in average thickness has fused xenoliths of a very quartzose sandstone to a clear glass (Fig 1) The picritic intrusion at Igdlorssuit on Ubekendt Ejland, where it is 10–12 ft thick, has largely liquefied the marginal 3–6 in of the contiguous zeolitic olivine-rich, basaltic lava (Fig 2), the liquefaction possibly having been facilitated by the fluxing action of water derived from zeolites But in the case of many otherwise similar ultrabasic minor intrusions, and in parts of the Soay, Scalpay and Igdlorssuit intrusions already mentioned, the effects of contact metamorphism are so slight that much lower temperatures are apparently implied One can only infer that in such cases some factor other than the inward diffusion of water, suggested by Clark and Fyfe, has been responsible for the relative lack of thermal metamorphism In seeking an explanation, it seems reasonable to refer to the manner and mechanism of emplacement of these intrusions The differences between their contact effects may in fact be due not

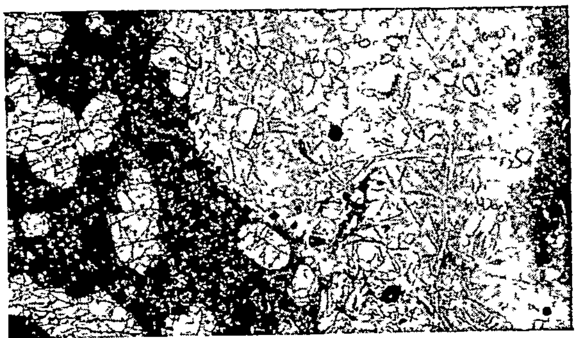

Fig 1 An ultrabasic contact (containing olivine phenocrysts) with a sandstone xenolith (containing small quartz relicts and acicular orthopyroxene) largely fused to form a clear glass (× 10)

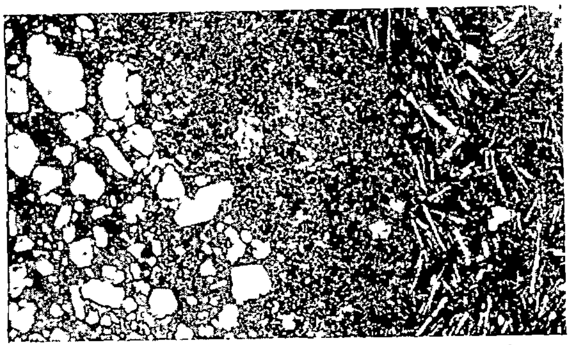

Fig 2 A later ultrabasic contact (containing non-skeletal olivine pheno-crysts) with rheomorphic, fused lava mixed with an earlier ultrabasic contact facies (containing skeletal olivines) (× 3)

to differences in the temperature of the intruding magma, but to differences in the period of time during which the adjacent country rocks were maintained at similar temperatures, the total heat effect at any one point being dependent, among other things, on the amount of magma that has passed that point and its rate of movement Variation within individual intrusions cannot be explained on this basis, but if ultrabasic magma, intruded at high temperature at one point, cooled and partially crystallized there before being translated to another position in relation to the country rock, it might well have much less thermal effect in its new position

Variability in the ultrabasic contact facies might be explicable by mechanisms similar to the above Development of skeletal olivines in the selvages of some intrusions, but not in others, could be accounted for by differences in rate of flow of magma In cases, such as those on Ubekendt Ejland (where ultrabasic selvages in which all the olivines are skeletal give place when traced laterally to selvages in which the olivines are predominantly non-skeletal), the second mechanism, namely cooling and partial crystallization at one point followed by translation to another, could apply In this way, the original, relatively quickly-congealed selvages with skeletal olivines could be dispersed and replaced by selvages characterized by non-skeletal phenocrysts (Fig 2) This does not diminish the significance of the development of skeletal olivines, which constitutes a much more common characteristic of ultrabasic contact facies than formerly suspected[5], as evidence of an ultrabasic liquid Nor does it imply that, where the marginal olivine is not skeletal, the degree of contact metamorphism should always be appreciably less

The direct experimental determination under hydrothermal conditions, by Clark and Fyfe, of the liquidus temperature of serpentinite, reinforces earlier deductions[3,6] which placed such temperatures considerably lower than had hitherto been regarded as possible An extension of this work should yield experimental results more closely applicable to the wealth of natural data now available

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METALLURGY

Fatigue under Random Loads

It is often necessary to deduce the endurance of metals under a random succession of loads of varying magnitude from the behaviour of specimens tested with stress cycles of constant amplitude One way of doing this is to use the cumulative damage law, which may be written

$$N \sum_i p_i/N_i = 1 \qquad (1)$$

where N is the number of cycles of random loading required to produce fracture, p_i is the fraction of these cycles with stress amplitude s_i, and N_i is the endurance of a specimen tested at a constant stress amplitude equal to s_i

This equation can be dangerously misleading Thus Freudenthal and Heller[1] tested specimens of aluminium alloy and of steel using up to six levels of stress applied in random succession The values of p_i were adjusted to give an exponential law $p_i \sim \exp(-h\, s_i)$ like that which describes the distribution of stresses in an aircraft from atmospheric gusts Freudenthal and Heller found life-times in this test about six times shorter than those given by equation (1) They proposed a more elaborate semi-empirical formula including interaction effects between the damage produced at the different stress-levels It seemed worth while to attempt a solution of this problem on the basis of the fatigue model proposed by me in previous communications[2,3]

According to this model notches are formed in the surface of the metal by randomly distributed slip The fraction f of notches which have depths between z and $z + dz$ after t cycles is given by

$$f = F \exp(-z/\alpha t)$$

where F is a slowly varying function of z and t, and α is a constant The total number of notches with depth greater than a critical value of z, say Z, is given by integration and will be of the form

$$A \exp(-Z/\alpha t)$$

where A is another slowly varying function, and has a large numerical value Now in a test at constant stress amplitude we suppose that all notches deeper than some fixed value of Z are liable to give rise to cracks, so that the probability of crack production per cycle can be written

$$PA \exp(-Z/\alpha t)$$

where P is a constant These cracks grow rapidly and soon lead to failure so that this expression can be taken as a mortality-rate for the specimens It is very small until t becomes of the order $Z/K\alpha$, where K is $\log_e(PA)$ and is taken to be about 15

Now suppose that tests are carried out at a series of different constant stress-levels Both α and Z will depend on the value of the cyclic strain ε As in a previous communication[3] we take α to be proportional to ε, and Z to be inversely proportional to ε Thus we put $\alpha_i = a\varepsilon_i$ and $Z_i = c/\varepsilon_i$, where a and c are constants Then the life-time of the specimen in a test at constant cyclic strain ε_i is given by

$$N_i\, \varepsilon_i^2 = c/(aK) = C^2$$

in accordance with Coffin's relation between the cyclic strain and the endurance[4]

Now we consider the case of a random succession of cycles with a fraction p_i having cyclic strain ε_i The law of growth of the notches will be essentially the same as in tests at constant ε except that the constant α must be replaced by an average value $\bar{\alpha} = a\sum_i p_i\varepsilon_i$

Thus the total number of notches of depth greater than Z after t cycles will be given by

$$A \exp\left(- Z/\bar{\alpha}t\right)$$

Now consider the probability that any fatigue cycle will generate a crack This is given by the product of the fraction p_i of cycles with strain value ε_i and the number of notches with depth greater than $Z_i = c/\varepsilon_i$ Hence the mortality-rate will now be

$$AP\sum_i p_i \exp\left(- Z_i/\bar{\alpha}t\right) \qquad (2)$$

and the life-time is determined by the value of t at which this sum ceases to be very small

In order to apply this relation to Freudenthal and Heller's tests we assume the validity of Coffin's law in order to obtain values of ε_i from the life-times N_i This leads to $\bar{\alpha} = a\,C\,\sigma$, where σ is written for the sum

$$\sum_i p_i/N_i^{1/2}$$

Similarly we can write $Z_i = (c/C)\,N_i^{1/2}$ Substituting these in the exponential part of (2) gives $\exp(-KN_i^{1/2}/\sigma t)$ bearing in mind that $c/(aK) = C^2$ Now for each term of the sum in (2) there is a value of t at which this term becomes large This value of t is given by $t_i = N_i^{1/2}/\sigma\ q_i$ where I have written q_i for $1 + (1/K)\log_e p_i$

Of the various t_i values, the least, which we call t_m, is the one which determines the life-time, because the corresponding term in (2) is the first to become large Thus on this theory the growth of the notches is determined by a mean value of α in which (in any event for the distributions used by Freudenthal and Heller) the low stress values are predominant The critical depth Z on the other hand, and therefore the point at which a notch becomes a crack, is determined by one of the stress-levels, usually a high one

The details of the calculation for one of Freudenthal and Heller's runs are shown in Table 1 This is the first of their runs with aluminium alloy specimens The s_i values are given as fractions of the ultimate tensile strength, and the N_i values are taken from the smoothed curve and formulæ given by Freudenthal and Heller It will be seen that whereas the lowest value of s makes the largest contribution to σ, the largest value of s is that which gives the smallest t_i and may therefore be supposed to be responsible for the ultimate failure of the specimen The experimental value of the life-time (Freudenthal and Heller's V_r') is 3 76 × 10^6, in quite good agreement with the smallest value of t_i, namely, 4 27 × 10^6 The life-time predicted by use of equation (1) is 1 49 × 10^7, which is thus grossly over-optimistic

Table 1 CALCULATION OF LIFE-TIME
(Run 22—aluminium alloy)

s_i	$N_i^{1/2}$	P_i	P_i / $N_i^{1/2}$		q_i	t_i	
0 35	34,000	0 822	2 42	10^{-5}	0 99	390	10^6
0 45	5,750	0 1456	2 53		0 87	76	
0 55	1,150	0 02664	2 32		0 76	17 35	10^6
0 65	490	0 00458	0 94		0 64	8 78	
0 75	255	0 0010	0 39		0 54	5 41	
0 85	158	0 000182	0 11		0 426	4 27	= t_m
		σ =	8 71	10^{-5}			

Similar calculations have been carried out for the other runs on aluminium alloy specimens The results are shown in Fig 1, where the experimental life-time V_r' is plotted against the value of t_m The mean value of the ratio V_r'/t_m is 0 85 with a standard deviation of 0 15 or 18 per cent, and the straight line in Fig 1 is drawn to correspond to this ratio

The corresponding calculations for Freudenthal and Heller's runs on steel specimens are complicated by the existence of a fatigue limit These results, and the application of the theory to continuous distributions of other forms such as the Gaussian noise source used by Head and Hooke[5], will be published later

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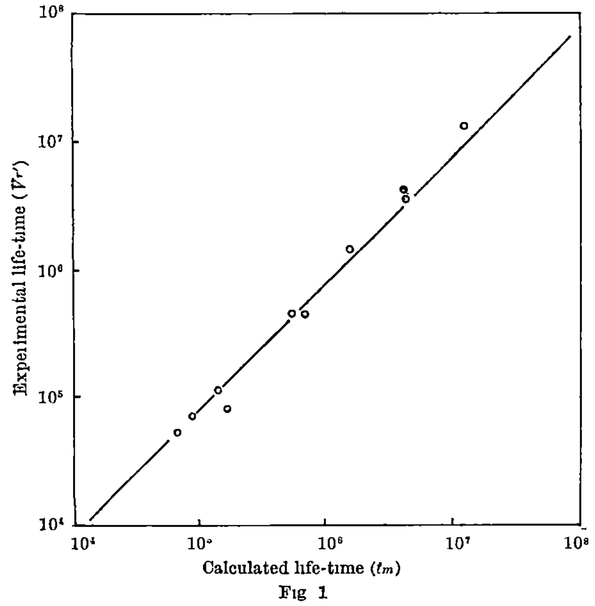

Fig 1

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CHEMISTRY

Formation of Active Nitrogen using Sources of Alternating Current and Direct Current

ACTIVE nitrogen has been generally investigated at pressures of several tenths of an mm The majority of workers have used either a condensed or electrodeless discharge to produce the active species[1] These two methods have been favoured to such an extent that little has been done to investigate other possibilities However, work on the active gas at pressures above several mm requires more efficient methods of production

In 1932, Kaplan[2] obtained active nitrogen by using a 1 kW, 25,000 V, alternating current source and inferred that this was the first time that the after-glow was obtained by means other than a condensed discharge More recently, Stanley[3] produced the active gas with a 10,000-V alternating current supply in a capillary arc and found that the after-glow obtained at high pressures (about 500 mm) differed markedly from the usual low-pressure spectrum Berkowitz *et al*[4] used a source of alternating current to investigate the nitrogen after-glow mass-spectrometrically

In the work reported here, part of an investigation of the chemical species produced by low-temperature plasmas, active nitrogen has been produced at 50 mm by both uncondensed alternating current and low-voltage direct current sources The equipment, similar to that described by Dehmelt[5], consisted of a conventional glass vacuum system and a 25-mm internal diameter quartz discharge tube in which a 3/32 in diameter tungsten cathode and 1/8 in diameter tungsten anode were mounted with a 30-mm gap The effluent from the discharge tube was pumped down a decay tube 1 m long and fitted with quartz windows for spectrographical observation A 5-kW, 600–1,300 V alternating current and 1 kW, 0–100 V direct current source were connected in series across the electrodes The alternating current was used to initiate the plasma, which was then sustained by direct current alone operating at 50–60 V and 2–2 5 amp A 0 8-Henry choke limited the alternating current to 4 amp, the alternating current discharge was first induced with the aid of a Tesla coil Argon was used as the main plasma medium with 1–2 per cent nitrogen added through a capillary leak Commercial cylinder nitrogen (Linde) was used directly without additional purification

At pressures greater than about 1 mm, it was observed that the characteristic yellow after-glow of the first positive system of nitrogen, the $B^3\Psi$–$A^3\Pi$ interaction, was produced when either the alternating current or direct current arc was operating The identity of the after-glows thus produced was first established with a direct-vision spectroscope and then confirmed with the aid of a prism spectrograph For purposes of comparison, Fig 1 shows spectrograms of the after-glow produced by the two different sources under identical conditions of flow, pressure, etc The spectrograms were taken with a small quartz Hilger, the Kodak *I–F* plate was calibrated with mercury lines which appear as the middle spectrum in Fig 1 In the top and bottom spectra, the two features at the extreme right are the 11,7(5804 Å) and 11,8(6251 Å) bands of the first positive system of nitrogen The remainder of the bands belong to the $v' = 0$ progression of the NOβ ($\beta^2\Pi$–$X^2\Pi$) interaction No γ-bands of nitric oxide impurity are detectable in the spectrogram

Several observations can be made regarding the spectra in Fig 1 The direct current power input of approximately 150 W produces a nitrogen spectrum at least several times as intense as an alternating current input of 3,000 W Since the intensity of the after-glow is proportional to the square of the concentration of nitrogen atoms, the supply of direct current appears to be at least 50–70 per cent more effective in dissociating nitrogen than a source of alternating current twenty times as energetic This tremendous difference in efficiency was checked in subsequent experiments by titration of the two streams of nitrogen atoms with nitric oxide[6] With nitric oxide injected into the after-glow through a capillary leak, the ratio of pressures required to reach an end-point for direct- and alternating-current was 1 46 Under the conditions of these experiments, the flow-rate of nitric oxide varies at least with the square of the fore-pressure[7], so that the concentration of nitrogen atoms in the direct current after-glow was at least twice that in the alternating current after-glow. The low-pressure plasma can thus be regarded as an extremely efficient source of active nitrogen at pressures above 1 mm

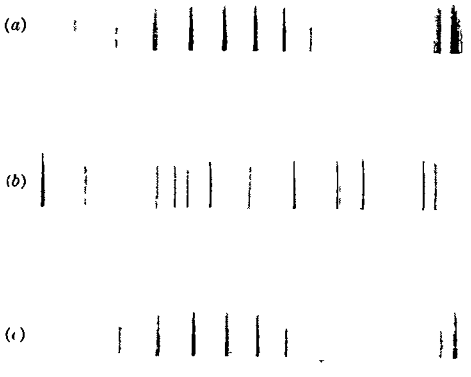

Fig 1 Spectrogram taken on a Kodak *I-F* plate with a small quartz Hilger Spectra (*a*) and (*c*) are 20-min exposures of the d c and a c after-glow, respectively The two features at the extreme right are first positive bands of nitrogen, the remaining bands belong to the NOβ system Line (*b*) is the mercury spectrum used in calibrating the plate

This work is being carried on with financial support from the Battelle Memorial Institute

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Microwave Spectrum of Fluoro-Acetonitrile

Fluoro-acetonitrile (FCH_2CN) is a molecule of some structural interest which is well suited to microwave study, all nuclei being well removed from the molecular centre of gravity in a structure of considerable dipole moment It should thus be possible to locate the atoms of the —CH_2CN group accurately with respect to the mass centre, by the method of isotopic replacement, and hence also to determine the position of the fluorine atom As part of such an investigation, we report an assignment of the spectrum of the principal isotopic form Transitions of the *R*-branch corresponding to the *A*-component of the molecular dipole have been identified for $J = 0 \rightarrow 1$, $1 \rightarrow 2$, $2 \rightarrow 3$ and $3 \rightarrow 4$, between 9 and 38 Gc/s, *Q*-branch lines for the same component, $J_{1,J} \rightarrow J_{1,(J-1)}$, have been assigned for $J = 6$, 9, 10, 11 and 12 Corresponding to the *B*-component of the dipole, lines of the type $J_{0,J} \rightarrow J_{1,(J-1)}$ have been measured for *J* values of 1–5

Analysis leads to the following rotational and centrifugal distortion constants, in Mc/s

$$A = 36{,}577\ 47 \pm 0\ 10 \qquad D_J = 0\ 0033 \pm 0\ 0007$$
$$B = 4{,}781\ 29 \pm 0\ 02 \qquad D_{JK} = -0\ 068 \pm 0\ 003$$
$$C = 4{,}339\ 75 \pm 0\ 02 \qquad \delta_J = 0\ 00056 \pm 0\ 00001$$

Nuclear quadrupole splittings due to nitrogen-14 are resolvable, and of approximately the anticipated magnitude They are at present being more accurately measured, as are Stark effect displacements for evaluation of the dipole moment components

Intensities of the lines should permit examination of further isotopic forms, and work on this is continuing in order to allow completion of the structure determination It may be noted, however, that, if the —CH$_2$CN group were assigned a structure close to that in methyl cyanide[1], the constants so far measured would require a carbon–fluorine distance of about 1 38 Å, and an angle FCC near 111° The present results are thus compatible with a carbon–fluorine distance similar to that in methyl fluoride[2], and a carbon–carbon distance similar to that in methyl cyanide Definite conclusions, however, must await further measurements

We thank the Department of Scientific and Industrial Research for a research studentship awarded to one of us (B E J)

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Rubeanic Acid as Stabilizer of Aqueous Solutions of Ascorbic Acid

Especially noteworthy among the stabilizers of ascorbic acid solutions are sulphydric organic derivatives such as glutathione, thiourea, methylthiouracyl, cysteine[1–3], 2-carbethoxy-mercapto-1-methylglyoxaline[2,3], dithioglycerin, thioglycolic acid[2], etc On the other hand acids have found application as agents reducing the rate of oxidation of ascorbic acid in solutions Among these oxalic acid plays an important part In 2 per cent and 1 per cent dilutions it is applied as a stabilizer of synthetic ascorbic acid in various solutions and of ascorbic acid in biological material

This suggested the use of rubeanic acid for preventing oxidation of ascorbic acid in aqueous solution Rubeanic acid is a diamide of thio-oxalic acid We wanted to investigate the stability of 0 01 per cent aqueous solution of ascorbic acid in the presence of rubeanic acid

Ascorbic acid solutions containing rubeanic acid were prepared, and the ascorbic acid content was assessed during storage Moreover, the same solutions were subjected to the effect of oxidizing factors, such as irradiation with a quartz lamp or introduction of cupric ions, the ascorbic acid content being measured all the time This was carried out by means of Tillmann's method, consisting in the reduction of 2,6-dichlorphenolindophenol Table 1 shows results obtained in determining the stability of ascorbic in rubeanic acid solutions

Table 1 Stability of 0 01 per cent Aqueous Solution of Ascorbic Acid in the Presence of Rubeanic Acid

Time of storage (hr)	Ascorbic acid content (per cent of initial content) in aqueous solution	in 0 01 per cent rubeanic acid	in 0 001 per cent rubeanic acid
—	100 0	100 0	100 0
0 5	82 0	99 2	98 3
1 0	49 9	98 3	97 6
2 0	33 3	98 3	96 0
3 0	16 6	97 6	96 0
4 0	13 3	97 6	94 4
23 0	0 0	92 8	80 0

In Table 2 are assembled the results obtained in assessing the stability of ascorbic acid solutions irradiated with a quartz lamp

Table 2 Stability of 0 01 per cent Aqueous Solution of Ascorbic Acid in the Presence of Rubeanic Acid on Irradiation with a Quartz Lamp

Time of storage (hr)	Ascorbic acid content (percentage of initial content) in aqueous solution	in 0 01 per cent rubeanic acid
—	100 0	100 0
0 5	69 7	98 3
1 0	51 3	98 3
2 0	21 0	98 3
3 0	9 1	97 6
4 0	3 3	97 6

Table 3 shows the ascorbic acid content in 0 01 per cent and 0 001 per cent solutions of rubeanic acid in the presence of copper ions (0 001 per cent and 0 0001 per cent copper sulphate)

Table 3 Stability of Ascorbic Acid in 0 01 per cent Solution, in the Presence of Rubeanic Acid and on Admixture of Copper Ions

Time of storage (hr)	Ascorbic acid content (percentage of initial content)				
	in 0 001 per cent CuSO$_4$	in 0 01 per cent rubeanic acid		in 0 001 per cent rubeanic acid	
		in 0 001 per cent CuSO$_4$	in 0 0001 per cent CuSO$_4$	in 0 001 per cent CuSO$_4$	in 0 0001 per cent CuSO$_4$
—	100 0	100 0	100 0	100 0	100 0
0 5	10 0	99 0	99 3	98 3	100 0
1 0	0 0	99 0	99 3	97 4	99 2
2 0	—	98 1	98 4	91 2	98 3
3 0	—	95 8	96 5	91 2	96 6
4 0	—	93 4	95 9	91 2	95 8
24 0	—	80 0	86 3		
48 0	—			66 9	72 3

From the results in Tables 1–3 rubeanic acid is seen to be a stabilizer of ascorbic acid The stability of the solutions is dependent on the amount of rubeanic acid added. Ultra-violet radiation and copper ions in solution, which are factors accelerating the oxidation of ascorbic acid, lose their activity in the presence of rubeanic acid

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Ligand Field for Chromic Ions in the Corundum Lattice

Recent work on absorption spectra and lattice spacings in the Al$_2$O$_3$–Cr$_2$O$_3$ solid solution[1–6] has been in sufficient agreement to warrant a fitting of the optical results to the crystal field-splitting diagram[7]. The relevant terms are shown in Fig 1, which also shows the transitions involved in the yellow and blue absorption bands

Assuming the Racah parameter B to be constant throughout the system, it is impossible to obtain a fit no matter what value of B is chosen An example is shown in Fig 2, with B = 650 cm^{-1} This value was chosen to give a fit at about 5 5 per cent Cr$_2$O$_3$, where the observed maxima for ruby (Graham[1]) and Cr(H$_2$O)$_6^{3+}$ (Tsuchida[8]) are identical.

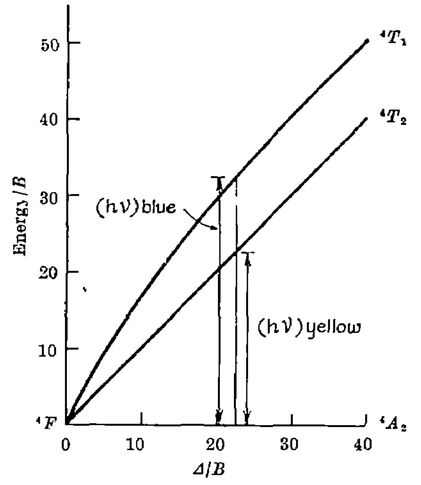

Fig 1 Energy-level diagram for the splitting of ^4F in an octahedral field

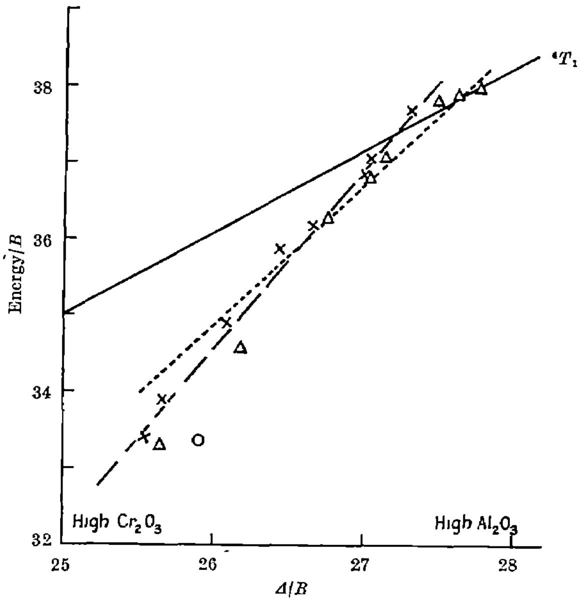

Fig 2 Experimental energy values derived for B = 650 cm^{-1} O, Graham, Δ, Schilly, ×, Schmitz-Dumont and Reinen, — —, Schultz

It is known that an increase in covalent bonding[9] and/or charge transfer[10] results in a decrease in B There is evidence[1] for the existence of bonding other than ionic in both Al_2O_3 and Cr_2O_3, and it can be shown that the proportion of such bonds increases as the chromium content decreases This follows from a consideration of cell volumes Thus if we assume ionic bonding and regular octahedral surroundings for the Al^{3+} and Cr^{3+} ions in pure Al_2O_3 and Cr_2O_3 respectively, we would expect an increase in unit cell volume of 10 35 per cent in passing from Al_2O_3 to Cr_2O_3, whereas the observed increase is 13 5 per cent The contents of the unit cell (on the basis of ionic volumes) have increased in volume by only 2 8 per cent, so that in the absence of other types of bonding one would expect distortions of the octahedron to accommodate a good deal of the increase calculated here The observed increase in cell volume is therefore much greater than that to be expected unless Al_2O_3 is more covalent in character than Cr_2O_3 The lattice spacings[1,2] tend to confirm this conclusion, as the effective radius of the chromium ion is greater at the alumina-rich end of the solid solution, perhaps indicating a greater amount of charge transfer Based on present ideas, it would be expected that B would increase as the chromium content increases

However, if we calculate the values of B required to fit the experimental and theoretical curves at all points, we find that B increases approximately linearly with decreasing Cr_2O_3 content or increasing Δ (Fig 3) Although there is some scatter between observers, the result is quite clear, and the variation of B is very large The results of Graham and of Schultz are closely linear, and the lines of best fit have been plotted in Figs 1–3 The accurate single crystal absorption data of Mandarino[4] on weighting the ordinary and extraordinary components in the ratio 2 3 according to the experimental result of Schultz[11] give a value of Δ 18 11 cm^{-1} for Al_2O_3 in excellent agreement with ref 1

The increase in Δ as the chromic oxide content decreases is easily explained on the basis of decreasing metal-ligand spacing, but the corresponding increase of B is anomalous Since the axial ratio is almost constant throughout the system, the trigonal component of the field is unlikely to account for the anomaly

Goodenough[12] points out that interactions between the ligands and the d-shell of the metal in the corundum structure are weak if there are three electrons or less in the d-shell, and postulates direct cation–cation coupling as the cause of antiferromagnetic ordering in these structures Chromic oxide itself is antiferromagnetic with a Néel temperature near room temperature, and it would appear that the overlap of the d-orbitals of adjacent Cr^{3+} ions which results in the antiferromagnetic ordering may also explain the absorption spectrum if the d-shells interact to some extent even in the dilute solutions[13] The decrease in B would then correspond to increasing cation–cation interaction as the chromium content increased This is again consistent with the structural results[1], as the metal-to-metal distance in Cr_2O_3, 2 58 Å , is significantly less than that in Al_2O_3, 2 73 Å In terms of the cation radius, the difference is even more marked

It is felt that the anomalous values of B and Δ obtained by one of us (J G) for pure Cr_2O_3 (the separate points shown in Figs 2 and 3) are associated with

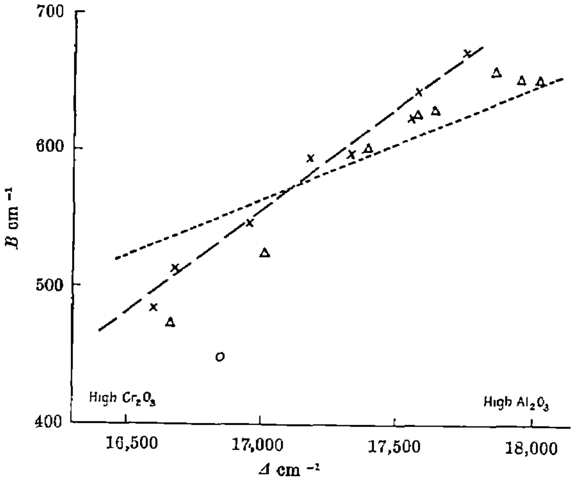

Fig 3 Variation of B with crystal field (details as for Fig 2)

antiferromagnetic ordering in this sample This would be expected to result in a discontinuous decrease in B, but the accompanying increase in Δ is not so easy to understand

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BIOCHEMISTRY

Biosynthesis of L-Ascorbic Acid : Missing Steps in Animals incapable of synthesizing the Vitamin

It has been reported[1] that cyanide greatly stimulates the synthesis of L-ascorbic acid from D-glucuronolactone by the liver microsomes of the rat By this method using cyanide it was found that either the liver or the kidney tissue of all the species examined which are known to be independent of an exogenous source of ascorbic acid was able to synthesize L-ascorbic acid, while the corresponding tissue of those which are dependent on such a source could not Whether the enzyme system concerned was present in the liver or the kidney tissue depended on the position of the species in the phylogenetic scale of evolution[2] This technique revealed further that, apart from man, monkey and the guinea pig, the India fruit bat (*Pteropus medius*) and the red-vented bulbul (*Pycnonotus cafer*) were unable to synthesize L-ascorbic acid, and this was confirmed by the production of experimental scurvy in the bulbul and its cure by the administration of L-ascorbic acid[3] This communication concerns the location of the genetic defect in all these species, which distinguishes them from those which can synthesize L-ascorbic acid

According to a scheme given elsewhere[4] indicating the probable biosynthetic pathway of ascorbic acid in those species which can make it, the following representation (Fig 1) may be made in regard to the substrates and the enzymes of the microsomes and of the soluble supernatant involved in this synthesis

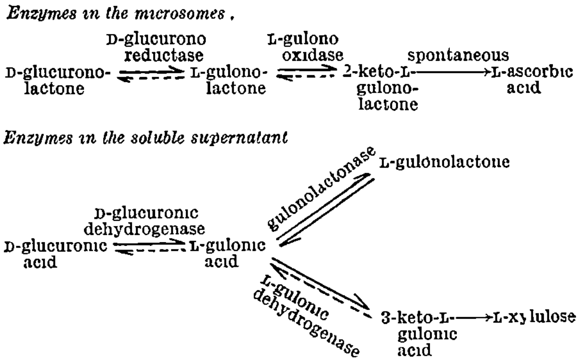

Fig 1 Schematic representation showing the substrates and the enzymes of the microsomes and of the soluble supernatant involved in the biosynthesis of L-ascorbic acid in animal tissue

Fig 1 indicates that with D-glucuronolactone as substrate, microsomal enzymes alone can convert it into L-ascorbic acid through L-gulonolactone and 2-keto-L-gulonolactone With D-glucuronic acid as substrate, however, the microsomes require the addition of the soluble supernatant which supplies the necessary enzymes to convert D-glucuronic acid into L-gulonolactone, which can be then converted into L-ascorbic acid by the microsomes We have previously reported[4] that the failure of the guinea pig to synthesize L-ascorbic acid was due to the absence of L-gulono oxidase, which oxidizes L-gulonolactone to 2-keto-L-gulonolactone Using the tracer technique this was also observed by Burns *et al* [5] and by Burns[6] in regard to man, monkey and the guinea pig

It has now been found, however, that not only L-gulono oxidase but also the other microsomal enzyme, D-glucurono reductase, which converts D-glucuronolactone into L-gulonolactone, is specifically absent from those species which cannot synthesize L-ascorbic acid This has been demonstrated by the fact that, under anaerobic conditions, incubation of D-glucuronolactone with liver or kidney microsomes from man, monkey, the bat, the guinea pig and the bulbul in the presence of cyanide did not result in the formation of L-gulonolactone whereas in similar experiments with microsomes from rat and goat livers, L-gulonolactone was produced as an intermediate L-gulonolactone was identified by paper chromatography of the deproteinized and concentrated incubated mixture using (*a*) ethyl acetate/acetic acid/water (3 1 3, v/v, upper phase, descending), (*b*) ethanol/acetic acid/water (8 1 1 v/v, descending), (*c*) acetone water (4 1 v/v, ascending) as the irrigating solvents The R_F values for D-glucuronolactone and L-gulonolactone were (*a*) 0 51 and 0 43, (*b*) 0 62 and 0 54, (*c*) 0 72 and 0 55 respectively The lactones were identified on the chromatogram by the hydroxamic acid method[4] L-gulonolactone has also been identified by Suzuki *et al* [7] as a product of anaerobic incubation of D-glucuronolactone with rat-liver microsomes in the presence of cyanide

The absence of D-glucurono reductase was further revealed in the following way The microsomes of the liver or kidney tissue (40 mgm protein) from all the aforesaid species which cannot synthesize L-ascorbic acid were incubated with D-glucuronolactone (20 μmoles) in the presence of sodium phosphate buffer (0 02 M, pH 7 0) and potassium cyanide (0 05 M) After incubation, the mixture was deproteinized with perchloric acid, neutralized with caustic potash and, after filtering off potassium perchlorate, freed from cyanide by acidification with N hydrochloric acid followed by evaporation to dryness under suction The residue was taken up in a minimum volume of water, neutralized and then incubated with goat liver microsomes, but no L-ascorbic acid was formed Had there been any formation of L-gulonolactone, it would have led to the synthesis of L-ascorbic acid in the absence of cyanide on addition of goat liver microsomes[4] A control experiment starting with L-gulonolactone (5 μmoles) instead of D-glucuronolactone resulted in the formation of L-ascorbic acid (0·5 μmole) indicating little destruction of L-gulonolactone in the process of removal of cyanide.

In the scheme given (Fig. 1), the supernatant enzymes, converting D-glucuronic acid into L-xylulose and into L-gulonolactone, have been found to be present in all the above-mentioned species requiring dietary ascorbic acid[8]. Thus there are two genetic blocks in regard to the synthesis of L-ascorbic acid in these species: (1) the absence of D-glucurono reductase so that D-glucuronolactone cannot be reduced to the intermediate L-gulonolactone, (2) the absence of L-gulono oxidase so that L-gulonolactone cannot be oxidized to the intermediate 2-keto-L-gulonolactone, even if the enzymes of the soluble supernatant bring about the conversion of D-glucuronic acid into L-gulonolactone. That these two enzymes are actually present but are masked by some microsomal inhibitory substance is considered unlikely, as the addition of the microsomes from these species to the solubilized enzyme from rat liver, prepared as described before[4], was not found to inhibit the synthesis of L-ascorbic acid by the latter.

Our thanks are due to the Indian Council of Medical Research for financing this work.

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Riboflavin and Liver Sulphydryl-Levels in Mice

DURING the course of a series of investigations of the sulphydryl content of mouse liver under varying experimental conditions, it was found that during a period of nine months there was a steady increase in the level found in control animals. The liver —SH content rose from 31·4 ± 1·7 μgm per 100 mgm wet weight to 34·3 ± 5·9 μgm per 100 mgm wet weight. In all cases the groups of animals used comprised a minimum of 10 Strong *A* male mice. This steady increase in control-levels was succeeded by a sharp fall to 27·7 ± 2·6 μgm per 100 mgm wet weight in a group of similar animals. Consideration of factors which might have been involved in this variation led to suspicion of the diet, particularly since, during the period in question, growth-rates in these animals had shown deviations from the expected pattern. Further examination of the diet question focused suspicion on the vitamin content, and especially on the constancy of the riboflavin-level. We were thus led into an investigation of the influence of dietary riboflavin on liver —SH levels.

All measurements of —SH levels recorded in the following paragraphs have been made by the method described by Calcutt and Doxey[1]. Whereas in the original use of this technique millivoltmeters were used for the potentiometric titrations these have now been replaced by transistorized titrimeters based on Stock's design[2]. These instruments have proved superior to millivoltmeters in operation as the settling time is negligible and scale movements are large and easily read.

All experimental work has been carried out with Strong *A* mice. These have been used in batches of the same age and, so far as possible, of approximately equal weights. This latter precaution has been taken in view of Calcutt's[3] finding of an inverse relationship between body weight and —SH content per unit mass of liver. The effects of addition and withdrawal of riboflavin from the diet have been examined and also the effects of riboflavin given by injection.

Addition of riboflavin to drinking water. Twenty mice were divided into two groups of ten each. While both groups were maintained on a normal pellet diet, one group received fresh water to drink whereas the second was given a saturated solution of riboflavin in tap-water. After four days the liver —SH content was estimated for all the animals. The group which had been given water had an —SH level of 33·9 ± 2·8 μgm per 100 mgm of wet weight of liver, while the corresponding figures for the group given riboflavin were 33·5 ± 2·6 μgm of —SH per 100 mgm of wet weight of liver. There is obviously no significant difference between the two groups. Since the solubility of riboflavin is only 0·01 per cent in water, the intake by the mice from this source would be small. Further, the actual absorption may have been even smaller, as mice eat and drink at separate times, and the evidence collected by Bro Rasmussen[4] indicates a requirement for the presence of protein for the adequate absorption of riboflavin. The slightly bitter taste of the solution may also have limited drinking by the mice in question. In the circumstances, the results obtained in this experiment are not surprising.

Withdrawal of riboflavin from the diet. Thirty male mice were placed on a synthetic diet obtained as a vitamin-free diet from British Drug Houses, Ltd., of London. To this, vitamins were added in the amounts used by Greenstein, Otey, Birnbaum and Winitz[5] in their experiments on synthetic diets. After 28 days, during which period the animals maintained their weight and health, ten were taken as controls and the liver —SH content was estimated. The remaining animals were transferred to the vitamin-free diet supplemented, as previously, with all vitamins except riboflavin. These animals were killed in batches of five at intervals for liver —SH measurements. The results are shown in Fig. 1, where it will be seen that the liver —SH content rose above the control-level seven days after withdrawal of the riboflavin.

Effects of riboflavin by injection. Thirty male mice were divided into two batches of 15 each. One lot received an intraperitoneal injection of 0·1 mgm of riboflavin in 1 ml. of distilled water each mouse. Liver —SH content was estimated on all animals, those that had received riboflavin being killed 1 hr. after the injection. The control animals had 34·5 ± 4·2 μgm of —SH per 100 mgm of wet weight of liver whereas the corresponding figure for the riboflavin-treated animals was 28·8 ± 4·75 μgm of —SH. In a similar series, using female mice, the treated animals were killed 18 hr. after the injection. This time the control —SH level was 38·6 ± 3·8 μgm per 100 mgm wet weight of liver and the level in the treated animals was 25·3 ± 5·0 μgm per 100 mgm wet weight of liver. As a further experiment a batch of male mice was injected, as previously, with an

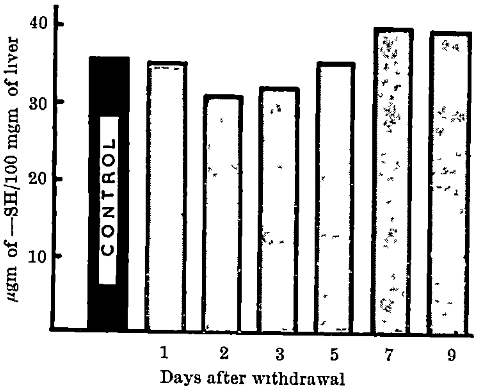

Fig 1 Effects of withdrawal of riboflavin from mouse diet on liver —SH levels

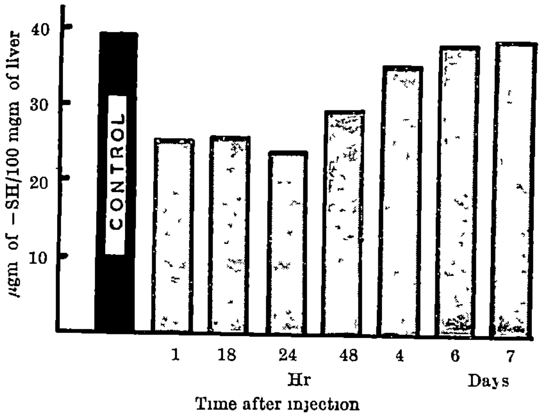

Fig 2 Effects of injected riboflavin on mouse liver —SH levels

aqueous solution of riboflavin These were killed in batches at daily intervals and the liver —SH level estimated Return to the control-level did not take place until six days after treatment

Tissue riboflavin-levels have often been associated with tissue protein content As a follow-up to the above experiments, which were performed on mice fed a high-quality diet, the effects of riboflavin on animals on an inferior diet have been examined Mice were fed rice and water only for ten days and then received an injection of 0 1 mgm of riboflavin in 1 0 ml of distilled water Estimates of liver —SH level showed that this remained depressed until six days after treatment, as in the previous experiment The results are shown in Fig 2

It is known that riboflavin plays a part in oxidation/reduction systems as a consequence of its participation in various enzyme systems The experimental results reported here now indicate that riboflavin may exercise a function in relation to the general oxidation/reduction status of the liver cell This activity would appear to be independent of the nutritional state of the animal, since there is no apparent distinction between the effect of injected riboflavin in well-fed or poorly fed animals From this it seems unlikely that the effect involves the riboflavin in conjunction with the proteins of the cell

The actual mechanism of the effect is at present unknown Iwaki[6] has shown that a direct combination of glutathione and riboflavin can occur, but even if this did happen the linkage would probably be broken by the more reactive mercurial used in the —SH estimation Riboflavin can act as an electron acceptor, and it is possible that, rather than any direct chemical combination of the vitamin with cellular components, we have an induced oxidation of various cellular components which, in turn, are responsible for maintenance of sulphur-containing groups in the reduced form It has in fact been suggested by Booth and Boyland[7] that riboflavin inhibits the microsomal hydroxylating system by causing the oxidation of reduced triphosphopyridine nucleotide, which is required for maintenance of —SH in the system

Recently, Hsu and Chow[8] have found that thiamine deficiency in rats leads to an elevation in the level of liver glutathione This finding, in conjunction with the results recorded above, suggests that dietary vitamin-levels, at least of thiamine and riboflavin, may take a major role in determining the biochemical status of the liver Further, it once more stresses the extreme importance of strict dietary control of animals used for biochemical work

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In vivo and *in vitro* Inhibition of Aromatic Hydroxylation by Riboflavin

Detailed and extensive investigations of the metabolism of polycyclic hydrocarbons have been carried out over many years, but it is only recently that information linking the metabolism of these agents with an enzymic system present in liver has become available It has been found that hydroxylation of the carcinogenic 3 4 benzpyrene[1] and the non-carcinogenic naphthalene[2] is brought about by an enzyme, or 'hydroxylase'[1], which occurs in the microsomal fraction of liver homogenates, this system requiring reduced triphosphopyridine nucleotide and molecular oxygen for activity One of us (K H H) has found that the same or a similar system is also active against pyrene and 1 2 benzanthracene The products obtained are similar to those obtained during *in vivo* metabolism This work will be reported in detail elsewhere

This hydroxylating system is susceptible to sulphydryl complexing agents, such as *p*-chloromercuribenzoic acid The information concerning the effects of riboflavin on liver sulphydryl-levels reported by Calcutt, Doxey and Coates (see previous communication) led us to further consideration of the effect of riboflavin on microsomal hydroxylation Previously, it had been found by Booth and Boyland[2] that flavine mononucleotide (FMN) is an effective inhibitor of the microsomal hydroxylation of naphthalene It seemed desirable, therefore, to see whether riboflavin also affected hydroxylation of other compounds

The pyrene hydroxylating system (active in the conversion of pyrene to 3-hydroxypyrene) was chosen for the first investigation The low solubility

Table 1 INHIBITION OF PYRENE HYDROXYLATION BY RIBOFLAVIN

Compound	Concentration	Percentage inhibition
'Beflavit'	10^{-3}	100
	10^{-4}	95
'Dummy' 'Beflavit'	10^{-3}	70
	10^{-4}	15
FMN	10^{-3}	60
	10^{-4}	20

of riboflavin in aqueous solutions led us to the use of a concentrated (5 mgm./ml.) commercial preparation made in a solvent base. This was 'Beflavit', prepared by Roche Products, Ltd., England. For control purposes the solvent base preparation was also used. The results, together with those obtained using FMN, are summarized in Table 1.

It is evident from these figures that the solvent base itself contributes to the inhibitory powers of 'Beflavit', but even when allowance is made for this, riboflavin itself appears appreciably more active than the FMN.

Identical findings were then obtained in a similar series of experiments based on the metabolism of 3 : 4 benzpyrene.

The effect of riboflavin on the hydroxylation of 3 : 4 benzpyrene *in vivo* was then examined. Ten mice were each given an intraperitoneal injection of 0.1 mgm. of riboflavin in water and, 1 hr. later, an intravenous injection of 0.6 mgm. of benzpyrene as a colloid in 1 ml. of water. A second group of ten mice each received a similar benzpyrene injection. This group acted as controls. The excretion of phenolic metabolites, predominantly 8-hydroxybenzpyrene, in the fæces was then followed over a period of 48 hr. The animals treated with the hydrocarbon alone excreted approximately 25 per cent of the total injected dose as phenolic derivatives during this period. The difference between this group and that which had received prior treatment with riboflavin was striking, for this group excreted less than 1 per cent of the dose as metabolites during the same period. The benzpyrene in this group was not excreted unchanged, since only trace amounts were detected in the urine and fæces. Whether it is stored or metabolized in a different manner is still to be determined.

This last experiment provides excellent confirmation of the *in vitro* experiments and also supports the view that the microsomal hydroxylating system observed *in vitro* is a true representation or model of the normal cellular process. Further, this last experiment is interesting in that this is the first time that it has been shown that the metabolism of benzpyrene may be influenced by dietary or other external agents.

It is not yet clear which part of the hydroxylating system is inhibited by riboflavin. It appears unlikely that riboflavin becomes bound to the enzyme system, since microsomes prepared from animals pretreated with riboflavin show undiminished activity as compared with control preparations, provided that they are washed several times with 1.15 per cent potassium chloride solution. This would suggest that the effect is on the coenzyme moiety rather than the enzyme itself.

Apart from the more obvious implications arising from this work in respect of the nutritional state of animals used in metabolic studies, there is the question of riboflavin and carcinogenesis by polycyclic hydrocarbons. Boyland[3] has given reasons for considering that carcinogenesis by polycyclic hydrocarbons is associated with the metabolism of these compounds. If this is true, then the influence of riboflavin on the metabolism of these agents should be reflected in the carcinogenic activity. Further evidence of possible effects of riboflavin in carcinogenesis is given in the finding that the hepatocarcinogen (4′ dimethylaminoazobenzene) is metabolized by the same, or a similar, microsomal enzymic system[4], whereas the carcinogenic action of this agent is inhibited by a high dietary riboflavin intake[5]. Further consideration of these problems will be given elsewhere.

We are grateful to Roche Products, Ltd., Welwyn Garden City, England, for the gift of samples of the solvent base in which 'Beflavit' is prepared.

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Effect of Insulin on the Phosphorus-32 Mononucleotide Labelling of the Isolated Rat Diaphragm in the Controlled Absence of Extracellular Glucose

EXTENSIVE work on glucose transport into the cell under the influence of insulin and on the metabolic consequences of this transport has been carried out on the eviscerated animal[1], the isolated rat diaphragm[2], the isolated perfused heart[3] and the isolated epididymal fat pad[4,5]. Insulin stimulation seems to involve not only an enhanced transport-rate, but also an increased intracellular distribution space as measured with non-metabolizable sugars[2] and amino-acids[6,7]. However, some recent reports[8,9] dealing primarily with incorporation of carbon-14 into proteins of rat diaphragm, in the absence of added glucose, demonstrated a striking insulin effect, which could not be explained by an increased accumulation of amino-acid precursors of the labelled protein. This effect may be correlated[10] with another report mentioning the action of insulin on endoplasmic reticulum proliferation in the fat pad[11]. Whatever the mechanism of action of insulin may be, stimulation of glucose transport, protein synthesis or any other metabolic event is bound to be reflected in the turnover of free mononucleotides and especially adenosine nucleotides, the role of which is essential in the activation of sugars, fatty acids[12] and amino-acids[13].

The present communication reports an effect of insulin on the labelling of free mononucleotides in the isolated rat diaphragm in the controlled absence of extracellular glucose. The isolated rat diaphragms were first washed, for 5 min., in Krebs–Ringer bicarbonate medium[14] at 37°, in an atmosphere of 95 per cent oxygen plus 5 per cent carbon dioxide. After removal and careful blotting of the diaphragms, they were incubated in fresh Krebs–Ringer bicarbonate medium with or without added glucose and insulin

Table 1 INFLUENCE OF INSULIN ON THE LABELLING OF PHOSPHATES IN THE ISOLATED DIAPHRAGM WITH OR WITHOUT GLUCOSE (NORMAL RAT)

	µgm phosphorus/100 mgm fresh weight				Specific activity (c p m /µgm) (percentage extracellular or intracellular phosphorus)			
Glucose (2 mgm /ml) Insulin	0 0	0 +	+ 0	+ +	0 0	0 +	– 0	+ +
Extracellular phosphorus	1 2	1 1	0 9	0 9	20,200	21,600	27,100	25,300
Intracellular phosphorus	23 3	28 6	24 6	28 6	1,540 % *E* 7 7	1,235 5 7	1,835 6 8	1,405 5 6
P C	15 8	12 9	20 2	17 7	634 % I 41	799 67	869 47	979 70
ATP	30 5	31 1	32 5	23	~ 421 % I 27	602 49	699 38	734 52
ADP	7 8	7 8	5 9	7 9	~ 289 % I 19	452 37	354 19	540 38
U + G	3 9	3 6	2 5	2 5	~ 370 % I 24	450 36 4	686 37	827 59
DPN	2 9	4 0	2 2	2 4	ε	ε	ε	ε
Res phosphorus	3 5	3 6	6 1	8 7	421 % I 27	477 39	648 35	624 44

~ = labile phosphate bonds

The washing and incubation were performed in a Warburg apparatus After 5 min gassing and 10 min pre-incubation, a known amount of phosphorus-32 was tipped in the incubation medium and the incubation continued for another 10 min The amount of glucose still present in the diaphragm at the moment the phosphorus-32 was added to the medium can be demonstrated (Fig 1) This amount has been determined by a chromatographic procedure After rapid weighing, the diaphragms were homogenized in ice-cold 10 per cent trichloracetic acid, the extract centrifuged, an aliquot taken for the estimation of inorganic phosphate and phosphocreatine and specific activities The remaining supernatant was processed using a chromatographic procedure, essentially identical to that described in a previous report[15] adenosine triphosphate (ATP), adenosine diphosphate(ADP), adenosine monophosphate (AMP), diphosphopyridine nucleotide (DPN), and the sum of uridine and guanosine phosphates (U + G) were thus separated, their amounts and their specific activities measured The 'residual phosphate' comprising mainly the phosphorylated intermediates of glycolysis has also been determined In all cases, the total phosphorus and radioactivity recovered after the fractionation procedure amounted to 90 per cent–100 per cent of the original phosphorus and radioactivity present in the trichloracetic acid extract It should be mentioned that the amount and specific radioactivity of intracellular inorganic phosphate has been calculated from the total inorganic phosphate and the extracellular phosphate present in the incubation medium assuming an extracellular space of 22 per cent as measured by Kipnis and Cori on the cut diaphragm[2]

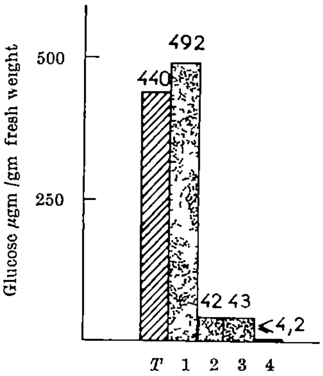

Fig 1 Glucose content of rat diaphragm incubated under various conditions *T*, Theoretical value for (1) (extracell space, 22 per cent), 1, washed 5 min , 37°, Krebs–Ringer glucose, 2 mgm /ml , 2, washed 5 min , 37°, no glucose, 3, washed 5 min , 37°, no glucose, insulin, 10 µgm /ml , 4, same as 3 plus incubation fresh medium 15 min

Table 1 shows a characteristic result obtained with normal rat diaphragms in absence or in presence of added insulin (10 µgm /ml) or glucose (2 mgm / ml)

From these results the following conclusions may be drawn (1) There seems to be no influence of insulin or glucose on the amount of the different phosphate fractions in the rat diaphragm except so far as the 'residual phosphate' is concerned The amount of this phosphate fraction increases in the presence of glucose and still more with glucose + insulin, as may be expected if it consists mainly of glycolytic intermediates (2) There is no influence of insulin or glucose on the rate of penetration of radioactive phosphorus into the diaphragm of the normal rat, as visualized by the ratio of the specific activities, namely, intracellular phosphate/extracellular phosphate (3) There is a striking enhancement of the labelling of phosphocreatine, ATP, ADP, uridine and guanosine phosphates by insulin in the presence or absence of added glucose The increase of specific activity of these fractions with respect to intracellular phosphates averages 50 per cent for phosphocreatine (PC), 80 per cent for ATP, and 100 per cent for ADP No significant labelling of ribose-linked phosphoryl groups such as AMP or DPN occurred within our limited times of incubation (4) In the presence of glucose there is a definite rise of the labelling of ATP both with or without insulin There is a slight glucose effect on the specific activities of phosphocreatine and the 'residual fraction' and a marked effect on the labelling of the uridine and guanosine phosphates But there is no influence whatsoever of glucose on labelling of ADP, whereas insulin exerts its most striking effect on the labelling of this substance

It is thus clear that insulin has a marked effect on the labelling of phosphorylated coenzymes even in the absence of any measurable amounts of glucose within or without the cell The exceedingly small amount of glucose found in the diaphragm with the conditions under which the present experiments were carried out could as well have arisen from the limited breakdown of labile esters like glucose-1-phosphate or of glycogen itself The marked effect of insulin on the labelling of the terminal phosphate group of ADP, which seems to be in equilibrium with the second phosphorylated group of ATP, is what one might expect to occur if amino-acid activation including a 'pyrophosphate split' of ATP (ref 13) and subsequent re-phosphorylation of AMP by an adenylate kinase system would occur However, many other metabolic events such as, for example, activation of fatty acids[12] and increased dephosphorylation of ADP by myokinase could equally well explain the increased formation of AMP

Finally, it must be borne in mind that although insulin does not appear to increase the penetration of radioactive phosphate into the cell of the diaphragm, a mechanism whereby insulin could promote penetration of phosphate into a restricted compartment of the muscle cell where re-phosphorylation of AMP to ADP and ATP normally occurs, thus increasing the actual labelling of the two terminal phosphorylated groups of ATP, cannot be excluded at present

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Effects of the Sulphur Amino-Acids on the Vitamin A Content in Liver of Rats

RECENTLY we reported[1] the effect of vitamin A on the cystine content in the skin of rats and suggested a causative relationship between vitamin A and sulphur amino-acids The present work was designed to investigate the effects of dietary supplementation of sulphur amino-acids on the vitamin A content in liver of rats

The test animals used were rats of both sexes of the Wistar strain weighing about 40 gm They were housed in individual compartments with screen bottoms and fed the basal diet deficient in sulphur amino-acid content as shown in Table 1

Table 1 COMPOSITION OF THE BASAL RATION

	Exp 1	Exp 2
Milled rice	60 gm	92 gm
De-fatted sovbean meal	26 ,,	6 ,,
Soybean oil	5 ,,	2 ,,
Dried yeast	5 ,,	0 ,,
Sodium chloride	0 ,,	1 ,,
Calcium carbonate	0 ,,	1 ,,
Salt mixture (Hegsted)	4 ,,	0 ,,
Supplements per rat daily		
Thiamine	0 μgm	20 μgm
Riboflavin	0 ,,	50 ,,
Vitamin A palmitate	100 ,,	1 ,,
α-Tocopherol	300 ,,	0 ,,

In the first experiment we investigated the effect of supplementary methionine, cystine, lysine and gelatin on the basal diet Five groups of rats were given different diets as described in Table 2 Rats in group 1 were fed the basal diet and used as control Groups 2, 3, 4 and 5 were given methionine, cystine, lysine and gelatin respectively. The amount of gelatin was so adjusted that the lysine content in it was equal to the amount of lysine used in group 4 During the four-week feeding period, the amount of diet was restricted to 6 gm per rat daily during the first week, and thereafter increased by 1 gm a week At the end of four weeks the animals were killed Pieces of liver from the median and left lateral lobes were removed immediately, weighed and saponified with alcoholic potassium hydroxide, and the amount of vitamin A extracted by di-ethyl ether was measured by the $\alpha\alpha'$-glycerol dichlorohydrin method[2] For determination of the sulphydryl activity of the liver, another weighed piece was homogenized and incubated with monoiodoacetic acid at 37° C for 1 hr and the liberated iodine in its deproteinized filtrate was measured by Mori's method[3] The absorption coefficient (E) obtained on a colorimeter was taken as the measure of sulphydryl activity

The result of the first experiment showed that the vitamin A content in liver of animals fed the methionine-supplemented diets, but not those given the lysine- or gelatin-supplemented diets, was strikingly elevated above that of the control Cystine supplementation showed a moderate increase in vitamin A content of liver over that of the control animals The sulphydryl activity showed close parallelism with vitamin A content, the highest value was obtained with a supplement of methionine, the next highest being that of cystine

In the second experiment we investigated the effect of methionine on the vitamin A content in liver of rats fed an inadequate supply of protein and vitamin A In this case, a basal diet of lower protein content than that in the first experiment was used and was given in conjunction with the liberal or small dosage of vitamin A, the small dosage being inadequate to allow accumulation of the vitamin in the liver Amount of supplement of the four groups and analytical data are shown in Table 3

The results of the second experiment showed that with the addition of methionine to the diet of low protein content, the differences in the content of vitamin A in livers were less pronounced but still significant, in other words, the vitamin content was higher than those of the unsupplemented regardless of the presence of an ample or minimum supply of vitamin A

Present findings indicate that the dietary supplement of sulphur amino-acids, especially methionine, elevated the vitamin content in the liver of rats, while lysine or gelatin increased it a little or not at all above that of rats fed the basal diet The basal and supplemented groups of rats were normal in general appearance and no apparent difference was observed between their growth-rates It is remarkable that the sulphur amino-acids alone had an increasing effect on the vitamin A content in liver This is in agreement with our suggestion[1] that vitamin A is involved in the metabolism of sulphur amino-acid

Concurrent with the increase of vitamin A content in liver, the sulphydryl activity in liver and muscle of rats fed a diet supplemented with methionine increased In this connexion, Flesch *et al* [4] noticed that vitamin A was able to unite with the sulphydryl

Table 2 EFFECT OF METHIONINE, CYSTINE, LYSINE AND GELATIN ON THE VITAMIN A CONTENT IN LIVER OF RATS
(Values shown with mean and standard deviation of five rats in each group)

Group No		1	2	3	4	5
Per 100 gm of diet	DL-Methionine (gm)		0 2			
	L-Cystine (gm)			0 2		
	DL-Lysine (gm)				0 2	
	Gelatin (gm)					4 3
Body-weight	Initial (gm)	40 4 ± 8 3	40 4 ± 6 6	40 2 ± 5 9	40 8 ± 5 4	40 8 ± 5 3
	Gain per day (gm)	2 0 ± 0 12	2 0 ± 0 11	2 0 ± 0 49	2 1 ± 0 12	2 0 ± 0 28
Liver	Weight/Body wt (per cent)	4 52	3 59	3 71	3 61	3 67
Liver	Vitamin A (μgm per cent)	309 ± 50	774 ± 141	494 ± 111	347 ± 40	306 ± 60
	Sulphydryl activity*	100 ± 27 6	152 ± 21 8	128 ± 28 2	125 ± 21 8	103 ± 18 4

* Relative value against group 1 by reading of colorimeter

Table 3 EFFECT OF METHIONINE ON THE VITAMIN A CONTENT IN LIVER OF RATS
(Values shown with mean and standard deviation of six rats in each group)

Group No		1	2	3	4
Vitamin A per rat daily (μgm)		1	1	100	100
Methionine per 100 gm diet (gm)		0	0 2	0	0 2
Body-weight	Initial (gm)	36 3 ± 2 1	36 5 ± 2 5	36 5 ± 2 5	36 5 ± 3 1
	Gain per day (gm)	1 86 ± 0 10	1 94 ± 0 07	1 96 ± 0 12	1 98 ± 0 10
Liver	Vitamin A (μgm per cent)	157 ± 52 6	231 ± 37 8	386 ± 45 0	424 ± 40 6
Muscle	Sulphydryl activity*	100 ± 16 5	146 ± 18 1	104 ± 15 7	157 ± 14 0

* Relative value against group 1 by reading of colorimeter

groups, possibly due to the unsaturated centres, and prevented their conversion into disulphide linkages Thus, our findings that there was a correlation between the increase of sulphydryl activity and vitamin A content are especially interesting

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Conservation of the Biological Activity of a Soluble Ribonucleic Acid

PREVIOUS investigations[1] carried out in one of our laboratories have shown that nucleic acids could be precipitated as quaternary ammonium salts, soluble in various organic solvents, and that they could be regenerated from these organic solutions as the sodium salts on addition of concentrated solutions of sodium chloride This treatment does not affect the infectivity of tobacco mosaic virus ribonucleic acid (RNA)[2]

We thought that it might be interesting to see if such a treatment could also be applied to soluble RNA (S-RNA) and to study its influence on the ability of S-RNA to accept amino-acids activated as amino-acyl adenylates

S-RNA was prepared from baker's yeast according to the technique of Monier *et al* [3] This S-RNA was precipitated by addition at 0° of trimethylhexadecylammonium bromide in the proportion of one quaternary ammonium group for one nucleotidic phosphorus The precipitate of quaternary ammonium ribonucleate, washed three times with distilled water, was soluble in absolute ethyl alcohol On the addition of an equal volume of 3 *M* sodium chloride solution, the S-RNA was regenerated as the sodium salt, and, after three washings with a mixture of equal volumes of ethyl alcohol and 3 *M* sodium chloride, it was redissolved in distilled water

The activity as valine-acceptor of the so-treated S-RNA was compared with that of a control S-RNA, under the following incubation conditions 100–150 μgm of S-RNA, 10 μmoles of adenosine triphosphate (ATP)-Mg^{++} and 0 333 μmole of ^{14}C-DL-valine (corresponding to 1 μc) were incubated in a final volume of 1 ml of *tris*-(hydroxymethyl)aminomethane hydrochloride buffer 0 025 *M* *p*H 7 6 containing 0 0025 *M* magnesium chloride, at 37° for 30 min , in the presence of a valine-activating enzymic preparation The enzymic preparation was the *S*4 fraction of rat ascites hepatoma cells, prepared according to Hecht *et al* [4] The S-RNA was precipitated and isolated, and its radioactivity measured under the conditions described by these authors[4]. The results obtained are summarized in Table 1

Table 1 ^{14}C-VALINE INCORPORATION RATE OF CONTROL S-RNA AND TREATED S-RNA

	C p m /mgm S-RNA	Incorporated valine (mμmoles)	
Control S-RNA	988 1,024	2 13 2 21	2 17
S-RNA precipitated by quaternary ammonium and regenerated	1,024 1,167	2 21 2 52	2 37

The biological activity of S-RNA was suppressed neither by the treatment with the quaternary ammonium salt nor by the dissolution in an organic solvent Instead, a slight increase of the specific activity was observed, in agreement with the chromatographic behaviour of the treated S-RNA and the control S-RNA on 'Ecteola'-cellulose columns[5,6]

The elution with a 0–1 *M* sodium chloride gradient of the control S-RNA showed evidence of a fraction which was eluted by low concentrations of sodium chloride and which was composed of oligonucleotides, inactive as amino-acid acceptors This fraction disappeared after precipitation of the S-RNA as quaternary ammonium salt and regeneration This precipitation would thus constitute a method of purification of S-RNA, which is equivalent, so far as the elimination of inactive oligonucleotides is concerned, to chromatography on 'Ecteola'-cellulose

In summary, after treatment with a quaternary ammonium salt, S-RNA can be dissolved in an organic solvent and regenerated from this solution as the sodium salt, without loss of its biological activity

Experiments are in progress to take advantage of this dissolution in an organic solvent and attempt a fractionation of S-RNA

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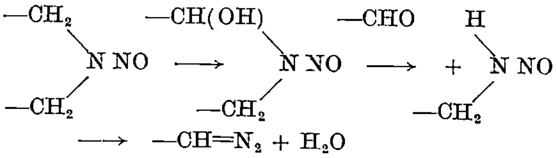
Mechanism of the Hepatoxic Action of Dialkylnitrosamines

Dimethylnitrosamine[1] and some other dialkylnitrosamines[2,3] cause centrilobular necrosis in rats Schoental and Rose[4] suggested independently that centrilobular necrosis was caused by diazo-alkanes formed by the metabolic oxidation of the nitrosamines in the liver *in vivo*[5,6] The sequence of reactions is presumed to be



On this sequence (*n*-butyl)(methyl)nitrosamine (BuMeN NO) should cause centrilobular necrosis, but (*tert*-butyl)(methyl)nitrosamine (Me_3C MeN NO) should not α-Oxidation of the *n*-butyl compound can give either diazomethane or diazobutane, according to which alkyl group is attacked In the *tert*-butyl compound the *tert*-butyl group cannot be attacked in the α position, and oxidation of the methyl group leaves a compound with no α-hydrogen atom, which cannot, therefore, yield a diazo-alkane

The two compounds were synthesized and tested on female rats (Porton Wistar strain) Both compounds were given intraperitoneally as solutions in normal saline

The *n*-butyl compound caused acute centrilobular necrosis in doses of 100–120 mgm /kgm in 1–2 days Doses of 60 mgm /kgm were without obvious effect This compound is about one-quarter as toxic as dimethylnitrosamine, and affects the liver in a very similar way

Twenty rats were treated with the *tert*-butyl compound Four died from the treatment they received, so the doses given were near the maximum tolerable Three survived 1,600 mgm /kgm given in divided doses over 4 days, and three others survived 1,400 mgm /kgm given over the same period The rest survived smaller doses given over shorter periods The survivors were killed at various times after the last dose—from the highest doses at 1, 3 or 10 days after Various lesions were observed on histological examination, but no centrilobular necrosis in any case Thus the *n*-butyl compound caused the typical lesion, but the *tert*-butyl compound did not, in accordance with the diazo-alkane theory

This evidence for the diazo-alkane theory is not, of course, conclusive It is possible that still higher doses of the *tert*-butyl compound might cause centrilobular necrosis Alternatively, perhaps the *tert*-butyl compound does not reach the sites in the liver at which metabolism takes place This last possibility is being investigated with nitrosamines labelled with carbon-14

I thank Dr P N Magee for carrying out the histological examinations, Dr A R Mattocks for preparing the specimens of nitrosamines, and Mr J Rose for technical assistance

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PHYSIOLOGY

Cytotoxic Effect of Aminoacetonitrile on Fibroblast Cultures and its Prevention by Histidine

The understanding of the ætiological factors involved in the curious poisoning arising from the ingestion of *Lathyrus odoratus* seeds has been greatly advanced by the identification of β-amino-propionitrile as the active moiety of the toxic principle and the recognition of the even more potent lathyrogenic property of aminoacetonitrile (AAN)[1,2] However, the site and nature of the primary metabolic disturbance responsible for the characteristic mesenchymal lesions produced by aminonitriles have not yet been elucidated The present investigation was designed primarily to explore the possibility of using tissue cultures as a prospective tool for studying under *in vitro* conditions the biochemical mechanism underlying the toxic action of AAN

The experiments were performed regularly with chick embryo fibroblasts grown in a modified *M*–150 medium of Morgan *et al* [3] consisting of Hanks's salt solution, a mixture of amino-acids and vitamins and fortified with 2 per cent calf serum In most experiments 3–4 days-old cultures were used and AAN was added with fresh medium following the removal of the initial culture fluid Under such conditions

Table 1 PREVENTION OF CYTOTOXIC ACTION OF AMINOACETONITRILE BY HISTIDINE

Amino-acetonitrile (μmoles per ml)	L-Histidine (μmoles per ml)	Time course of cytotoxic effect (hr)				
		24	48	72	96	120
0	0	—	—	—	—	—
0	0 25–4	—	—	–	—	—
0 7	0	+	+ +	+ + + +	+ + + +	+ + + +
0 7	0 25	—	—	+ + +	+ + + +	+ + + +
0 7	0 50	—	—	+ +	+ + +	+ + + +
0 7	1 00	—	—	—	—	+ +
0 7	2 00	—	—	—	—	—
1 0	0	+ + + +	+ + + +	+ + + +		
1 0	1 00	—	+ +	+ + + +		
1 0	2 00	—	—	+		
3 0	0	+ + + +	+ + + +	+ + + +		
3 0	1 0	+ +	+ + + +	+ + + +		
3 0	2 0	+ +	+ +	+ + +		
3 0	4 0	+	+ +	+ +		

Suitable dilutions of aminoacetonitrile hydrosulphate and histidine hydrochloride neutralized with NaOH were added to 3-day-old cultures along with the replacement medium *M*-150 (ref 3) Incubation (at 37° C) was continued and the cultures were examined microscopically at the intervals indicated All experiments were performed in 5-replicate tubes

Key — No effect, +, scattered appearance of rounded cells but cell-sheet intact, + +, incipient loosening of cell texture, + + +, pronounced fragmentation of cell sheet, large number of loose rounded cells, + + + +, disintegration of cell sheet complete, rounded cells and cell debris

AAN (100 μgm per ml) produced a series of progressive alterations manifested by swelling of the nuclei, rounding of the cells and enlargement of their volume until the fibroblastic morphology was no longer recognizable concurrently, fragmentation of the cell-sheet became increasingly pronounced and finally complete disintegration of the culture took place at the end of 2–3 days after the addition of AAN With higher levels of AAN (150–400 μgm per ml medium) the course of the toxic phenomenon was considerably accelerated and short-circuited

The noxious effect of AAN was most prominent in young, actively proliferating fibroblasts and appeared to decrease with increasing age of the culture This observation may be correlated with the striking enhancement of AAN toxicity occurring in fibroblast cultures supplemented with microgram quantities of triiodothyronine, triiodothyronine has been shown previously to stimulate considerably the metabolic rate of chick fibroblasts[4] Both findings seem to suggest that high metabolic activity is essential for poisoning to occur It may be also relevant to mention in this connexion that the susceptibility to experimental lathyrism is highest in weanling rats and declines sharply with increasing age of the animals[5]

Mouse fibroblasts exhibited an essentially similar response to AAN although their sensitivity was of a somewhat lower order than that of the chicken fibroblasts On the other hand, two epithelial lines tested (kindly supplied by Dr J F Morgan), namely, human kidney cells and the *FL* strain of human amnion cells, proved to be refractory to AAN even at doses threefold higher than those lethal for chick fibroblasts

Of particular interest was the observation that addition of an enzymic hydrolysate of lactalbumin to the culture medium (10–20 mgm per ml) resulted in a significant delay or even complete prevention of the toxic phenomenon induced by AAN under the usual test conditions The latter finding, which appears to bear directly on the mode of action of AAN, may be related to the repeated observations that the course and severity of the *Lathyrus* poisoning in rats can be modified considerably or attenuated by maintaining the animals on protein-rich diets[6,7]

The protective effect of lactalbumin could be duplicated by raising the amino-acid concentration in the culture medium Furthermore, out of 20 natural amino-acids tested singly or in various combinations, only histidine overcame the cytopathic action of AAN As shown in Table 1, L-histidine at 2 × 10^{-3} *M* gave complete protection against 7 × 10^{-4} *M* AAN When tested against graded amounts of AAN, the effective dose of histidine varied in a roughly parallel manner with the changing levels of AAN

Further work revealed that D-histidine is equally effective as its natural (L-) enantiomorph in counteracting the toxicity of AAN Histamine and imidazole, on the other hand, failed to exhibit a protective effect

It may be concluded, therefore, that the ability of histidine to prevent the cytotoxic action of AAN is probably unrelated to its role as an essential amino-acid since the latter property is known to be stereospecific with respect to the L-configuration[8] The possible relationship of the protective property of histidine to its well-known metal-binding capacity[9] is being examined

Addendum Recent experiments revealed that the cytotoxic action of AAN on fibroblasts requires the presence of traces of copper ions in the culture medium In the light of the latter finding, the protective effect of histidine appears to be attributable to its copper-chelating property

We acknowledge the assistance of Miss M Faierman

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Incorporation of ^{14}C-glycine by Sub-Nuclear Preparations from Rat Liver Nuclei

IT has been shown previously[1] that rat liver nuclei isolated in 0 25 *M* sucrose solution will incorporate ^{14}C-glycine into protein *in vitro* The possibility of non-specific exchange of glycine was eliminated[2] It was shown that radioactivity was retained after re-incubation of labelled nuclei for 2 hr in a 200-fold excess of unlabelled glycine The incorporation of glycine by nuclear preparations was inhibited by 2,4-dinitrophenol (DNP), azide, and anoxia

The ability to incorporate glycine was retained after the nuclear preparations had been subjected to various treatments Suspending the nuclei in water, freezing and thawing this preparation up to four times, or even freeze-drying and resuspending the nuclei in water did not reduce the ability to incorporate by more than 30 per cent Treatment with a detergent, 'Triton *X* 100', was no more effective Microscopic examination showed these treated nuclei to be structurally intact

In a further attempt to disrupt the nuclei, the preparation was subjected to sonic disintegration Microscopic examination showed that complete disintegration was obtained after 15 min at 20,000 oscillations per sec using a Mullard ultra-sonic disintegrator This sonicate incorporated ^{14}C-glycine without any loss of activity compared with the intact nuclei (Table 1)

Table 1

Preparation	Counts/min at infinite thickness
Intact nuclei	192
Sonically disintegrated nuclei	204
Supernatant after 6 3 × 10^6 g_{av} min *	171
Supernatant after 4 41 × 10^7 g_{av} min	156
Residue after 4 41 × 10^7 g_{av} min	380

For incorporation of 2-^{14}C-glycine into nuclear protein the system used was sodium phosphate buffer, *p*H 7 4, 20 m*M*, NaCl, 20 m*M*, ^{14}C-glycine, 1 μc , 1 5 ml of a sub-nuclear suspension in 0 25 *M* sucrose, water to 2 5 ml , temperature, 38° C , for determination of radioactivity, see Rees and Rowland (ref 1)

* Centrifugal force developed at the mid-point of the tube

The sonicate was centrifuged at 105,000*g* for 60 min in a 'Spinco' model *L* ultra-centrifuge at 0° C Using the same incubation medium as for intact nuclei, the supernatant was able to incorporate ^{14}C-glycine and this incorporation was not inhibited by DNP or anoxia Centrifugation of this supernatant for 7 hr at 105,000*g* yielded a pellet which on re-suspension in 0 25 *M* sucrose very actively incorporated ^{14}C-glycine The supernatant also retained ability to incorporate (Table 1)

Sub-nuclear particles have been prepared from calf-thymus nuclei by extraction with *tris* buffer (*p*H 7 1)[3] These particles incorporate amino-acids but, in contrast to the preparations described in this paper, they require a system containing the nuclear *p*H 5 enzymes[3], adenosine triphosphate, guanosine triphosphate and a source of generating adenosine triphosphate

This work was financed by a grant from the British Empire Cancer Campaign

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Fate of Tritiated Noradrenaline at the Sympathetic Nerve-Endings

Previous work in this Laboratory has shown that tritiated adrenaline and tritiated noradrenaline are taken up and retained in certain tissues for long periods of time[1,2] It was also demonstrated that chronic denervation of sympathetic nerves resulted in the decreased ability of denervated tissues to take up administered tritiated noradrenaline and adrenaline[3] These observations indicated that sympathetic nerve-endings selectively took up and retained circulating catecholamines This property enabled us to label the nerve-endings with tritiated noradrenaline and to study its fate on liberation from sympathetic nerves

In the experiments described here cats were given tritiated noradrenaline, the splenic nerve stimulated in the manner described by Brown and Gillespie[4], and the tritiated noradrenaline and its metabolites measured in the venous outflow Because of the relatively large amount of tritiated noradrenaline present in the blood supplying and leaving the spleen, this organ was isolated and perfused with blood from a donor cat that was free of radioactive material Cats were given 200 μc /kgm DL-^{3}H-noradrenaline (20 mc /mgm) in the forelimb vein Three hours later the animals were anæsthetized with pentobarbitone and given 3000 U S P units of heparin The small and large intestines were tied off and removed The blood vessels between the spleen, stomach and omentum were tied and cut Polyethylene tubing ('*PE* 200' Clay-Adams) was inserted into the superior mesenteric vein and the tubing clamped The superior mesenteric artery was tied off close to the aorta and cut, and the splenic artery was separated from the connecting tissues Polyethylene tubing ('*PE* 20') filled with heparinized saline was inserted into the artery branching from the splenic artery to the stomach and was used to inject drugs into the spleen A second cat received pentobarbitone and heparin, and its femoral artery cannulated with polyethylene tubing ('*PE* 200') and connected to the splenic artery of the first cat The blood supplied from the donor cat was replaced by blood obtained from a third cat The portal vein of the first cat was tied and cut, and the tubing in the mesenteric vein opened After the remaining connexions were tied and cut, the spleen was placed in a saline water bath at 37° C The splenic nerve was placed on a dipolar platinum electrode and was coated with paraffin The splenic nerve was stimulated with 10 V for a duration of 1 msec a stimulus and a frequency of 10 or 30/sec An equal number of

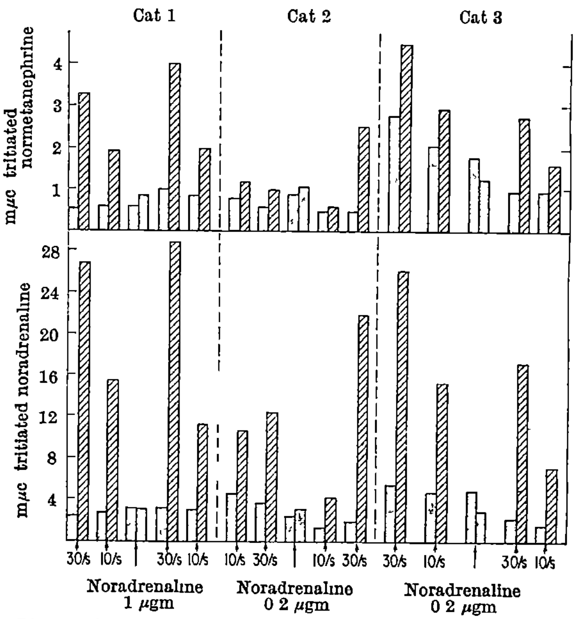

Fig 1 Release and *O*-methylation of tritiated noradrenaline after stimulation of the splenic nerve Cats were given tritiated noradrenaline intravenously and the spleen was prepared as described in the text Splenic nerves were stimulated at a frequency of 10 or 30/sec Cats 1 and 3 received a total of 300 stimuli for each period and cat 2 received 200 stimuli Blood was collected 2 5 min before and 2 5 min during and after stimulation from cats 1 and 3 and 2 0 min for each period from cat 2 Non-radioactive noradrenaline was injected into the splenic artery Each bar represents tritiated noradrenaline or tritiated normetanephrine in mμc found in each period Stippled bars are tritiated noradrenaline and tritiated normetanephrine measured before stimulation, and striped bars are tritiated noradrenaline and tritiated normetanephrine during and after stimulation

stimuli was given in all cases. A Grass stimulator, model *S 4B*, was used. Blood was collected for 2.0 or 2.5 min before and 2.0 or 2.5 min during and after stimulation. There was a 7-min interval between each group of stimuli. Blood was collected in graduated centrifuge tubes containing two drops of heparin solution and the hæmatocrit was determined. An aliquot of plasma was deproteinized with 10 ml of 0.4 *N* perchloric acid and tritiated noradrenaline was determined as described in refs. 1, 2. Tritiated normetanephrine was estimated on the effluent from the alumina column by adsorption on 'Dowex 50' (ref. 5). Results are expressed as mμc tritiated noradrenaline released or tritiated normetanephrine formed in each period.

After each stimulation there was a marked increase in the concentration of tritiated noradrenaline in the venous outflow (Fig. 1). A greater amount of the tritiated catecholamine was released after 30 stimuli/sec than after ten stimuli. During each stimulation period there was a contraction of the spleen. The release of tritiated noradrenaline, however, was unrelated to this contraction since a similar contraction induced by the administration of non-radioactive noradrenaline directly into the splenic artery did not increase the outflow of tritiated noradrenaline (Fig. 1). There was a small amount of tritiated noradrenaline released spontaneously without stimulation.

After each stimulation there was a concomitant increase in tritiated normetanephrine (Fig. 1). The amount of tritiated normetanephrine found was directly related to the amount of tritiated noradrenaline released. The de-aminated metabolites (3,4-dihydroxyphenylglycol and 3,4-dihydroxymandelic acid) in the venous outflow were also determined[5]. The amount of these compounds found were small (0.2 mμc/period) and were not elevated during stimulation.

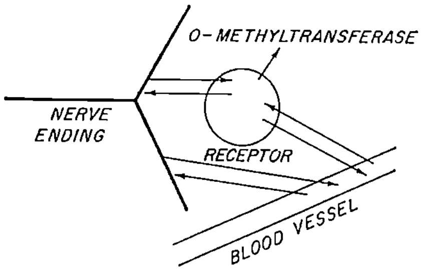

Fig. 2. Fate of noradrenaline at the sympathetic nerve ending

Our earlier work has shown that sympathetic nerve endings or neighbouring structures take up and bind circulating catecholamines[1-3]. In this communication we have demonstrated that stimulation of these nerves releases tritiated noradrenaline which was taken up previously from the circulation. This indicates that the neurohumour, noradrenaline, can be taken up from the blood-stream, bound and then released on stimulation. The *O*-methylated metabolite tritiated normetanephrine was also elevated after each series of stimulations.

It can be concluded that the noradrenaline liberated from the nerve endings reacts with the receptor, and a part is discharged into the bloodstream, a part *O*-methylated by catechol-*O*-methyl transferase[6] and a part bound again at the sympathetic nerve endings. The fate of noradrenaline at the sympathetic nerve ending is shown schematically (Fig. 2).

We wish to thank Mr. R. W. Patrick for his competent technical help.

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Phenylalanine as Inhibitor of Transport of Amino-Acids in Brain

In phenylketonuria the tissues are unable to metabolize phenylalanine to tyrosine, resulting in abnormally high levels of phenylalanine in body fluids, together with a number of disorders of tyrosine metabolism[1,2].

It has been shown that certain amino-acids can interfere with the transport of other amino-acids in tissues[3,4]. Thus it is possible that the high levels of phenylalanine in phenylketonuria might produce a similar type of interference and result in abnormal metabolism of free amino-acids generally.

To determine whether such an interference might occur, the effect of phenylalanine on the uptake of five amino-acids by brain tissue has been investigated, using an *in vitro* technique described elsewhere[5]. Slices of rat brain were agitated for 1 hr. at 37° C. in Krebs's bicarbonate saline which contained the amino-acids under investigation. The concentration of the particular amino-acid being investigated was then estimated in tissue and suspending medium by a colorimetric method[6-8]. There was no evidence that phenylalanine when present in equimolar amounts interfered with the estimation of any of the other amino-acids; at a concentration ten-times higher it interfered only with the estimation of arginine, but to a negligible extent. Recoveries of appropriate amounts of tyrosine added to tissue initially agitated for 1 hr. in medium free of amino-acid were found to be low (87–88 per cent), values found for tyrosine in tissue have therefore been corrected by the corresponding factor. Recoveries of the other amino-acids were satisfactory. The *p*H of the suspending medium (critical in this type of experiment[5]) was measured at the end of all experiments and lay within the range 7.3–7.4. The mean blank value in tissue fluid of each amino-acid being estimated was not greater than 0.35 m*M*.

Results have been expressed in the form of the 'concentration difference' (concentration of amino-acid in tissue fluid minus concentration of amino-acid in suspending medium), which is an indication of the net effectiveness of the transport mechanism for the particular amino-acid being investigated.

Table 1 shows that phenylalanine not only interferes with transport into brain tissue of all the amino-acids investigated, but also that it inhibits uptake of tyrosine considerably more than that of any of the other four amino-acids. It follows that the high phenylalanine-level of phenylketonuria probably causes a similar interference with movements of tyrosine and, to a smaller extent, of other amino-

Table 1 EFFECT OF PHENYLALANINE ON TRANSPORT OF AMINO-ACIDS

Amino-acid	Concentration difference developed between slices and suspending medium (m*M*)		Inhibition by L-phenylalanine (per cent)
	When present alone	In the presence of an equimolar amount of L-phenylalanine	
L-Proline	8 48 ± 0 73 (16)	7 71 ± 0 67 (16)	9
L-Histidine	15 6 ± 1 1 (12)	9 0 ± 1 6 (20)	42
L-Arginine	2 70 ± 0 56 (16)	1 47 ± 0 32 (15)	46
L-Ornithine	3 44 ± 0 31 (12)	1 82 ± 0 14 (12)	47
L-Tyrosine	3 88 ± 0 32 (13)	1 15 ± 0 10 (12)	70

Initial concentration of each amino-acid in suspending medium 2 m*M* Figures shown for concentration difference are mean and standard deviation with the number of samples in parentheses Experimental period, 1 hr 37° C

acids in brain and other tissues of the body The considerable interference with tyrosine transport would be of greater significance in those tissues such as brain and intestinal mucosa which transport tyrosine the most actively[9] With tissues in which there does not appear to be active transport of tyrosine[9], movement of tyrosine would probably not be affected, as has already been shown with liver[10]

I wish to thank Prof N L Edson for giving me facilities in the Department of Biochemistry, and the Medical Research Council of New Zealand for financial support

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Stability of Dextran during Prolonged Storage

So far as we are aware, no measurements relating to the stability of clinical dextran solutions kept for several years have been published Two dextran solutions made in the United States, *A* and *B*, and two made in Great Britain, *C* and *D*, were examined in 1954, and again after storage for 5 yr at 4° C Where applicable the methods of testing described in the British Pharmacopœia[1] were used The optical rotation of each dextran was determined and used in calculating the concentrations of solutions for the viscosity measurements (Table 1) Dextran *D* had undergone the most change, the changes in the others were relatively small

The renal excretion of these dextrans was measured in rabbits (Table 2) The greater part of the dextran was excreted on the first day, less than 3 per cent on the second and less than 1 per cent on the third day Two, three or four rabbits were used for each determination and the daily results averaged, Table 2 records the totals for 3 days after injection The results for dextran *A* suggest a change in molecular composition, but no corroboration of this was found in the other measurements

Table 1

Dextran	Optical rotation (*a*)	Intrinsic viscosity at 25° C 1954	Intrinsic viscosity at 25° C 1959
A	195 1	0 249	0 248
B	197 7	0 254	0 251
C	201 2	0 394	0 388
D	198 1	0 362	0 326

Table 2

Dextran	Percentages of injected dose excreted 1954	Percentages of injected dose excreted 1959
A	37 2	47 6
B	43 1	39 1
C	13 5	14 6
D	15 9	15 4

The retention of each dextran in the plasma was determined by taking the average of daily estimations in the same groups of rabbits (Table 3) During five years of storage little if any change had occurred in the dextrans affecting their retention in the circulation

Table 3 PLASMA CONCENTRATION OF DEXTRAN AS PERCENTAGE OF CONCENTRATION 10 MIN AFTER INJECTION

Dextran		10 min	Day 1	2	3	4	5	6	7
A	1954	100	28 8	11 7	0 4	0 0	0 0	0'0	0 0
	1959	100	29 6	15 2	2 25	0 8	0 0	0 0	0 0
B	1954	100	29 2	13 8	3 6	0 0	0 0	0 0	0 0
	1959	100	30 0	14 1	2 0	0 6	—	—	0 0
C	1954	100	56 8	34 8	12 3	3 8	0 7	—	0 0
	1959	100	61 0	40 9	18 3	10 0	—	—	0 6
D	1954	100	60 5	40 2	22 8	12 8	5 1	—	0 8
	1959	100	56 5	38 7	22 0	—	—	3 3	1 5

The difference in molecular composition of American and British dextrans is well illustrated by these results The lower average molecular weight of the American dextrans is associated with shorter retention in the circulation and greater renal excretion The British dextran, of higher average molecular weight, would induce greater aggregation of red cells *in vitro*[2] and possibly *in vivo*, too

From our observations we conclude that during the 5-yr period there was little, if any, change in the molecular composition of these dextran solutions and none that would be noticeable in clinical use It is probable that any limitation of the storage 'life' of dextran solution will be imposed by defects in the container and its closure rather than by any instability of dextran solution itself

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PHARMACOLOGY

Effects of 7-Chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepin-4-oxide on Mitochondria from Rat Liver and Brain

ALTHOUGH little is known about the relations between *in vivo* drug action and the effects of a drug on metabolic processes *in vitro*, it has been confirmed that psychopharmacological materials in many cases act on oxidative reactions in the mitochondrial respiratory chain The two different drugs, amytal and chlorpromazine, both, for example, have inhibi-

Table 1 EFFECT OF CMPB ON UPTAKE OF OXYGEN, RESPIRATORY CONTROL AND PHOSPHORUS/OXYGEN RATIO OF LIVER AND BRAIN MITOCHONDRIA

The phosphorylation medium consisted of phosphate-buffer, *p*H 7 4, 20 m*M*, *tris*-buffer, *p*H 7 4, 20 m*M*, adenosine diphosphate, 2 m*M*, substrate, 10 m*M*, except β-hydroxybutyrate, 30 m*M*, and malate together with pyruvate, 1 m*M*, glucose, 28 m*M*, and hexokinase in excess Each tube contained mitochondria from 100 mgm (glutamate), 135 mgm or 200 mgm (brain) wet-weight tissue, and sucrose, 50 m*M* In the case of respiratory control adenosine diphosphate and hexokinase were omitted The final volume was 1 ml The vessels were incubated for 15 min at 30°

Substrate	m*M* CMPB	Liver			Brain		
		Uptake of oxygen (μatoms)	Respiratory control (μatoms)	P/O	Uptake of oxygen (μatoms)	Respiratory control (μatoms)	P/O
Glutamate	0	2 3	0 1	2 2			
Glutamate	1	1 3	0	2 2			
Malate	0	3 6	1 4	2 1			
Malate	1	2 5	1 0	2 0			
β-Hydroxybutyrate	0	2 1	1 6	1 7			
β-Hydroxybutyrate	1	1 6	1 3	1 5			
Succinate	0	4 9	2 7	2 0	3 2	1 9	1 0
Succinate	0 4	4 6	3 0	1 8	3 4	2 6	0 8
Succinate	2 0	4 7	3 2	1 7	3 3	3 3	0 5
Pyruvate + malate	0	2 9	0 7	2 6	1 6	0 4	1 9
Pyruvate + malate	0 4	2 5	1 0	2 5	1 7	0 3	1 8
Pyruvate + malate	2 0	1 4	0 8	2 5	1 1	0 6	1 4

tory effects on the flavoprotein in the respiratory chain[1,2] Therefore it was of interest to test whether the new drug, 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepin-4-oxide (CMPB, used as hydrochloride)[1] acts on mitochondrial reactions in a similar manner

CMPB, a drug of a new chemical group, has been tested in concentrations up to 1 m*M* With higher concentrations a precipitate is formed at the physiological *p*H 7 4 Rat liver mitochondria were prepared according to the method described by Ernster and Low[3] Rat brain mitochondria were isolated in 0 25 *M* sucrose containing 1 m*M* ethylenediaminetetra-acetic acid (EDTA)[2] between 1,400 and 12,000*g*

As shown in Fig 1, CMPB is a potent swelling agent on liver mitochondria The swelling effect, however, could be prevented completely by 1 m*M* EDTA (Fig 1*b*), which is in accordance with earlier findings on a variety of other swelling agents[4] Lehninger[5] has shown that mitochondrial swelling produced by various agents in a buffered saline medium, but not in 0 3 *M* sucrose, could be reversed by adenosine triphosphate and magnesium In Fig 1*C* it can be seen that in a potassium chloride medium, swelling induced by CMPB could be reversed partially by adenosine triphosphate alone and more effectively by adenosine triphosphate with magnesium Using brain mitochondria, no swelling could be obtained even with higher concentrations of CMPB (2 m*M*), however, 20 m*M* phosphate, too, which causes a rapid swelling of liver mitochondria, had no effect on brain mitochondria A resistance of brain mitochondria against swelling agents has been observed recently by Spirtes[6]

The effect of CMPB on mitochondrial oxidation and phosphorylation and the respiratory control is shown in Table 1 There is a distinct inhibition of the oxidation-rates with substrates linked with diphosphopyridine nucleotide (DPN), however, no inhibition is found using succinate as substrate Whereas the phosphorylation with the DPN-linked substrates is almost unaffected, with succinate there is a slight but obvious depression of the ratios of phosphorus to oxygen Similar results are obtained concerning the respiratory control Only with succinate a marked loss of the respiratory control could be detected With brain mitochondria analogous results were obtained

To find out the site of action of CMPB, the 2,4-dinitrophenol (DNP)-induced adenosine triphosphatases of both types of mitochondria were examined, because it has been suggested, from the DNP-induced adenosine triphosphatase, to occur only at the first phosphorylation step at the flavin region[7] As shown in Table 2, the DNP-induced adenosine triphosphate is markedly depressed by CMPB This effect is even higher with brain mitochondria Amytal, which is known to have an inhibitory effect on the flavoprotein of the respiratory chain[1], stimulates the effect of CMPB

From these observations it is suggested that CMPB has two points of action at the respiratory chain of mitochondria It seems to inhibit the redox reactions of the flavoprotein However, the reactions which transfer the high-energy phosphate at the flavoprotein level probably are not influenced by this drug, because of the small uncoupling effect on the phosphorylation with DPN-linked substrates compared with succinate Low[7] has proposed a scheme of the DNP-induced adeno-

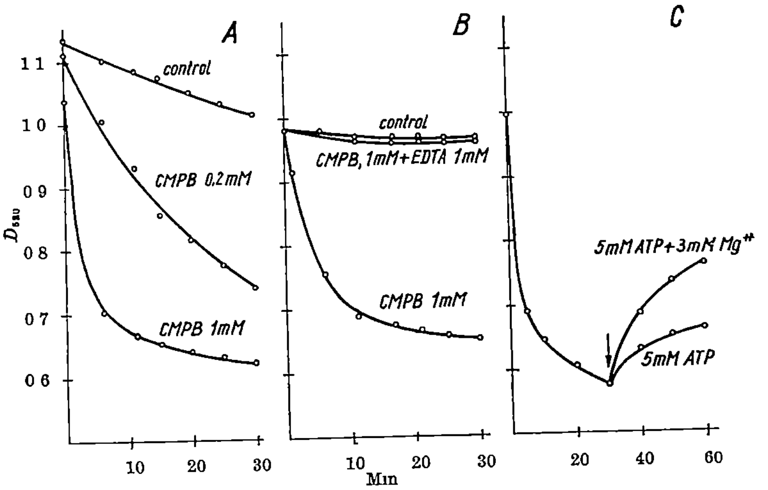

Fig 1 Effect of CMPB on swelling of liver mitochondria *A*, effect of various concentrations, *B*, prevention of swelling by EDTA, *C*, reversal of swelling by adenosine triphosphate and magnesium The swelling medium consisted of *tris*-buffer *p*H 7 4, 0 02 *M* in 0 3 *M* sucrose (*A* and *B*), or in 0 125 *M* potassium chloride (*C*) Mitochondrial swelling was carried out in 1-cm tubes at 520 mμ at room temperature

Table 2. EFFECT OF CMPB ON THE DNP-INDUCED ADENOSINE TRIPHOSPHATASE

Each tube contained mitochondria from 100 mgm (liver), or 200 mgm (brain) wet-weight tissue, adenosine triphosphate, 5 m*M*, *tris*-buffer, *p*H 7·4, 5 m*M*, DNP, 0·1 m*M* in 1 ml. 0·25 *M* sucrose, and was incubated for 20 min. at 30°

Additions	m*M* CMPB	μmoles inorganic phosphate liberated	
		Liver	Brain
	0	2·05	1·59
	0·1	1·95	1·34
	0·5	1·76	1·22
	1·0	1·53	0·89
Amytal, 10^{-3} *M*	0	1·92	1·43
Amytal, 10^{-3} *M*	0·1	1·65	1·35
Amytal, 10^{-3} *M*	0·5	1·34	1·14
Amytal, 10^{-3} *M*	1·0	1·19	0·76

sine triphosphatase reaction. In agreement with that scheme it may be suggested that the reaction between the flavoprotein and the reduced diphosphopyridine nucleotide may be the one inhibited by CMPB. The second point of action of this substance seems to be the phosphorylation steps between cytochrome *b* and the cytochrome oxidase, which is suggested from the uncoupling effect on succinate-linked phosphorylation.

No marked difference between the effects on mitochondrial reactions from brain and liver has been found. This seems to indicate that the effects shown here may have a common meaning as applied to mitochondria from different origins. Although we are not able to explain the special psychopharmacological action of this drug *in vivo* by the results obtained *in vitro*, it is of interest to note that chlorpromazine, a chemically completely different compound, which has similar pharmacological effects, acts on mitochondria in a surprisingly analogous way[8-10].

CMPB, available under the trade name 'Librium(R)', was a gift from the Deutsche Hoffmann-La Roche AG.

We thank Mrs. Brigitte Kuhn for skilful technical assistance.

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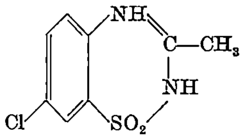
A Non-diuretic Benzothiadiazine with Anti-hypertensive Properties

IN recent years the benzothiadiazine diuretics have become the drugs of choice for treating mild to moderate hypertension. Although their anti-hypertensive effect is relatively weak, they are quite non-toxic and are well absorbed orally. The mechanisms by which these agents reduce blood pressure in hypertensive individuals are not known. Some investigators[1,2] have implicated natriuresis as the primary event, whereas others[3] have suggested that the concentration of circulating pressor agents is reduced. That benzothiadiazine diuretics do antagonize *in vitro* intestinal and aortic contractions has already been demonstrated[4,5]. Consequently, the possibility exists that these agents directly affect the responsiveness of vascular smooth muscle. It was, therefore, considered of interest to try to dissociate the anti-hypertensive and diuretic actions of these compounds and, further, to intensify the former action while minimizing the latter.

Accordingly, several types of benzothiadiazine analogues were synthesized by Dr. J. G. Topliss, M. H. Sherlock and Dr. N. Sperber of the Chemical Research Division, Schering Corporation, and afterwards evaluated for anti-hypertensive and diuretic activities. It soon became apparent that certain structural modifications of the benzothiadiazine nucleus produced a class of compounds that lowered arterial blood pressure acutely in laboratory animals. The most critical structural change involved the elimination of the sulphamyl group from the benzenoid moiety. A representative member of this des-sulphamyl benzothiadiazine series which has received considerable attention in our laboratories is 3-methyl-7-chloro-1,2,4-benzothiadiazine-1,1-dioxide (*SRG* 95213). This agent has virtually no natriuretic or diuretic activity. Indeed, it caused slight sodium and urine retention in acute experiments on hydrated rats.



In acute work on more than 100 anæsthetized, normotensive cats and dogs, intravenous injections of *SRG* 95213 (2·0–10·0 mgm./kgm.) caused an immediate and prolonged fall in arterial blood pressure. In contrast, benzothiadiazine diuretics, in doses as high as 40·0 mgm./kgm., produced little or no change in blood pressure under the same experimental conditions. In chronic work on hypertensive dogs (Goldblatt renal preparations) and rats (active deoxycorticosterone acetate and metacorticoid hypertension), daily oral administration of 5·0–20·0 mgm./kgm. of *SRG* 95213 resulted in a gradual and sustained reduction in blood pressure followed by a gradual recovery to pre-treatment-levels after the drug was discontinued (Figs. 1 and 2).

In other experiments designed to elucidate the mechanisms whereby this agent produces its characteristic effects, it was observed that *SRG* 95213-induced hypotension in anæsthetized cats was not blocked by previous administration of atropine, phentolamine, hexamethonium, chlorpheniramine, reserpine or by transection of the cervical spinal cord. In addition, direct injections of *SRG* 95213 into the femoral, renal and coronary arteries of anæsthetized dogs caused immediate and prolonged increases in the rate of flow of blood through each vascular bed. The results of this work suggest that *SRG* 95213 does not primarily influence nervous pathways or humoral factors in producing its hypotensive effect, but rather induces a direct peripheral vascular relaxation. *In vitro* experiments on rat and rabbit aortic strips tend

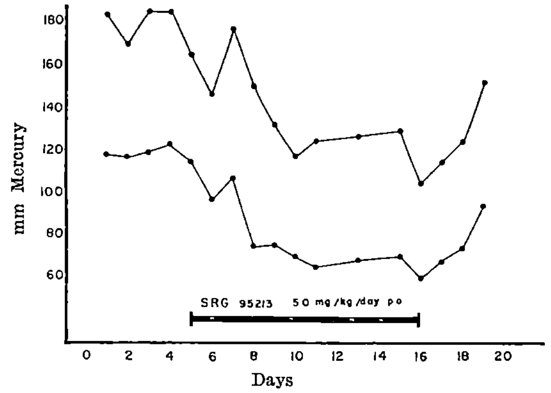


Fig 1 Anti-hypertensive effect of *SRG* 95213 on the systolic and diastolic pressures of one renal hypertensive dog

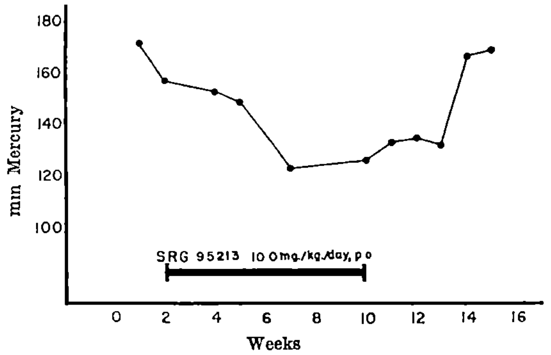


Fig 2 Anti-hypertensive effect of *SRG* 95213 on the mean systolic blood pressure of 9 DOCA hypertensive rats

to support this thesis by demonstrating *SRG* 95213 antagonism to contractions induced by several different classes of vasoactive agents, for example, norepinephrine, angiotensin and serotonin

In summary, it has been shown that *SRG* 95213, although structurally similar to the benzothiadiazine diuretics, is, in fact, non-diuretic but does reduce blood pressure in normotensive as well as hypertensive animals Preliminary clinical observations in hypertensive individuals have, so far, confirmed the results obtained with animals It is therefore not unreasonable to assume that *SRG* 95213 represents a true separation of the anti-hypertensive and diuretic properties associated with benzothiadiazine diuretics The possibility does exist, however, that *SRG* 95213 possesses an anti-hypertensive action unlike that of the benzothiadiazine diuretics and, therefore, may be representative of a new class of anti-hypertensive agents

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RADIOBIOLOGY

Effect of γ-Radiation on *Escherichia coli* grown in Deuterium Oxide Medium

IT has been shown by several authors, for example by Alper and Gillies[1], that the nature of the growth medium has an effect on the radiation sensitivity of bacteria with regard to their ability to divide and form colonies A simple, and yet considerable, change in the growth medium is the substitution of D_2O for H_2O It was found that, on transferring cells from normal minimal medium (NH_4Cl 2 gm , Na_2HPO_4 6 gm , KH_2PO_4 3 gm , NaCl 3 gm , $MgCl_2$ 10 mgm , Na_2SO_4 26 mgm , glucose 5 gm per litre) to the same made in 99 5 per cent D_2O, the cells grew, after a long lag period, with a generation time of 4 hr at 37° C , which is about five times the normal Similar results were found by Borek and Rittenberg[2] Such cells, after growth, must possess very considerable substitution of deuterium for hydrogen, with consequent modification of hydrogen-bonded molecular structures as well as any changes due to the increased rotational moment of inertia of water The greatly reduced rate of division could be due to slowed enzyme kinetics, to less efficient protein synthesis, or to some modification in the DNA, among other reasons Since it is likely that radiation action, as regards cell division, is concerned with the DNA or its organization, it seemed of interest to see whether cells grown in D_2O showed any differences in radiation sensitivity

The results of two separate experiments, in which cultures grown in H_2O and D_2O were simultaneously irradiated in a cobalt-60 source, are shown in Table 1

Table 1

Dose (r)	Survival (per cent) D_2O First experiment	Survival (per cent) H^2O	Survival (per cent) D_2O Second experiment	Survival (per cent) H_2O
5,000	15 0	8 0	28	23
10,000	4 8	2 1	5 1	3 9
15,000	1 5	$3 7 \times 10^{-1}$	0 7	1 1
20,000	$2 1 \times 10^{-1}$	$1 6 \times 10^{-1}$	$1 1 \times 10^{-1}$	0 26
25,000	$2 5 \times 10^{-2}$	$3 5 \times 10^{-2}$	$3 7 \times 10^{-2}$	$1 1 \times 10^{-1}$
30,000	$7 1 \times 10^{-3}$	$1 1 \times 10^{-2}$	$4 4 \times 10^{-3}$	$2 8 \times 10^{-3}$

Both types of bacteria are inactivated, to a first approximation, according to the relation $\ln(n/n_0) = -(\text{constant})\ (\text{dose})$, when n is the number of surviving colony-forming units for a given dose, and n_0 is the number present for no dose The average 37 per cent doses, for which $\ln(n/n_0) = -1$, are 3,040 r for H_2O bacteria and 3,230 r for D_2O The difference is not significant.

In these experiments the bacteria were diluted in H_2O medium and plated on H_2O agar Since the return transition to H_2O might be thought to affect the colony count, the colony counts so found were checked against the turbidity of the culture, which is measured in D_2O The plate counts matched the expected turbidity. Microscopic observations showed no visible alteration in the bacteria grown in D_2O It is believed, therefore, that the colony counts made in this way are significantly related to survival Whether cells plated on D_2O agar would show a difference cannot be said here

In addition to these experiments the relative radiation sensitivity while the cells were adapting to D_2O was measured, and the results are shown in Table 2

These results involve separate observations from the previous series The variation in D_{37} is probably only

Table 2 RELATIVE RADIATION SENSITIVITY OF BACTERIA WHILE ADAPTING TO D_2O

Time (hr)	Control colony count	D_{37} (roentgens)
0	$1\ 4 \times 10^6$	3,000
16	$1\ 8 \times 10^6$	4,100
23	$1\ 6 \times 10^6$	6,000
39	$9\ 0 \times 10^6$	4,900
47	$4\ 3 \times 10^7$	3,700

significant as regards the 23-hr stage, just before the cells began to grow and divide in the new medium This kind of behaviour has already been reported for normal cells by Stapleton[3] and cannot be ascribed to any special effect in D_2O

These experiments suggest that the DNA in deuterium-grown bacteria is not markedly different, using radiation sensitivity as an indicator, from that in normally grown bacteria The considerable modification in growth-rate, therefore, still offers a challenge in interpretation

Acknowledgement is made of the assistance of Mrs Anne Porter in the bacterial assays

The work was aided by the U S Atomic Energy Commission

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Specificity of Plant Feeding in Mosquitoes as determined by Radioactive Phosphorus

THE development of resistance to insecticides in mosquito populations has made it increasingly apparent that alternative methods of control are required In this connexion, the plant-feeding habits of mosquitoes might offer a possible approach to control by biological means If specificity could be proved in the plant-feeding habits of any mosquito species, the extermination of such plants might lead to the control of that species

It is generally assumed in entomological literature that mosquitoes, especially the males, feed on nectar from flowers In work using phosphorus-32 on the feeding habits of arctic mosquitoes, West and Jenkins[1] claimed that, under laboratory conditions, both males and females of *Aedes communis* DeGeer ingested the juices and nectar from flowers of several species of arctic plants The present communication gives results of preliminary experiments, done in the Biology Branch of Atomic Energy of Canada, Ltd , Chalk River, Ontario, to investigate the plant-feeding habits of *Aedes aegypti* L reared in the laboratory and of native *Aedes* species from this area

In these experiments, the flowers and leaves of 24 different species of native plants were labelled with phosphorus-32 solutions at a concentration of 20 μc /ml Preliminary tests with cotton wicks, using different concentrations of phosphorus-32 (as $H_3{}^{32}PO_4$) in 5 per cent dextrose solution, had proved that this concentration gave easily detectable counts in both male and female mosquitoes, which fed on the dextrose solution

So far as possible, plants found in bloom around the breeding places of mosquitoes were used for the experiments These were brought into the laboratory and their stems were cut short under water The flowers and/or leaves of these plants were placed in 2-ounce bottles with the ends of the stems, wrapped in cotton, immersed in 30 ml of phosphorus-32 solution The mouths of the bottles were covered with cheese cloth to prevent the mosquitoes from coming in direct contact with the radioactive solution Without exception, the flowers and leaves of these plants took up appreciable amounts of radioactivity, reaching in some species about 25,000 count/min /cm ^{2} of leaf However, the presence of radioactivity in the nectar was not specifically determined

Larvæ of native mosquitoes were collected from temporary pools and swamps and reared together to the adult stage in the laboratory Three species of *Aedes* were identified in these collections These were *Aedes implacabilis* (Walker), *Aedes dianteus* H D and K , and *Aedes punctor* (Kby) The native species were tested as a mixed population and separate tests were carried out with pure cultures of *Aedes aegypti*

For each feeding experiment 30 adults, including both males and females approximately one to two days old, were removed from the stock emergence cage to small laboratory cages (about 11 in × 11 in × 26 in) having sliding plastic fronts These mosquitoes, previously fed on 5 per cent dextrose solution, were left for 24 hr without food before the start of any experiment After a 24-hr period of confinement with the plants, the mosquitoes were killed, washed in three changes of distilled water, dried between filter papers, and mounted under cellulose tape on counting planchets This method of mounting was also used for the plants where, in most cases, the phosphorus-32 uptake of whole flowers and unit samples of leaves (1 cm ^{2}) were measured

Counts of radioactivity were made with a Geiger–Muller end-window tube (3 1 mgm /cm ^{2}) and a Marconi scaler All counts were corrected for background and decay of phosphorus-32 The plants used are listed in Table 1

Table 1

Acer rubrum L
Amelanchier bartramiana (Tausch) Roemer
Amelanchier canadensis (L) Medic
Amelanchier laevis Vieg
Clintonia borealis (Ait) Raf
Comptonia peregrina (L) Coulter
Corydalis sempervirens (L) Pers
Corylus sp
Cypripedium acaule Ait
Epigaea repens L
Erythronium americanum Ker-Gawl
Fragaria americana (Porter) Britton
Geranium bicknelii Britton
Osmunda cinnamomea L
Prunus depressa Pursh
Salix humilis Marsh
Spiraea latifolia (Ait) Borkh
Streptopus roseus Michx
Taraxacum officinale Weber
Trillium grandiflorum Michx
Vaccinium angustifolium Ait
Viburnum alnifolium Marsh
Viola conspersa Reichereb, J E Smith

Results of these laboratory tests showed that feeding had taken place on only three species of plants, namely, *Viola conspersa*, *Trillium grandiflorum*, and *Spiraea latifolia* All mosquitoes confined with these three plant species became radioactive, the average counts of the mosquitoes are shown in Table 2

Table 2

	Aedes aegypti	Native *Aedes* sp
Viola conspersa (American dog violet)	517 counts/min	114 counts/min
Trillium grandiflorum (white trillium)	315 ,,	8 ,,
Spiraea latifolia (meadow-sweet)	54 ,,	32 ,,

An important finding in these laboratory experiments was that, in all cases, the mosquitoes confined with flowers alone did not receive any radioactivity, on the other hand, placing leaves with the respective flowers of these three species of plants or the use of leaves only gave positive results This evidence indicates that, under the conditions of these experiments, mosquito feeding was confined to plant juices from the leaves

These results differ from those of West and Jenkins on *Aedes communis*[1] and emphasize the need for further work on the plant-feeding habits of mosquitoes

We are indebted to Dr Marshall Laird, Institute of Parasitology, McGill University, for suggesting the problem, to Dr A S West, Zoology Department, Queen's University, for help in planning the experiments and identifying the mosquitoes, and to Dr A M Moore, Biology Branch, Atomic Energy of Canada, Ltd , for identification of plants

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Effects of Irradiation on the Incorporation of Amino-Acid into Normal and Regenerating Rat Liver

The biosynthesis of proteins has been studied in normal rat liver[1,2] and in regenerating liver of partially hepatectomized rats[3] Although the effect of ionizing radiations on the incorporation of amino-acids into proteins in normal rat liver has been investigated[4,5], to our knowledge it has not been investigated in regenerating rat liver The present report presents a comparison of the effects of X-rays on the potential capabilities of livers of normal and partially hepatectomized rats to incorporate amino-acids into their liver proteins in relation to the dose and time of exposure to radiation after hepatectomy

In the first of two series of experiments, 20 Sprague Dawley rats, each weighing 80–100 gm , were used Three groups, each consisting of 5 rats, were exposed to 500, 1,000, or 2,000 r of total-body X-irradiation, whereas the remaining five rats served as the non-irradiated control group In the second of these series, 20 rats, each weighing 200–250 gm , were partially hepatectomized[6] under ether anæsthesia Four groups, each consisting of four rats, were given total body exposures of 1,000 r 2, 4, 6, and 18 hr after hepatectomy, whereas the remaining hepatectomized animals served as the non-irradiated control group In both series of experiments the radiation was delivered by an X-ray tube operated at 250 kV and 15 mA. The radiation was filtered through 0 5 mm of copper and the radiation field was rendered uniform by means of a parabolic mirror, measuring in depth 14 2 cm at the centre The dose-rate was 50 r /min All rats were starved after X-irradiation, but had free access to water In the second series, food was withdrawn after hepatectomy

Each rat in the first series of experiments received an intraperitoneal injection of 3 6 × 10^6 counts per min (c p m) (3 0 μmoles) of leucine-U-^{14}C 3 hr after X-irradiation, whereas the rats in the second series received 6 0 μmoles of leucine-U-^{14}C 22 hr after operation All animals were killed 3 hr after the injection From each animal, 3 gm of liver were removed and homogenized in a Dounce all-glass homogenizer at 0° C for 3 min in 15 ml of a 0 35 *M* sucrose medium containing 0 025 *M* potassium chloride, 0 035 *M* potassium bicarbonate and 0 004 *M* magnesium chloride Each homogenate was centrifuged at 105,000*g* at 0° C for 2 hr in a preparative 'Spinco' ultracentrifuge and the clear supernatant fraction was removed and filtered through 8 layers of fine cheese cloth To 1 0 ml of the supernatant fraction 5 0 ml of 5 per cent trichloracetic acid (TCA) was added and the resulting precipitate sedimented by centrifugation was washed twice with 5 per cent TCA, alcohol and ether respectively, as described by Devi *et al*[7]. In a few instances, the precipitate was heated at 90° C. for 15 min in 5 per cent TCA, then washed and counted as before The isotope concentration of the precipitate was not changed by this manipulation Radioactivity was determined with a windowless gas-flow counter using aluminium planchets 12 5 mm in diameter on to which an infinitely thin layer (less than 5 mgm) of the aforementioned precipitate had been placed The counting error did not exceed 3 per cent

Table 1 INCORPORATION OF LEUCINE-U-^{14}C INTO LIVER PROTEINS OF NORMAL AND PARTIALLY HEPATECTOMIZED IRRADIATED RATS

Experimental series	Radiation dose (r)	Time of X-irradiation after hepatectomy (hr)	^{14}C activity (c p m /mgm of protein)	
			Range of values	Mean of values ± *S E*
Non-hepatectomized rats (Series I)	0	—	400–600	510 ± 37
	500	—	460–650	570 ± 36
	1,000	—	500–700	606 ± 39
	2,000	—	550–800	680 ± 46
Hepatectomized rats (Series II)	1,000	2	600–780	702 ± 30
	1,000	4	640–760	708 ± 22
	1,000	6	650–710	686 ± 14
	1,000	18	790–1,050	938 ± 44
	0	—	860–1,120	1,018 ± 45

The results of both series of experiments are summarized in Table 1. Evidently the intact rat liver differs qualitatively from the hepatic tissue remaining after partial hepatectomy in so far as the effect of X-irradiation on the incorporation of leucine-U-^{14}C into liver proteins is concerned Thus X-irradiation at 1,000 or 2,000 r is followed by an increase in the isotope concentration in the proteins of the non-hepatectomized rat liver, whereas in the case of regenerating liver exposed to 1,000 r a decrease in the isotope concentration in liver proteins is found if the animals are irradiated within 6 hr after hepatectomy After this time period, the exposure of the hepatectomized rats to X-rays is without effect on amino-acid incorporation The time period after hepatectomy during which the partially hepatectomized liver exhibits evidence of radiosensitivity is probably not characterized by a change in cell number since it precedes that period during which the DNA concentration increases, a pre-requisite condition for an increased rate of mitosis[8] It is conceivable that during the first 6 hr after hepatectomy conditions exist which either limit the supply of amino-acid-activating enzymes or of enzymes required for transfer of the activated amino-acids to microsomal ribonucleic acid and protein Either condition would reduce the rate of utilization of amino-acids for protein synthesis In interpreting the significance of the observed changes in the hepatectomized, X-irradiated liver, it must be remembered that there are several uncontrolled variables in the experimental conditions prevailing here for example, the variation in elapsed time between X-irradiation and the injection of ^{14}C-leucine, and the effect of starvation on the irradiated as compared with that on the non-irradiated rat livers The contribution of these factors is probably small compared with that of the X-irradiation

In the non-hepatectomized rat, radiation-induced changes are limited to the 1,000 r and 2,000 r dose-level, at the dose-level of 500 r the increase is not statistically significant Although the mechanism underlying the increased incorporation of carbon-14 into the proteins of normal livers of the irradiated animals remains unknown, two possible factors may be operating (*a*), a contraction of the leucine pool may occur, thus reducing the isotope dilution of the injected labelled leucine, and/or (*b*), a translocation of ^{14}C-labelled protein from radiosensitive sites to the less sensitive hepatic tissue[9]

In conclusion it may be stated that the normal rat liver, with cells in the inter-mitotic phase, and regenerating liver, many cells of which are in the pre-mitotic phase, seem to respond differently to the action of ionizing radiation in so far as their ability to incorporate ^{14}C-labelled leucine into protein is concerned

We thank Mr K I Altman for his interest in this work and for his many helpful suggestions

This work was supported, in part, by a grant from the United States Atomic Energy Commission

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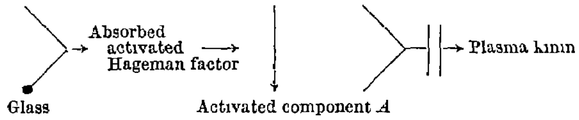
HÆMATOLOGY

Role of Hageman Factor in the Activation of Vasodilator Activity in Human Plasma

HAGEMAN factor is a clot-producing substance which is not found in the plasma of patients with Hageman trait[1] The coagulation of blood obtained from patients with Hageman trait is grossly abnormal, although hæmostasis is usually unimpaired The defect in blood in Hageman trait may be corrected *in vitro* by the addition of partially purified Hageman factor[1,2] Margolis[3,4] has shown that Hageman factor is required for the appearance in glass-treated human plasma of a permeability factor and of a pain-producing and smooth muscle-contracting substance believed to be a plasma kinin Three separate stages have been proposed for the release of plasma kinin when plasma was treated with glass



In this scheme Margolis[3] has suggested that activated component *A* and the permeability factor are the same substance Since the permeability factor and the glass-activated proteinase (activated component *A*) have not been distinguished clearly from plasma kallikrein[5], work was undertaken to determine whether Hageman factor might be related to the activation of plasma kallikrein Conceivably, component *A* might be identified with kallikreinogen, which on activation becomes plasma kallikrein In turn, plasma kallikrein could act through component *B* to release kallidin from kallidinogen[6]

Citrated plasma from patients deficient in Hageman factor and plasma from normal individuals were prepared in silicone-coated tubes and kept frozen until used 'Celite' activation was accomplished by the addition of 1–2 mgm 'Celite Filter-cel' (Johns Manville)/ml of plasma and gentle agitation for 10 min at 25°, acetone activation was accomplished by the addition of 0 25 ml of acetone/ml of plasma and by standing at 25° for 4–6 hr, and trypsin activation by the addition of 1 mgm crystalline trypsin/ml of plasma and by standing at 25° for 15–20 min [7] The vasodilator activity in the preparations, a measure of kallikrein activity, was determined by measuring the increase in blood-flow following their injection into dogs[5] The results in Table 1, recorded in Frey units of human plasma kallikrein, are averages of two or more determinations The vasodilator activity obtained by all methods of activation was probably mediated through a proteinase, since the addition of soybean trypsin inhibitor after activation prevented the increase in blood-flow

'Celite' induces clot-promoting activity in plasma containing Hageman factor but lacks this effect in plasma deficient in this substance[8] In these experiments, 'Celite' released small quantities of vasodilator activity from normal plasma With plasma deficient in Hageman factor, no activation occurred Similarly, vasodilator activity did not appear in Hageman factor-deficient plasma treated with acetone, a technique thought to destroy inhibitors and thus permit the activation of kallikreinogen by kallikreinogenase Trypsin activation, on the other hand, is thought to attack the kallikreinogen molecule directly This method liberated vasodilator activity both in normal and in Hageman factor-deficient plasma The addition of partially purified Hageman factor[9] to the Hageman factor-deficient plasma gave it the ability to produce vasodilator activity upon treatment with 'Celite' or acetone The preparation of Hageman factor contained approximately 1 5 times as much Hageman factor as that found in 1 ml of plasma and was deficient in other recognized clotting factors and in plasminogen, from which Hageman factor is readily separable[1,10,11] Higher concentrations of activated Hageman factor than those used in this work were capable of producing vasodilator activity in dogs presumably by activation of the dogs' plasma kallikrein However the results varied from one dog to another when compared with the response obtained with human plasma kallikrein and did not appear to give the same dose-response relationship

Table 1 EFFECT OF HAGEMAN FACTOR ON THE RELEASE OF VASODILATOR ACTIVITY IN HUMAN PLASMA

Method of activation	Frey units of plasma kallikrein activated in Normal plasma	Hageman plasma	Hageman plasma + Hageman factor*
None	<0 03	<0 03	<0 03
'Celite'	0 13	<0 03	0 5
Acetone	6 1	<0 6	9 3
Trypsin	10 7	5 4	—

All results calculated per ml of starting plasma

* Partially purified Hageman factor gave no detectable vasodilator activity with acetone activation, and <0 15 Frey unit of activity with 'Celite' activation

These experiments are compatible with the hypothesis that the plasma of patients with Hageman trait is not deficient in kallikreinogen but lacks either kallikreinogenase itself or some prior enzymic step required for its activation by acetone or 'Celite' Whether the vasodilator activity released from human plasma by the various methods of activation is due to the release of a single proteinase cannot as yet be unequivocally stated However, plasmin, because of its relatively low potency in producing vasodilatation[5], cannot be one of the proteinases involved Similarly, the question of the identity of plasma kallikrein with the permeability factor and the glass-activated proteinase in human plasma cannot as yet be resolved The results provide evidence that a common mechanism is involved in their release, but this does not exclude the possibility that the enzymes themselves may be different Certainly, however, the simplest hypothesis would be that they were the same substance Keele[11] has reported that salivary and urinary kallikrein form kallidin in the absence of component *B* and in the presence of soybean trypsin inhibitor It would be of interest to know whether plasma kallikrein, which is inhibited by soybean trypsin inhibitor, produces kallidin by a process dependent on component *B*

This work was supported in part by grants from the National Heart Institute, National Institutes of Health, U S Public Health Service, and the American Heart Association

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Presence of Two Iron-Transport Proteins in Serum

THE iron-binding protein transferrin was isolated in crystalline state as early as 1952, and it has been generally accepted that this was the only iron-containing protein in serum under normal conditions

Using electrophoresis in starch supporting medium according to Kunkel and Slater[1] and iron-59 determinations after electro-plating, it is possible to detect minor variations in the electrophoretic pattern which are not in agreement with this point of view The experiments were performed on human beings and the results are given in Table 1

When serum and ^{59}Fe are incubated for 15 min and investigated by electrophoresis, 11 7 per cent of the ^{59}Fe is found in front of the transferrin containing β-globulin It must be noted that no activity is found in the γ-globulin

Table 1. PERCENTAGE OF TOTAL ^{59}FE ACTIVITY

	Sample	Albumin – α_1-Globulin	α_2-Globulin	β-Globulin	γ-Globulin
	In vitro				
1	serum + ^{59}Fe	6 0	5 7	88 2	0 1
	In vivo				
2	Serum + ^{59}Fe injected	2 6	3 3	94 1	0 0
3*a*	Serum + ^{59}Fe injected 15 min after injection	5 5	1 3	93 2	0 0
3*b*	25 min after injection	3 0	1 8	95 2	0 0
4*a*	Transferrin + ^{59}Fe 15 min. after injection	10 0	7	81 3	1 5
4*b*	25 min after injection	9 4	6 4	82 0	2 2
5*a*	^{131}I-transferrin Rabbit	0 1	0 6	84 8	14 5
5*b*	Human	0 2	0 0	97 2	2 6

When serum from a patient is incubated with ^{59}Fe and re-injected, the serum from the patient shows the same type of pattern It is of interest to note that two samples taken after an interval of 10 min show the same relative distribution although the activity has decreased by 25 per cent from the first to the second sample

If the ^{59}Fe is incubated with transferrin (Behringwerke) we find again the same pattern with a splitting of the ^{59}Fe into more than one protein fraction.

The electrophoretic findings are in many ways identical with the effect of a protein–protein interaction This could, however, be shown not to be the case If iodine-131-labelled transferrin is injected into a rabbit or in a man, ^{131}I is found only at the expected place for transferrin and not where the fast fraction of the ^{59}Fe was found

The results can therefore only be taken to indicate that serum contains a protein which is not identical with transferrin and which has the ability to bind ^{59}Fe reversibly

The constant relative size of the fast fraction in experiments 4*a* and *b*, where the iron was injected bound to transferrin, makes it probable that this smaller fraction may even represent an intermediary stage between transferrin and the erythroblasts

The presence of such an intermediate in serum has been suggested by the work of Najean, Ardaillou and Bernard[2] They showed that the rate of iron transfer from transferrin to reticulocytes could be augmented by adding serum to the system The active component was non-dialysable and thermolabile

In the ghosts of erythrocytes from animals injected with ^{59}Fe Falbe-Hansen and Lothe[3] found an iron-containing fraction which could not be identified with known iron proteins This fraction appeared to be an intermediate stage in iron transport as it could only be found during the first hours after the ^{59}Fe injection. The possibility of identity between these two fractions is being investigated

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Genetical Evidence for Synthesis of Transferrin in the Fœtus

Clear-cut evidence that the fœtus synthesizes its own plasma proteins is scant It has been demonstrated by *in vitro* experiments that the liver of a fœtus of three months gestation can incorporate glycine labelled with carbon-14 into proteins resembling albumin, α_1-globulin, α_2-globulin, and β-globulin of human plasma[1] These investigations also showed that metal-binding β-globulin (presumably transferrin) was capable of being synthesized by fœtal liver Other workers have established that the fœtus synthesizes haptoglobin by finding some new-born children with types of haptoglobin that were absent in their mothers[2,3]

In the course of a survey of 212 cord blood sera by starch-gel electrophoresis, genetical evidence was obtained indicating that the fœtus produces its own transferrin

The sera, each with sufficient hæmoglobin added to saturate the haptoglobin completely, were tested by Smithies's technique of starch-gel electrophoresis[4], using Poulik's discontinuous buffer system[5] The resultant gels were sliced horizontally, one half stained with amido black 10*B* to identify protein bands, and the other half with a dilute benzidine stain to locate the haptoglobin–hæmoglobin complexes[6]

Three of the 212 cord sera contained two protein bands in the transferrin region The faster migrating of these two bands corresponded to the usual transferrin designated as *C* In each case, the mobility of the slower-migrating band was identical to that of the D_1 transferrin of a reference standard These three new-born children were therefore of the heterozygous transferrin type C/D_1 The remaining 209 cord blood sera contained only one band in the transferrin region, which had the mobility of *C* transferrin These new-borns were considered to be homozygous (*Tr C/C*) Two of the *Tr* C/D_1 new-born children were negroes, while the third was of Caucasian ancestry Other examples of slow-migrating transferrins (which as a group are designated '*D*' transferrins) have been found in negro populations and Australian aborigines[6]. The three mothers of the *Tr* C/D_1 new-born children had the usual, homozygous transferrin type (*Tr C/C*), while their husbands were all heterozygous (*Tr* C/D_1) (Fig 1) In one of the negro families and in the Caucasian family, autoradiography with iron-59 performed[7] on fresh sera of the infants and their parents verified that the presumptive transferrin bands were truly iron-binding Since each of the three mothers lacked the D_1 transferrin, it is evident that transplacental passage from mother to fœtus could not account for the D_1 transferrin in the cord blood, and consequently these new-born children must have synthesized the D_1 transferrin themselves.

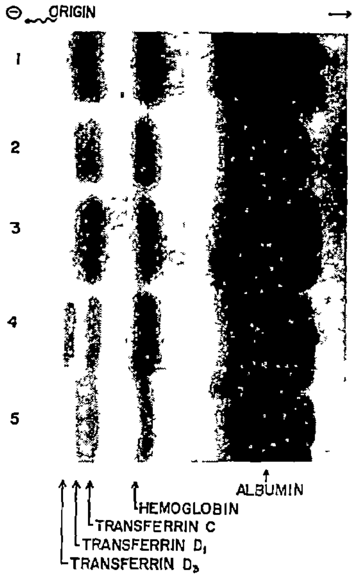

Fig 1 Portion of starch-gel electrophoretic patterns of family with new-born of *Tr* C/D_1 pattern, run in parallel with reference standards 1, New-born, *Tr* C/D_1, 2, reference standard, *Tr* C/D_1, 3, mother, *Tr C/C*, 4, reference standard, *Tr* C/D_3, 5, father, *Tr* C/D_1

We thank Dr Eloise Giblett, of Seattle, Washington, for supplying us with *Tr* C/D_1 and *Tr* C/D_3 reference standard sera This work was supported by grants from the John A Hartford Foundation, Inc , and the National Heart Institute (*H*–4706)

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IMMUNOLOGY

Rapid Preparation of Fluorescent Antibodies using Gel-Filtration

In the preparation of fluorescent antibodies the excess dye is generally removed by dialysis for 5–6 days (ref 1) In order to avoid a time-consuming dialysis and to decrease the risk of destroying antibody activity we have used gel-filtration[2] in the preparation of a number of fluorescent antibodies, the titres of which were afterwards compared with those of dialysed samples A preliminary report of the method has already been published[3]

After the conjugation of serum or a serum fraction with fluorescein isothiocyanate (Sylvana Chemical Co , Orange, New Jersey) as described by Riggs *et al*[4], one portion was dialysed for six days at 2° C against a saline-phosphate buffer (0 15 *M* sodium chloride in 0 01 *M* phosphate, *p*H 7 0) with two daily changes, and another portion was subjected to gel-filtration One ml of fluorescein-treated material was put on a 2 × 5 cm column of gel-filter substance 'Sephadex *G*–25' (AB Pharmacia, Uppsala, Sweden), previously washed with the saline-phosphate buffer During elution, which was performed with the same buffer, two yellow zones appeared The fast-moving one contained the conjugated proteins, while the unbound dye was retarded partly by the gel-filtering effect and partly by adsorption to the 'Sephadex'. The strongly coloured protein fraction was collected and used for the subsequent comparison with the material prepared by dialysis Generally, a two-fold dilution

Table 1 EFFECT ON ANTIBODY ACTIVITY OF DIALYSIS AND GEL-FILTRATION

Experiment No			Antibody titres (dilution × observed titre)			
			Original serum or serum fraction	Fluorescein-labelled material		
				Without further treatment	Gel-filtered fraction	Dialysed fraction
1	Anti-*A* in pooled group *O* serum	I	1 48	1 48	1 48	
		II	1 16	1 12	1 12	1 12
2	Separated 19 *S* anti-*A*	I	1 128	1 80	1 72	
		II	1 128	1 80	1 72	1 80
3*a*	7 *S* anti-*D*	I	1 128	1 160	1 160	
		II	1 256	1 80	1 80	1 20
3*b*	7 *S* anti-*D*	III	1 128	1 20	1 36	No detectable activity
4	Rabbit anti-human γ-globulin	I	1 3200	1 2000	1 2000	
		II	1 6400	1 8000	1 8000	1 4000

I, Titrated immediately after gel filtration, II, titrated after preparation of the dialysed fraction, III, titrated two days after completion of the dialysis

Anti-*A* activity was determined using a 2 per cent suspension of type A_1 red cells in saline In experiment 1 cells from different donors were used in I and II, which precludes direct comparison between the two series In the other experiments I and II were titrated under similar conditions using fresh cells from the same donors

Anti-*D* activity was titrated using a 3 per cent suspension of papainized group *O D*-positive red cells in saline

Antiglobulin activity was determined on slides using a 5 per cent suspension in saline of four times washed group *O D*-positive red cells previously incubated with the anti-*D* serum used in experiment 3

The dilution compared with the original serum or serum fraction was 1 2 5 for the fluorescein-labelled material without further treatment and for the dialysed fraction, and 1 4 5–1 6 25 for the gel-filtered fraction

occurred during the passage through the column and, as expected, the *p*H of the alkaline protein solution changed from 9 0 to 7 0 The elution was usually completed in 10–15 min Antibody titres were determined immediately after gel-filtration as well as after storage for 6 days at 2° C -4° C , when the dialysed samples were also titrated For comparison, on both occasions the original serum or serum fraction was titrated as well as the fluorescein-labelled material without removal of excess dye

The following antibodies were investigated

(1), Anti-*A* in pooled serum from three group *O* donors (2), Anti-*A* separated from pooled human sera by starch-block electrophoresis[5] The anti-*A* active fraction behaved as a 19 *S* γ-globulin in density gradient ultracentrifugation[6] and on diethylaminoethyl-cellulose (DEAE)[7] (3*a*) and (3*b*), Anti-*D* of the incomplete type in the serum from an Rh-immunized woman The antibody behaved as a 7 *S* γ-globulin in ultracentrifugation and on DEAE-cellulose (4), Rabbit anti-human γ-globulin No 72 from Behringwerke A G , Marburg, Germany (5), Neutralizing antibody against chicken erythroleukæmia virus (CEV) Convalescent serum was obtained from a cock which had survived infection with CEV and had then been further inoculated subcutaneously four times with CEV at intervals of about one month The serum was collected 48 hr after the last CEV dose had been given The globulin fraction used was precipitated by adding ammonium sulphate to half saturation

The results of the titrations are presented in Table 1, which shows that no significant loss of activity was caused by the coupling with fluorescein isothiocyanate Nor did any significant changes in titre occur during the later removal of excess dye by either gel-filtration or dialysis in the experiments with anti-*A* (1 and 2) or anti-human γ-globulin (4) Anti-*D* (3 *a* and *b*), however, was less stable, and some loss of activity occurred during storage of the fluorescein-treated material Compared with the fluorescein-treated starting material the gel-filtered preparation showed no significant decrease in titre, whereas the dialysed fraction had a lower activity

In the experiments with CEV antibody, blood smears from a chicken with erythroleukæmia were fixed with methanol for 10 min , stained for 30 min with the fluorescent antibody solutions to be compared, and washed with saline-phosphate buffer With this procedure the stem-cells (erythroblasts) showed a specific fluorescence in the cytoplasm, whereas other cells belonging to the red cell series were not stained No qualitative difference was observed between the dialysed and the gel-filtered preparations The staining properties of various other gel-filtered conjugates containing other antibodies, as well as other fluorescent dyes, have been qualitatively equal to those prepared by dialysis

In a separate semi-quantitative experiment, fixed blood smears were stained with serial two-fold dilutions of CEV antibody, half of each slide being stained with a gel-filtered and the other half with a dialysed antibody solution of equal titre The highest dilution at which fluorescence could still be seen was 1 64 for both preparations

Thus it can be concluded that gel-filtration is superior to dialysis in the preparation of fluorescent antibodies mainly because of its extreme rapidity and also, to some extent, because it involves less risk of damage to labile antibodies

This work was supported by grants from the Swedish Medical Research Council and the Swedish Cancer Society.

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BIOLOGY

Fluctuations in Hormone Activity in Brachyura Larvæ

ALTHOUGH similarities between the control of some chromatophores in larval and adult Crustacea have been noted[1], relatively little is known of the presence of chromatophorotropic substances or their development in larval Brachyura The three zoeal stages and one megalops stage of *Sesarma reticulatum*, reared from hatching to the first crab and beyond, in controlled laboratory conditions, provide an excellent source of material to test for the presence of a *Uca* black-dispersing substance in all stages of larval development and to determine if the activity of this substance during larval development is associated with the larval moult and inter-moult periods

Eye-stalks were dissected from ten zoeæ at the following intervals first zoeæ immediately after hatching, and thereafter at 12-hr intervals up to 72 hr , second zoeæ 6 hr after moulting, 18 hr , 30 hr , 42 hr , 54 hr and 65 hr , third zoeæ 2 hr after moulting, 13 hr , 25 hr , 37 hr , 44 hr and 55 hr , megalops 12 hr after moulting, 24 hr and 48 hr The eye-stalks were placed in a glass dish, the excess water removed with filter paper, and the tissues homogenized with a ground glass rod The homogenate was then diluted in 0 5 ml of filtered sea water which was boiled and centrifuged The supernatant fluid was injected into 10 adult *Uca* from which both eye-stalks had been removed 12–14 hr previously Each adult *Uca* received 0 05 ml of the extract Using the chromatophore index in which the number 5 indicates complete dispersal of the *Uca* black chromatophores and 1 signifies complete contraction[2] the chromatophore stage of the adult crabs was determined at intervals of 15, 30, 45, 60, 90, 120 and 150 min after injection The sums of the activities during the total period of dispersion[3] for the intervals noted previously were then compared to determine if fluctuations in hormone activity of the tracts were associated with a physiological state within the moulting cycle of the larvæ

To compare possible differences in technique between successive experiments, eye-stalk extract from adult *Uca* (10 pairs/2 25 ml) was injected into a separate series of 10 de-stalked *Uca* at the same time as the larval eye-stalks were assayed Sea water controls, 0 05 ml per adult *Uca*, were used with each experiment Initially, muscle from the abdominal segments of the larvæ was injected into a third series of test *Uca* Larvæ in each experiment were taken from one egg-mass to avoid possible genetic differences and eliminate variations in size of the larvæ Where possible, injections were made during the same period of the day in order to avoid diurnal differences in the sensitivity of the black chromatophore response of the recipient *Uca*

The *Uca* black-dispersing substance was found in the unstalked eyes of the first zoeal stage and in the eyestalks of all remaining larval stages of *S reticulatum* Dispersal of the black chromatophores of test *Uca* was not observed following injection of abdominal muscle from the larvæ

At the time of hatching the total activity of the hormone was lowest During the next 36 hr the activity increased, but was again reduced at the interval preceding the first moult The activity increased during the inter-moult period, was reduced prior to, and following, the second moult, and increased again during the intermoult period of the third zoeal stage This same pattern, lower activity before and after moulting followed by an increase in total activity, was observed for extracts of eye-stalks of the megalops Zoeæ which were delayed in moulting normally showed a decrease in total activity at the time when moulting would be expected but, if ecdysis did not follow, the total activity of the hormone increased again

The observed fluctuations in total activity of the hormone cannot be attributed solely to size of the moulting zoeæ since the main reduction in activity occurred prior to moulting, in other words, without any change in size The differences do not show any association with changes in light or phases of the tide It is conceivable, therefore that the fluctuations in activity are associated with moulting or the physiological processes which precede and follow moulting Although the eye-stalk of the adult Brachyura is known to contain the major site of production of the moult-inhibiting hormone it has not been established when these organs develop functionally or if they control moulting in the larval stages Even if different hormones are responsible for the control of colour changes and the control of moulting, several hypotheses are possible on the basis of these results (1), the *Uca* black-dispersing substance located within the eye-stalks of the larvæ fluctuates because of changes in titre associated with moulting but is not directly responsible for, nor involved in, the actual moulting process, or (2), if the endocrine organs within the eye-stalks of the larval stages are responsible for production or storage of several hormones, including the moult-inhibiting hormone, the reduction of the titre of the *Uca* black-dispersing substance may reflect the reduction in titre of the moult-inhibiting hormone also

The reduced titre is followed by ecdysis, a subsequent increase in concentration of the moult-inhibiting hormone, and another larval intermoult period This pattern of decreasing activity or titre prior to moulting and increasing activity after moulting would be repeated through all larval moults and carried over into the crab stages

The assistance of Miss Joanne O'Connell is gratefully acknowledged The work was supported by the Office of Naval Research, *NR* 104–194

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Axenic Cultivation of an Enchytraeid Annelid

THE monoxenic cultivation of *Enchytraeus fragmentosus* Bell, 1959[1] (that is, the rearing, through successive generations in serial subculture, of this micro-annelid in the company of one[2,3] other species —in this case, *Escherichia coli*), was reported by us here[4] some months ago At that time our next goal was its axenic cultivation (that is, growth and reproduction, generation after generation, in complete isolation from living organisms—or parts thereof—belonging to other species[2,3]) We can now report success over a period exceeding 2 months at this date, with indications that such cultivation can be sustained indefinitely

After our earlier report[4], *E fragmentosus* has continued to grow monoxenically with little difficulty and is now in its sixth set of serial cultures For our first axenic cultures we used a few of the numerous worms in an old (231-day) monoxenic culture originating with one of the 3 fragments into which the initially monoxenized organism had divided[4]

Axenization was accomplished by rinsing the worms 3 times in sterile distilled water, followed by their transfer to 10 ml antibiotic solution (1,000 units potassium penicillin *G* and 100 γ streptomycin sulphate per ml), after 1 5 hr the worms were moved to a second antibiotic rinse, and, 50 min later, 7 were inoculated on to 1 5 per cent nutrient agar slants in 16-mm test-tubes, one worm per tube, the following day, 20 hr later, 5 active worms (2 had died) were transferred to individual test-tubes containing the experimental medium None of the 7 transiently occupied nutrient-agar slants developed bacterial colonies

The experimental medium was initially biphasic (1), a semi-solid base of 1 5 per cent nutrient agar, autoclaved in test-tubes 16 mm wide, allowed to cool to 48° C, and then, while still liquid, supplemented with a sterile heated (lamb) liver extract (HLE), prepared, according to a strict recipe[5] of relatively mild heating (53° C for 6 min), from liver homogenized with equal parts (w/v) distilled water The HLE was incorporated to give a 16 × dilution, uniform dispersal being achieved by gentle shaking of each tube before the agar hardened as a slant (2), A liquid phase of much smaller volume—HLE (diluted × 16), added later at 0 1 ml /tube After inoculation with antibiotic-treated worms, the experimental tubes were kept almost horizontal until evaporation left negligible free liquid—then, on the 32nd day, they were placed upright

Of the 5 tubes one showed contamination with *E coli* on the third day, one gave no visible evidence of contamination until the 32nd day (with an unidentified organism—not *E coli*—of undetermined origin), and the remaining 3, by all evidence to date, received, and continue to harbour, axenized worms In 2 of these 3 tubes the isolated worms grew to large size and began to fragment on the 36th day, yielding, within 1–2 days, 8 and 9 pieces, respectively Sub-cultures into the same medium were made on the 39th day with 5 of the offspring from one tube, one piece of worm/new tube Other sub-cultures have been initiated since Most F_1 pieces, whether left in the original tubes or sub-cultured, have recently reached adult size, and, so far, 3 worms of the former group and 1 of the latter have fragmented, the earliest, in an original tube, starting during the 29th day following the onset of first generation reproduction F_2 worms are growing, and further F_1 fragmentation appears imminent

To our knowledge, *Enchytraeus fragmentosus* is the first axenically grown annelid, being of further special interest as the only metazoon of extensive regenerative powers to be so maintained through successive generations Methods for such axenic culture of a few arthropods (insects, with the exception of one micro-crustacean) and nematodes have been known for from several to many years[3], and of a rotifer for a few months[5] HLE has now proved useful in work with members of three major invertebrate taxa *Caenorhabditis briggsae*[3] (class Nematoda, phylum Aschelminthes), *Lecane inermis*[5] (class Rotifera, phylum Aschelminthes), and *E fragmentosus* (class Oligochaeta, phylum Nematoda) For the axenic maintenance of the second and third of these, HLE has so far been a crucial ingredient in all media The preparation used for *E fragmentosus* was sterilized by Seitz-filtration, which, curiously enough, has consistently yielded HLE's inadequate for the rotifer, *L inermis* (the latter growing only with HLE filtered through 'Millipore' membranes)

With *E fragmentosus* under axenic cultivation on a complex organic (oligidic[2,3]) medium one may now reasonably set out to determine this annelid's absolute nutritional requirements

We thank the National Science Foundation for partial support, through grants *G*–6018, *G*–13138 and *G*–15034

The technical assistance of D JoAnne Ferral, Larry G Harris and Douglas L Getchell is gratefully acknowledged

Note added in proof *E fragmentosus* is now in its tenth set of serial cultures In certain tubes some or all worms show tumorous growth So far this phenomenon has been observed only when enchytraeids have been trapped in liquid—as when an excess of moisture collects, or is introduced[6], as a meniscus at the base of the slant, or when a worm is transferred into water or HLE (3 days is the shortest time so far observed with slants, 2 days with water)

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Life-cycle of an Acanthocephalan Parasite of the Eider Duck

ABOUT 190 species of Acanthocephala occur in birds, but the life-histories are known only in five species In all known cases the life-cycle is indirect

Thom and Garden[1] reported an outbreak of disease among eider ducks caused by acanthocephalan parasites, and Harrison[2] also found them in eider ducks in Orkney Rayski[3] analysed the systematic position of these Acanthocephala and suggested that they are *Profilicollis botulus* (syn *Polymorphus botulus*) In the Ythan estuary, these parasites are known to be responsible for a seasonal epidemic with high mortality every year, this is also known to occur in the Dutch island of Vlieland (Van den Broek, personal communication)

In 1960, 98 dead eiders (*Somateria mollissima* (L)) were collected in the Ythan estuary, 48 of which were unfit for examination Of the 50 dissected, all except two were found to be infected with 100–750 specimens of *P botulus* This suggests that the intermediate host must be common in the locality of the habitat of the duck and must constitute a large proportion of the diet of the eider

Samples of a wide range of the food of the eider taken from the Ythan estuary (which is brackish) were examined but with no positive result The material examined was as follows *Hydrobia ulvae, Littorina littorea, Retusa alba, Mytilus edulis, Corophium volutator, Crangon vulgaris, Gammarus zaddachi-salinus, G zaddachi-oceanicus, Marinogammarus marinus* and *Cancer pagurus*

Petrochenko[4], in his monograph on Acanthocephala, quotes a paper by Belopolskaya in which she states that the intermediate host of *P botulus* is *Gammarus locusta* We therefore concentrated on searching for these larvæ in a large number of different species of *Gammarus* from the habitat frequented by eiders, but with no success For several months attempts were made to infect gammarids obtained from the south coast of the Firth of Forth near Edinburgh Collecting areas were chosen which were not known to be frequented by eiders Laboratory-bred gammarids were also used in these experiments Gammarids greedily devoured both eggs and worms containing eggs They did not become infested, though in some cases eggs were seen in the alimentary canal several days later, hatching had not taken place

Eventually, a single specimen of the common shore crab *Carcinus moenas* was collected and fed with a worm containing a large number of eggs of *P botulus* Two days later the crab was dissected and about a dozen acanthors were seen in process of hatching in the alimentary canal Eventually 10 crabs collected from the same area were fed with eggs and worms containing eggs Five crabs died in the space of 3–5 weeks after infection and were found to contain large numbers of developing acanthellæ One crab died 50 days after infection, showing a large number of acanthella stages Four crabs were killed and examined 62 days after infection and two of these contained fully developed cysts containing acanthellæ (cystacanths) and two others had a large number of acanthellæ

From these experiments it was clear that the crabs had been hyper-infected, and at the same time it was obvious that full development of *P botulus* takes place in *C moenas*

Since then, 68 shore crabs from the Ythan estuary have been examined, and 29 of them were found to be infested with cysts containing acanthellæ of *P botulus*, the numbers varying from 1 to 9 in each crab When these were examined microscopically and the proboscis of the larvæ was compared with the proboscis of adults, it was found that number, arrangement and shape of hooks were identical The cystocanths were situated in the region immediately under the heart, and either above the hind gut or on both sides of it The very high rate of infestation in the shore crab in the Ythan estuary explains the common occurrence of this acanthocephalan parasite in eider duck

This is the first record of the crab *Carcinus moenas* acting as intermediate host in the life-history of acanthocephalan parasites

Unfortunately, Belopolskaya's paper is not available to us, and we have been unable to find out on what grounds she concluded that the larvæ found in *G locusta* were really the early stages of *P botulus*

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ENTOMOLOGY

Intracellular *Rickettsia*-like Micro-organisms in Certain Ticks

Little attention has been paid to the intracellular *Rickettsia*-like micro-organisms in acarines Perhaps the first approach to a comprehensive survey on these organisms in ticks was that of Cowdry[1], although a few earlier observations were made[2-4] Cowdry reported the occurrence of intracellular micro-organisms, of rickettsial nature, in the malpighian tubules and in eggs of several species of ixodid and argasid ticks He pointed out that, being present in eggs and unfed larvæ, the organisms were transmitted by inheritance, and postulated a symbiotic function Further work on the morphological appearances of such micro-organisms and their distribution in the malpighian tubules and oocytes of some of these tick species were developed by Buchner[5], Mudrow[6] and Jaschke[7]

Of the several species of *Argas*[8], however, it appears that only *A persicus* has been shown to possess intracellular *Rickettsia*-like micro-organisms Recent work[9] describes in detail for this tick the nature and distribution of the organisms in the malpighian tubules and in its oocytes at different stages of development, their recognition and description being based on histochemical methods and electron microscopy

Suitor and Weiss[10] also recently reported mainly serological and cultural aspects of this organism, isolated from the malpighian tubules, but not, it appeared, from the oocytes, and proposed from their description the name *Wolbachia persica* S and W

The present communication reports on intracellular micro-organisms of similar rickettsial appearance in the ticks *Argas vespertilionis*, *A transgariepinus*, and *A boueti*, collected from bat-infested places in the Abu-Rawash area near Cairo, Egypt In these ticks, as in *A persicus*, the organisms are found in almost all cells of the malpighian tubules and in oocytes In the malpighian tubule cells, the organisms are mostly present in groups of variable size, though single coccoid and short rod forms have been seen

In the oocyte cytoplasm, the organisms appeared in smaller groups, and were only demonstrable after combined ribonuclease enzyme treatment and Giemsa staining[9] or after Feulgen–Schiff's technique

Further work on the nature of the organisms in these species of *Argas*, including examination by electron microscopy is in progress, the work includes a survey also of representatives of other tick genera

I wish to thank Prof D S Bertram, director of this Department, for his valuable suggestions and helpful guidance in the work

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Biological Species confused under the Name *Culicoides austeni* (Carter, Ingram and McFie)

The age-grading techniques at present widely used in the study of biting Diptera having been found applicable to *Culicoides* in Gambia, they were used systematically with material referred to *C austeni* (Carter, Ingram and McFie) As a result it became apparent that material referable on morphological grounds to this species showed pronounced biological differences correlated with differences in its larval habitat Hopkins[2] and Nicholas[3] reared this species from rotting banana litter and Gambian material from this habitat agreed with theirs in requiring blood-meals throughout life for maturation It never mated under laboratory conditions and field observations showed that mating normally occurred in crepuscular swarms Far more abundant in Gambia, however, was a form of this species which, while morphologically identical, differed in having its larval habitat in the salt-water mangrove swamps of the river estuary. The salt-water form proved to be autogenous in the first ovarian cycle, maturing its first batch of eggs at the expense of the larval fat body without taking a blood-meal Swarming was never observed in this species and was evidently not obligatory since it mated freely without it under laboratory conditions, even as a single pair in a glass vial

Since *C austeni* s str was described from a coastal area (Accra, Ghana) the possibility of its being the salt-water form is quite strong The soil-breeding form, presumably that studied by the Nigerian workers and implicated in transmission of filariasis, was synonymized with *C milnei* Austen by Nicholas *et al*[4] *C milnei*, however, also occurs in Gambia and is clearly distinct from both forms of *C austeni* lat in having fully separated eyes as stated by Carter *et al*[1] in their original description of *C austeni*

Salt-water *C austeni* exhibits a pronounced 14-day periodicity due to synchronization of emergence with spring tides As a result of this, the age composition of the population varies cyclically, primiparæ biting for the first time, predominating at times of peak density and higher age groups at times of low density Correlated with this rhythm, a marked decrease in survival-rate after the third oviposition was apparent when the dissection results were substituted into the total numbers The immature stages have not been reared through from egg to adult, but enough is known to make it clear that the life-cycle cannot be completed between one spring tide and the next With a hatching time of two days, a fourth instar larval life of 4–5 days and a pupal life of 3 days the probability is that it can be completed in two full tidal cycles Taken together, these two factors imply the existence of two populations, ovipositing and emerging in alternate spring-tide periods, between which a considerable degree of isolation exists

Also breeding in the mangrove swamps is a third species of the group which can be separated morphologically from the two forms referred to above by virtue of its marked melanism This form, which is certainly a taxonomically separate species, is considered to be very much more closely related to salt-water *C austeni* than either is to the soil breeding form Like salt-water *C austeni* it is autogenous in the first ovarian cycle, mates without swarming and also comprises two alternating populations The two salt-water forms breed in different parts of the mangrove swamp, salt-water *C austeni* in the areas dominated by *Avicennia nitida* and the related species in parts of the zones characterized by *Rhizophora racemosa* and *R mangle* The peaks of emergence exhibited by these two species are usually out of phase with each other by several days, presumably in association with the different soil-levels and times of inundation of their respective habitats

For the taxonomy of this material I am indebted to Dr W W Wirth of the United States National Museum, Washington, D C The advice is gratefully acknowledged of Dr I A McGregor, director of the Medical Research Council Laboratories in Gambia, with whose permission these results are published

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Sorghum Midge, *Contarinia sorghicola* (Coq.), in West Africa

BARNES[1] listed the dates of the first definite records of the sorghum midge, *Contarinia sorghicola* (Coq), in various African countries as Sudan (1930), Nyasaland (1950), Uganda (1951), Aden Protectorate, Gambia, Kenya and Tanganyika (1952), Nigeria (1953) He suggested that these records probably reflect the fact that only in recent years have economic entomologists in Africa been able to turn their attention from cash crops to food crops, and that the midge was well established in these territories before it was first reported

In 1958 Matthee and Oberholzer[2] reported the first appearance of the midge in South Africa and supported the alternative explanation of these records, namely, that the midge was introduced into Africa from America and has gradually spread southwards from the Sudan

In an attempt to determine whether or not the midge was introduced to, and is spreading through, the African continent, we have begun an investigation of herbarium material of the genus *Sorghum* collected in Africa during the past century Herbarium specimens are examined for symptoms of midge damage, and, where this is suspected, spikelets are removed for dissection under the microscope Cecidomyiid larvæ and pupæ have been dissected from material collected more than thirty years ago, and, although larvæ and young pupæ cannot be identified beyond their genus, specific determinations can be made on the adult characters which are displayed by fully developed pupæ

A preliminary survey of the West African section of the genus *Sorghum* in the Kew Herbarium has revealed a wealth of material from which the following new records of *C sorghicola* have been made (Table 1)

Table 1 NEW RECORDS OF *Contarinia sorghicola* (COQ) IN WEST AFRICA

Country	Locality	Host	Date of collection of host
Northern Nigeria	Zaria	*S guineense*	1929
	Munchi	*S guineense*	1929
	Sokoto	*S caudatum*	1929
Gold Coast	Northern Territories	*S guineense*	1931
	—	*S guineense*	1930

In addition, larvæ of the genus *Contarinia*, which are almost certainly *C sorghicola*, were obtained from *S margaritiferum* collected in Sierra Leone (Port Lokko) in 1929

The oldest *Sorghum* collections, made in 1859, yielded no specimens of midge, possibly because these collections are less representative, and in poorer condition than the more extensive collections made in the 1920's and 1930's It is therefore impossible, at this stage, to determine whether the midge has or has not been introduced to Africa, since the new records presented here will easily fit into a pattern of initial introduction to West Africa, followed by distribution eastwards to the Sudan and then southwards to South Africa

However, it is obvious that Barnes was correct in suggesting that the sorghum midge might have been overlooked by economic entomologists in Africa

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Effect of Age and Pregnancy on the Tolerance of Tsetse Flies to Insecticides

It has been found that, in the laboratory, old female *Glossina morsitans* Westw show a considerably greater tolerance to some chlorinated hydrocarbon insecticides than do the young flies of both sexes, and in particular that pregnant flies show an exceptional degree of tolerance Complete log-dosage/probit-mortality regression lines have been determined for dieldrin (Table 1)

Dieldrin was applied in solution in lighting kerosene as a constant volume (0 022 µl) to the mesothorax of flies which had emerged from wild pupæ and had been given a blood feed the previous day Pregnant flies were defined somewhat arbitrarily as those more than 50 mgm in weight at the time of testing, and fertilized flies were those less than 50 mgm in weight and more than 21 days old They were a mixture of those insects which were in an early state of pregnancy or had recently larviposited or aborted All lines were parallel, and all differences given in Table 1 are significant at $P = 0$ 05 (ref 1) Old females also show significantly increased tolerance to DDT and γ-BHC, but males do not develop tolerance as they age These tests have been extended to *G swynnertoni* Aust caught as wild adults, and pregnant females were found to be nine times as tolerant as males to DDT

That breeding females are up to nine times as tolerant as young flies is of obvious practical significance In a recent paper, Yeo and Simpson[2] have given a theoretical forecast of the final results to be expected from repeated applications of a non-residual insecticide to wild tsetse populations, basing their calculations on a constant kill attained at each application The kill is usually assessed from catches of males, and for the first one or two applications, of females They discuss the results of a number of experiments in aerial control The apparent kill for both sexes is normally more than 90 per cent, that for males being the higher, but the final reduction at the end of the experiment is frequently consistent with a kill of about 60 per cent[2] It is suggested that this is because the major part of the measured mortality falls on young flies, the old breeding females escaping These form a small proportion of catches because the mean life of wild females is only 1–2 months[3] and because they come most readily to man when young[4] Thus a high kill may be recorded without any great reduction in the breeding population This will, of course, fall as the old flies die from natural hazards, but the result will be to delay the expected reduction in breeding and increase the value of the later applications above that assigned by Yeo and Simpson's analysis It is also necessary to re-examine the conclusion that an inter-treatment interval close to the pupal period is the best At the appropriate season in Central Tanganyika, the mean temperature is about 24° C [5], the pupal period about 31 days and the mean age at deposition of the first larva 23 days[6] Thus by the end of the third week of life the female fly is pregnant and tolerant of insecticide This means that, with an inter-application interval of 4 weeks[2], all females emerging in the week after an application are potentially tolerant of the next

A second consideration concerns tests for insecticidal resistance For these, care must be taken to test only males or females of known age

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Table 1 Toxicity Data for *G morsitans* to which Dieldrin was applied topically in Kerosene Solution 72 hr Mortality, corrected for Controls (ref 7)

Sample	Mean age (days)	Mean weight (mgm)	LD_{50} (µgm) and 95 per cent fid limits		Ratio and 95 per cent fid limits	
Young males*	4	29 2 ± 3 1	0 0017	0 0013–0 0022	1	
Fertilized females	32	43 3	0 0065	0 005 –0 0085	3 8	2 6– 5 6
Pregnant females	35	56 6	0 016	0 012 –0 021	9 4	6 4–13 8

* Young females of this batch were not tested, but in other batches there was no significant sexual difference in susceptibility (ref 8)

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Increase in Distribution of *Deraeocoris olivaceus*

The study of animal introductions and their fate provides an object lesson in ecology[1] Some, as the leafhopper *Graphocephala coccinea*, become permanent elements of the British fauna, others, as the tingid *Stephanitis rhododendri*, achieve an initial spread but later contract, still others, such as the rhopalid *Liorhyssus hyalinus*, can do no more than maintain an occasional very temporary colony in Britain The present communication concerns a conspicuous mirid bug, *Deraeocoris olivaceus*, which it is timely to map after a space of ten years It occurs on *Crataegus* (hawthorn), usually becomes adult during July, and is collected by beating, its biology is known in broad outline[2]

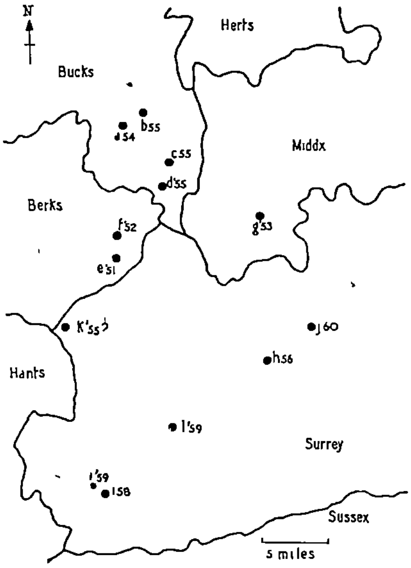

Fig 1 Distribution of *Deraeocoris olivaceus* (Hemiptera, Miridae) in southern England, showing year of first capture at each locality For names of localities see text

The bug was found for the first time in Britain in July 1951 at Silwood Park, Sunninghill, Berks, by Sands[3] It is known in Europe from Greece and the Balkans to France and the Low Countries but is absent from the Baltic lands and Scandinavia Fig 1 shows known occurrences with the year of first capture, based on published[4] or unpublished[5] records The localities, all in Berks, Bucks, Middx or Surrey, are (*a*) Burnham Beeches, (*b*) Hedgerley Park, (*c*) Langley Park, (*d*) Ditton Park, (*e*) Silwood Park, (*f*) Windsor Forest, (*g*) Hounslow Heath, (*h*) Bookham Common, (*i*) Witley and Elstead Commons, (*j*) Epsom Common, (*k*) Camberley, (*l*) Shalford Many of these localities are well known to collectors, and it is unlikely that the bug would be overlooked for more than a year or two

During the past few years the Herts–Middx and Hants–Surrey borders have been searched for the insect but so far without a capture in either Herts or Hants It seems, therefore, that Fig 1 represents the present distribution, based on records during the decade 1951–60, fairly accurately Recent checks have shown that the bug is still present at its earlier known stations Spread of the insect from its 1951–55 area is thus shown to have been slight, save towards the south where its advance seems to be on a broadening front The host-plant is common throughout southern England, so any restraint imposed on it is likely to be climatic No braconid parasitism has so far been detected although its congeners in Britain are known to be attacked

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MICROBIOLOGY

New Method of preparing *Corynebacterium pyogenes* Toxoid with High Anti-hæmolysin Titre in Rabbits

Corynebacterium pyogenes is generally grown in Robertson's medium[1] when small quantities are prepared and in derivatives of this medium for vaccine production Producing this medium for vaccines is an expensive time-consuming operation, so it was decided to investigate the possibilities of using fat-free milk as the basic medium Strains used throughout the work were those recently isolated from bovine lesions and used at present for vaccine production

To a 10-l 'Pyrex' bottle was added 7 l of fresh skimmed milk with a natural *p*H of 6 8, this being particularly important since milk with a higher acidity was found to coagulate on autoclaving The *p*H was raised to 7 4 using 40 per cent caustic soda To this was added 100 gm of calcium carbonate for controlling acidity during incubation Stoppered with cotton wool, the bottle was autoclaved at 5 lb /sq in for 30 min At a higher temperature caramelization of the lactose was found to occur resulting in a dark filtrate After cooling to 37° C the medium was inoculated with 10 ml of a 48 hr active unwashed culture of *Corynebacterium pyogenes* grown in Robertson s medium, with a hæmolysin titre of 1 640 A sterile rubber stopper fitted with an air filter and a length of glass tubing which almost reached to the bottom of the bottle replaced the cotton wool During the 48-hr incubation period at 37° C sterile air was passed slowly through the medium, carbon dioxide was also tried without equal success, as a cloudy filtrate resulted After 24 hr coagulation and digestion of the casein had begun, and after 48 hr all that remained was a small floccular precipitate and pale-yellow supernatant, colourless in small quantities The resultant supernatant was clarified afterwards through kieselguhr and sterile-filtered through Berkfield candles, there was very little loss of fluid during filtering The hæmolysin titre obtained by this method was 1 1,000 The filtrate was toxoided by the addition of 0 5 per cent formalin and this was incubated at 37° C over a period of 2 days 0 5 ml was injected intravenously into two mice which showed no toxic reaction after 24 hr The *p*H was then altered from 5 8 to 6 8 using 40 per cent caustic soda, and 'Thiomersalate' (British Drug Houses) in the final dilution of 1 10,000 was used as preservative 'Thiomersalate' was used in preference to phenol or tricresol as the latter preservatives tended to precipitate the proteins, causing an opacity in the toxoid which increased in density with time and high storage temperatures

Rabbits used in this work were tested for natural immunity before inoculation and those possessing antibodies were not used Their sera were titrated against 5 and 10 minimal hæmolytic dose of *Corynebacterium pyogenes* toxin Ten rabbits were injected with 10 ml of the toxoid intramuscularly with another injection of 15 ml ten days later and 20 ml 10 days after that The animals developed no signs of shock after injection nor was any rise in temperature noted, thus proving that milk pyrogens had been destroyed The animals were bled 14 days after the last injection On titrating the rabbit sera against 10 minimal hæmolytic dose of *Corynebacterium pyogenes* toxin 0 00156 ml of sera inhibited hæmolytic activity, controls were set up This figure represents thirty-two times the potency standard for *Corynebacterium pyogenes* set down by the British Veterinary Codex After 2 months of retesting, the sera showed no appreciable drop in titre I feel this method could be extended to other aerobic and anaerobic proteolytic organisms used for production of vaccine

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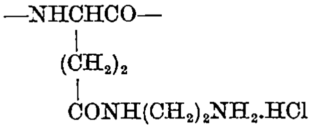
Antibacterial Synthetic Polypeptide Derivatives

As already stated[1], some synthetic polypeptide derivatives exhibit considerable antitubercular activity These antibacterial polypeptide derivatives were prepared from poly-α-aminodicarboxylic-acid-esters (for example, polymethyl-α-poly-L-glutamate, polymethyl-α-poly-DL-aminoadipate) and from polyaminodicarboxylic-acid-anhydrides (for example, anhydro-poly-aspartic-acid) with diamines (ethylenediamine, β-dialkylethylenediamines)[2]

The hydrochloride of the polypeptide derivative which was prepared from polymethyl-α-poly-L-glutamate with ethylenediamine, in particular, was examined, and is now produced by Chinoin Chemical and Pharmaceutical Works Co , Budapest, for clinical experiments under the name of 'Polyanthin' The characteristic feature of the structure of 'Polyanthin' can be given in the formula below, the unit represented is the main component of the polymeric material



$$\begin{array}{l}\text{—NHCHCO—}\\ \quad\ \ |\\ \quad (CH_2)_2\\ \quad\ \ |\\ \quad CONH(CH_2)_2NH_2.HCl\end{array}$$

The microbiological activity of 'Polyanthin' against different micro-organisms was further examined The examinations, the results of which are summarized in Table 1, were performed as follows the strains given in Table 1, were inoculated in bouillon media and incubated at 37° C for 24 hr With 0 1 ml quantities of these cultures, bouillon media containing various amounts of 'Polyanthin' (see first column of Table 1) were infected The controls were infected media with no 'Polyanthin' and non-infected culture media with 'Polyanthin' The degree of growth of the micro-organisms characterized by crosses in Table 1 was visually evaluated after incubation at 37° C for 24 hr

As seen in Table 1, 5–20 γ/ml of 'Polyanthin' restrains the growth of *Staphylococcus aureus haemolyticus* (Wood), and also of the penicillin- and sulphamide-resistant variations of *Staphylococcus aureus haemolyticus* isolated from diseased persons, of *Bacillus subtilis, Mycobacterium friburgensis* and *Mycobacterium smegmatis* There was no effect against bacteria belonging to the Gram-negative group, like *Proteus vulgaris* and *Escherichia coli*, even in concentrations as high as 300 γ/ml 'Polyanthin' seems to be active above all against those strains to which glutamine is necessary for the initiation of growth[3]

After the favourable results of the preliminary investigations clinical tests of 'Polyanthin' were also carried out 'Polyanthin' was used, so far only for local treatments, particularly in obstetrics and gynæcology, as well as prophylactically and in cases of developed suppurations It was used in obstetrics for the treatment of episiotomies and injuries of the vagina and cervix, in gynæcology after different operations of the vagina and abdomen in cases where the healing of the wound was disturbed

A solution, containing 1·3 gm 'Polyanthin' in a litre of water, was used for the different treatments either in the form of rinsings or for the soaking of gauze strips applied as tampons and covers The controls are similar cases as those treated later with 'Polyanthin', but taken from the time before the testing of 'Polyanthin' had begun These control cases were treated with wound powders ('Ultraseptyl-urea' (Chinoin Chemical and Pharmaceutical Works Co , Budapest), 'Dermaphorin' (United Works of Pharmaceutical and Dietetic Products, Budapest)) and wound ointments containing cod-liver oil

From Table 2 it is seen that when 'Polyanthin' is used for the treatments the primary healing of

Table 1

Polyanthin (γ/ml)	*Staphylococcus* (Wood)	*Staphylococcus*, penicillin-resistant	*Staphylococcus*, sulphamide-resistant	*Bacillus subtilis*	*Proteus vulgaris*	*E coli*	*Mycobacterium friburgensis*	*Mycobacterium smegmatis*
5	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
10	—	+ + + +	+ +	—	+ + + +	+ + + +	+ + + +	+ + + +
20	—	—	—	—	+ + + +	+ + + +	—	—
50	—	—	—	—	+ + + +	+ + + +	—	—
100	—	—	—	—	+ + + +	+ + + +	—	—
300	—	—	—	—	+ + + +	+ + + +	—	—

Symbols —, no growth, + +, partial growth, + + + +, full growth

Table 2

	Cases treated with 'Polyanthin'				No of cases	Control cases Healing	
	Treatments		Healing				
	Prophylactin	In case of suppuration	P/I*	P/II†		P/I*	P/II†
Obstetrics	155	81	231 (97 9%)	5 (2 1%)	236	161 (68 2%)	75 (31 8%)
Gynæcology	63	65	124 (96 9%)	4 (3 1%)	128	81 (63 2%)	47 (36 8%)
Total	218	146	355 (97 5%)	9 (2 5%)	364	242 (66 5%)	122 (33 5%)

* P/I, healed per primam † P/II, healed per secundam

vounds is 97 5 per cent, while without 'Polyanthin' he primary healing of wounds is only about 66 5 per ent

Further investigations for the extension of the linical applicability of 'Polyanthin' (for example, uberculosis, surgery, general medicine) are in rogress

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Nature of Colicine Receptors

Colicines are unique among antibiotics in being specifically adsorbed on to the surface of bacteria in a manner analogous to antibodies and bacteriophages Besides being removed from solution by adsorption on sensitive bacteria[1], they can be inactivated by extracts of sensitive, but not of resistant, organisms[2] The nature of colicine receptors on the bacterial surface has not so far been elucidated This communication describes some preliminary observations on the subject

Absorption experiments were undertaken, using two of Fredericq's strains, namely *Shigella sonne P*9, producing colicine *S*3, and the colicine-sensitive *Escherichia coli C*6 When colicine was mixed with preparations of the sensitive *E coli*, each killed by a different procedure, then centrifuged and the residual colicine assayed, a number of properties of the receptor sites were demonstrated They were unaffected when the organisms were killed by heat at 65° C for 30 min , by exposure to 0 3 per cent formalin or 2 per cent phenol, but were destroyed by heating at 100° C for 1 hr or exposure to 80 per cent ethanol

The residual colicine was titrated by the agar diffusion technique developed by E van Horn (personal communication) By this technique small differences in colicine titre can be detected, and results may also be obtained with a more than hundred-fold dilution of the original colicine Partial absorption of colicine could thus be detected but, in fact, in the absorption experiments described here either all or none of the colicine was removed from the solution

The results obtained with the absorption experiments were corroborated by assaying the colicine on agar plates inoculated with a mixture of viable and killed organisms The inoculum consisted of 5–10 × 10^6/ml viable organisms of the sensitive *E coli*, mixed with a suspension of 5–10 × 10^8/ml organisms of the same *E coli* killed by the methods used in the absorption experiments The killed suspensions in which the receptor site had been destroyed did not materially alter the size of the inhibition zones, those in which the receptor sites were intact adsorbed colicine and hence the inhibition zones were significantly reduced in size The amount of reduction was comparable with that obtained by an inoculum of viable cells, substituted for the killed organisms[3]

From these observations it appears that the receptor, at least for this particular colicine, resembles most closely a flagellar or, to a lesser extent, a fimbrial antigen *E coli C*6 is non-motile, which lends particular interest to the finding of a receptor substance on its surface having the properties of a flagellar antigen

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MISCELLANEOUS

A Simple Flattener for holding Filter Papers on Planchettes for Radioactive Counting

The use of a 'Millipore' chimney and filter paper provides a convenient means of collecting radioactive material for measurement of its activity A major disadvantage of this system arises from the curling of these papers by the precipitated materials and the allied difficulties of fitting them in the planchette The use of doubly gummed 'Scotch' tape provides only a partial solution to this problem, and if the material on the filter paper is to be analysed chemically afterwards, the tape adhesive may interfere The use of liquid adhesives and, in the case of 'Millipore' filters, of solvents also does not solve this problem The amount of collected material is limited by the point at which the precipitate will flake and not hold to the paper Recently we devised a simple spring mechanism to hold the paper in the carriers

The precipitates are collected on the surface of hardened small-pore filter paper (for example, Whatman No 42) or modified cellulose papers ('Millipore' filters) and dried in a desiccator The flatness of the resulting material is a function of the amount of precipitated material collected The diameters of the papers are slightly smaller than the inside diameter of the planchettes used (Nuclear-Chicago sample pan 1¼-in in diameter) The papers are fixed inside the planchette by use of a 330° arc of stainless steel spring wire No 24 gauge, so made that the arc has a diameter ¼ in greater than the planchette (Fig 1) The wire is placed within the planchette and on the paper by compressing it with the thumb and forefinger and placing the closed end against the wall of the planchette The spring is then permitted to expand slowly below the level of the planchette side wall, and final adjustment is made with a pair of forceps

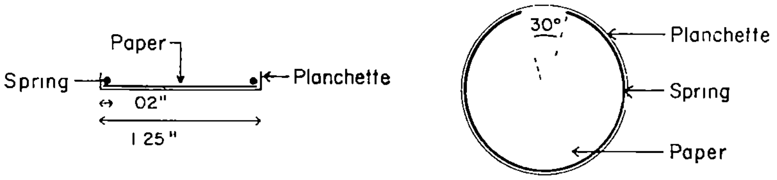

Fig 1 Details of arrangement of the spring flattener

We have found these uniquely successful in holding the papers and their contents flat, they are simple to use and easy to clean They will withstand brief washing in dichromate cleaning solution, may be autoclaved, and can be made by most machine shops A convenient material, though not stainless steel, is commercially available piano wire Thicker wire will provide additional strength where required

This work was supported in part by U S Public Health Service grants *H*–3174 and *E*–3854

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Chemico-Linguistics: Computer Translation of Chemical Nomenclature

I WISH to report what is believed to be the first successful mechanical translation of chemical names into chemical formulæ Tsukermann and Terentiev[1] have indicated their intention to work on this problem

Programming the initial experiments was simplified by imposing several restrictions Simulated tests by manual methods have been successfully performed without these restrictions (*a*) longest carbon chains allowed are ten carbon atoms in length, (*b*) maximum length of chemical names is 59 characters, (*c*) elements other than carbon, hydrogen, oxygen, nitrogen, sulphur, bromine, chlorine, fluorine and iodine were eliminated

The most significant aspect of this research is the linguistic study of chemical nomenclature which preceded, for several years, the development of the computer programme[2]

In effect, a recognition grammar for organic chemistry has been written which can be used not only for mechanical indexing but also for teaching chemical nomenclature Using Harris's methods[3,4] for grammatical analyses both the morphology and syntax of chemical nomenclature were analysed Morphemes, allomorphs, homonyms, etc , were identified as well as their roles in the generation of molecular formulæ

The difficult problem of calculating hydrogen values was overcome by application of the following formula, which is a modification of Soffer's[5] formula for determining double bonds and cyclic structures

$$H = 2(\Sigma M_C - \Sigma M_{db} + 1) + \Sigma M_N - \Sigma M_X$$

where M_i is an expression indicating the summation of the computational values represented by morpheme class i For example, M_C is the number of carbon atoms implied by all morphemes in the chemical names which contain carbon This completely resolved the tedious, though solvable, problem of constructing more complex rules for hydrogen calculation which are required when one attempts to follow the 'natural' inclination to record methyl as CH_3, amino as NH_2, etc

It is the ultimate objective of this work to generate accurate molecular formulæ for all chemical names and also to display and print ideographs, that is, structural diagrams Once the initial linguistic problems have been completely resolved, this can be done It has already been shown that computers can be instructed to draw such diagrams[6,7] However, neither Opler nor Waldo began with uncoded chemical names It is one thing to programme a computer to draw a diagram for a known specific chemical In this case, a human coder works from a diagram drawn by someone and calculates a cipher that is used by the computer to draw the same diagram It is another matter to translate, without human intervention, from chemical names to structural diagrams

The most difficult problem one encounters when trying to include all methods of naming chemicals, rather than just systematic International Union of Pure and Applied Chemistry names, is the ambiguity of different ring-numbering systems While this does not affect the ability to calculate molecular formulæ, it does affect the ease of creating entirely accurate diagrams In such cases the computer will render an invaluable service in reminding the chemist that several alternatives must be considered It may also remind authors to stipulate whether they are following International Union of Pure and Applied Chemistry or some other system

From a linguistic point of view, it is an interesting observation that the basic language of all naming systems in organic chemistry is essentially the same While two chemists will name the same compound differently, both will be able to draw the same structural diagram In this sense, the use of different systems corresponds to the problem of handling dialects rather than treatment of separate distinct languages In addition, the complete understanding of the mechanisms for translating the language of organic chemistry provides useful experimental data for the more general problem of mechanical translation If we cannot solve the problem of translating chemical nomenclature, there would seem to be little hope for translating natural languages such as English with a machine

The following chemical compounds (Table 1) illustrate the capabilities of the present computer programme While a *Univac* I was used for this experiment, the programme can be modified without too much difficulty for smaller computers such as the I B M 650 (ref 8)

Table 1

Chemical name	Molecular formula
1,4-*bis*[*bis*(3-Diethylaminopropyl)amino]butane	$C_{32}H_{72}N_6$
2-[(2-Aminopropyl)ethylamino]ethanol	$C_7H_{18}N_2O$
8-Hydroxy-6-octene-2,4-diynenitrile	C_8H_5NO
1,3,3,4,4-Pentachloro-2-methyl*cyclo*butene	$C_5H_3Cl_5$
2-Phenyl-2,4,6-*cyclo*heptatriene-1-one	$C_{13}H_{10}O$
7-(2,4,5-Trichlorophenoxy)heptanoic acid	$C_{13}H_{15}Cl_3O_3$
Ethyl 2-cyano-5-phenyl-2,4-pentadienoate	$C_{14}H_{13}NO_2$
2-Naphthyl-2-*cyclo*hexen-1-one	$C_{16}H_{14}O$
Diphenyl-3-butynol	$C_{16}H_{14}O$

I wish to thank Dr J O'Connor and Mr C Meunch for their help in writing this programme, Dr C Borkowski for fruitful discussions on problems of mechanical translation, and Prof N Rubin, who acted as my chemical 'informant'.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, October 16

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S W 1), at 5 30 p m—Mr E Sherlock "Advances in the Chemistry of Phosphorous Insecticides"

UNIVERSITY OF LONDON (at King's College, Strand, London, W C 2), at 5 30 p m—Dr F A E Pirani and Dr G F Elliott "Ballistic Missiles and Nuclear Weapons"* (First of seven lectures in War Studies Further lectures on October 23, 30, November 6, 13, 20 and 27)

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London W C 1), at 6 p m—Prof A Rodger "Occupational Versatility and Planned Procrastination" * (Inaugural Lecture)

INSTITUTE OF METAL FINISHING (at the Northampton College of Technology, St John Street, London, E C 1), at 6 15 p m—Mr S G Clarke "Chromate Passivation of Brass"

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W C 2), at 6 45 p m—Mr C F Price "Experimental Techniques Developed for Determining the Performance of Fuel Elements in Nuclear Reactors" (Chairman's Address)

Monday, October 16—Wednesday, October 18

INSTITUTE of METALS (in the Assembly Hall of the Royal Commonwealth Society, Craven Street, London, W C 2), at 9 30 a m on Monday and 9 a m on Tuesday and Wednesday—International Conference on "The Metallurgy of Beryllium"

Tuesday, October 17

UNIVERSITY COLLEGE, LONDON (in the Anatomy Theatre, Gower Street, London, W C 1), at 1 15 p m—Dr P J Newbould "Nature Reserves in Britain Can We Afford Them?"*

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W C 2), at 6 p m—Discussion on "The Place of Digital Computers in the Teaching of Electrical Engineers" opened by Dr P D Aylett

Wednesday, October 18

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S W 7), at 5 p m—Scientific Papers

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W C 2), at 5 30 p m—Dr J S Forrest "Research and Transmission" (Chairman's Address)

BRITISH INSTITUTION OF RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m—Mr J W Nichols and Mr A C MacKellar "Some Recent Developments in Marine Navigational Aids"

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S W 1), at 6 p m—Meeting on "Volatile Corrosion Inhibitors" Mr E Ll Evans "Principles", Mr R I Barraclough "Practical Applications"

INSTITUTE OF INFORMATION SCIENTISTS (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W C 1), at 6 15 p m—Sir Lindor Brown, F R S Presidential Address

INSTITUTE OF WOOD SCIENCE (at Carpenters Hall, Throgmorton Avenue London, E C 2), at 7 p m—Mr P O Reece "The Establishment of Safety Factors and Stresses for Timber".

OIL AND COLOUR CHEMISTS' ASSOCIATION (at Manson House, 26 Portland Place, London, W 1), at 7 p m—Dr L M Baraken "Solvents in the Surface Coatings Industry"

SOCIETY FOR ANALYTICAL CHEMISTRY BIOLOGICAL METHODS GROUP (at the Chemical Society Burlington House, Piccadilly, London, W 1), at 7 p m—Miss Maureen Wilson "The Use of Bacteriophages in Epidemiology"

Thursday, October 19

UNIVERSITY COLLEGE, LONDON (in the Anatomy Theatre, Gower Street, London, W C 1), at 1 15 p m—Dr M Mary Douglas "Cult and Ordeal as Techniques of Sorcery Control in the Belgian Congo" *

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W 1), at 5 p m—Discussion of a paper by Mr J T M Taylor on "Mining Practice on the Kolar Gold Field, India"

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W 1), at 5 p m—Scientific papers by Dr Anna B Hastings and Dr H G Vevers

UNIVERSITY OF LONDON (at Westfield College, Kidderpore Avenue, London, N W 3), at 5 15 p m—Mrs Grace Wyndham Goldie "Television in Society" *

LONDON PLANETARIUM (in collaboration with the ROYAL SOCIETY and the ROYAL ASTRONOMICAL SOCIETY, at the London Planetarium, Marylebone Road, London, N W 1), at 5 30 p m—Dr Joseph Needham, F R S "Astronomy in Classical China"

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m—Prof A S Parkes, F R S "External Factors in Mammalian Reproduction" * (Second of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

SOCIETY OF INSTRUMENT TECHNOLOGY (at the Royal Institution Albemarle Street, London, W 1), at 6 p m—Sir George Thomson, F R S "The Inspiration of Science" (First Thomson Lecture)

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the SOUTH-EAST ESSEX TECHNICAL COLLEGE SCIENTIFIC SOCIETY, at the South-East Essex Technical College, Longbridge Road, Dagenham, Essex), at 7 p m—Dr S I Levy "Chemical Patents"

CHEMICAL SOCIETY (at Burlington House, Piccadilly, London, W 1), at 7 30 p m—Scientific Papers

BRITISH INSTITUTE OF RADIOLOGY (in the Reid-Knox Hall, 32 Welbeck Street, London, W 1), at 8 p m—Ordinary Meeting followed by Mr F G Spear "Questioning the Answers" (Presidential Address).

UNIVERSITY OF LONDON (in Swanley Hall, Withersdane, Wye College, near Ashford Kent), at 8 15 p m—Prof J D Bernal, F R S "Science in History".

Thursday, October 19—Friday, October 20

INSTITUTION OF CHEMICAL ENGINEERS, GRADUATES AND STUDENTS SECTION (at the Royal Overseas League, St James's, London, S W 1)—Symposium on "Scale-up and Pilot Plants"

Friday, October 20

BRITISH PSYCHOLOGICAL SOCIETY, OCCUPATIONAL PSYCHOLOGY SECTION (in the Department of Psychology, Birkbeck College, Malet Street, London, W C 1), at 1 p m—Dr R A M Gregson "Operational Research, Psychological Information and Managerial Decisions"

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W 1), at 5 30 p m—Mr T A Brock "Petroleum Products in Cosmetics".

Saturday, October 21

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 3 30 p m—Mr E Milne-Redhead "A Plant-Collecting Expedition to Southern Tanganyika" *

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned

SCIENTIFIC OFFICER (male, with a good honours degree), for genetical, physiological and breeding research with cereals—The Secretary, Plant Breeding Institute, Trumpington, Cambridge (October 21)

MAP CURATOR/LIBRARIAN (preferably with qualifications in geography and librarianship) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Sheffield (October 23)

RESEARCH PHYSICIST, Senior Scientific Officer (with an honours degree together with several years appropriate experience) at the Defence Standards Laboratories, Finsbury, near Adelaide, South Australia, for basic studies relating to the effects of light, X- and gamma-radiation on photoconductive materials and the physics of electrophotography—The Senior Representative (AV 125/6), Department of Supply, Australia House, Strand, London, W C 2 (October 26)

ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, The University, Nottingham (October 28)

LECTURER or DEMONSTRATOR (Assistant Lecturer) IN PHYSIOLOGY—The Registrar, The University, Leeds 2 (October 31)

LECTURER or ASSISTANT LECTURER IN BIOCHEMISTRY—The Registrar, The University, Sheffield (November 4)

JUNIOR RESEARCH FELLOW (with a good first degree in zoology) IN LOCUST PHYSIOLOGY IN THE DEPARTMENT OF ZOOLOGY, University College of Rhodesia and Nyasaland, for work on the water relations of locusts—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (November 8)

READER IN CHEMICAL PATHOLOGY at the Postgraduate Medical School of London—The Academic Registrar, University of London, Senate House, London, W C 1 (November 8)

LECTURER IN PHILOSOPHY at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, November 15)

LECTURER (with special interest in plant physiology or morphology or taxonomy) IN THE DEPARTMENT OF BOTANY, University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W C 1 (November 15)

LECTURER/SENIOR LECTURER IN ORGANIC CHEMISTRY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall London, S W 1 (Australia and London, November 17)

LECTURER (with a good honours degree and experience in research or teaching, and preferably qualifications in the history and philosophy of science) IN THE SCHOOL OF PHILOSOPHY, University of New South Wales—The Agent-General for New South Wales, 56 Strand, London, W C 2, and the Appointments Section University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (November 17)

SENIOR LECTURER (with a higher degree or equivalent qualifications) and a LECTURER (with an honours degree) IN THE SCHOOL OF NUCLEAR ENGINEERING, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W C 2, and the Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia November 17)

Lecturer and an Assistant Lecturer in Inorganic Chemistry—The Registrar, The University, Nottingham (November 22)

Lecturer (with an honours degree in chemistry and preferably a higher degree, and experience and a special interest in modern physical methods) in the Department of Chemistry, Newcastle University College—The Agent-General for New South Wales, 56–57 Strand, London, W C 2, and the Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (November 24)

Chair of Pharmacology in the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (November 30)

Keeper (with an appropriate university degree, and preferably museum experience and/or a knowledge of herbarium work) of the Department of Botany—The Secretary, National Museum of Wales, Cardiff (November 30)

Lecturers (2) in Physiology, and a Lecturer in Pharmacology at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (November 30)

Senior Lecturer in Meteorology, and a Lecturer in Glaciology at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, November 30)

Assistant Experimental Officer/Experimental Officer (with a degree or H N C in chemistry or equivalent), for work mainly concerned with the analysis of foods and feeding-stuffs of tropical origin—The Director, Tropical Products Institute (D S I R), 56 Gray's Inn Road, London, W C 1, quoting Ref YE 709/9

Assistant Lecturer, Grade B (with a good honours degree in mathematics and preferably some teaching or research experience), in Mathematics—The Clerk to the Governors, South-East Essex Technical College, Longbridge Road, Dagenham, Essex

Entomologist (with a good honours degree in biology, with entomology as a special subject, and not less than two years postgraduate research experience in entomology) at the West African Institute for Trypanosomiasis Research, to carry out laboratory and field investigations on tsetse flies, at Kaduna, Northern Nigeria—The Crown Agents 4 Millbank, London, S W 1, quoting Ref M3B/53514/NF

Junior Technical Officer (with G C E at "A" level in two science subjects, one of which should be botany or biology), for work in a section investigating the cytogenetic effects of radiation—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berks

Lecturer (with a good honours degree in physics and preferably a Ph D degree, and preferably experimental research experience in some aspects of plasma physics and an interest in high-current discharge work) in the Department of Natural Philosophy—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C I

Librarian (with a knowledge of chemistry or physics) in the Department of Chemical Engineering and Chemical Technology—Prof A R Ubbelohde, F R S , Imperial College of Science and Technology, London, S W 7

Postdoctoral Fellow, for research in flame spectroscopy or a related subject—Dr A G Gaydon, F R S , Chemical Engineering Department, Imperial College, London, S W 7

Postdoctoral Fellow, for research in modern concepts between structure and biological activity or organic molecules, organic synthesis and stereochemistry—Prof B Belleau, Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada

Research Assistant (Graduate), to undertake cell counts as part of an investigation on the growth of cereal seedlings—The Secretary, Agricultural Research Council Unit of Plant Physiology, Imperial College of Science and Technology, London, S W 7

Scientific Officer (male or female, with an honours degree in science, pharmacy or biology) in the Blood Transfusion Service, for duties which will consist of technical work in the Blood Transfusion Service Laboratories—The Director, Blood Transfusion Service, Roby Street, Manchester 1

Senior Laboratory Technician, Grade 1 (with appropriate qualifications and experience)—The Head Master, King Edward VI School, Southampton

Statisticians (2) (with good qualifications in mathematics and statistics) to engage in the development of biometric techniques for medical research and the application of electronic computers to medical problems—The Secretary, Marischal College, The University, Aberdeen

Technician at the Marine Biological Station, Port Erin—The Registrar, The University, Liverpool 3, quoting Ref CV/N/M B S.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Institute of Personnel Management Job Analysis By H E Roff and T E Watson Pp 40 7*s* 6*d* Supervisory Training By J R Armstrong Pp 51 7*s* 6*d* (London Institute of Personnel Management, 1961) [148

Department of Scientific and Industrial Research National Physical Laboratory Notes on Applied Science No 22 Capacitors, Inductors and Resistors at Radio Frequencies By Dr T I Jones Pp iv+24 (London H M Stationery Office, 1961) 1*s* 9*d* net [148

Britain's Cultural Crisis By M C Goodall Pp 4 (Hove, Sussex M C Goodall, 16 King's Gardens, 1961) [158

C I B A (A R L), Ltd Technical Notes, No 224, (August, 1961) Boat Building by Yarecraft—Colf-moulded and Hard-Chine Hulls Bonded with "Aerolite" 300 Pp 8 (Duxford C I B A (A R L), Ltd , 1961) [158

Royal Observatory Bulletins No 27 Three-colour Photometry in the Southern Hemisphere—NGC 6383, NGC 6405 and Standard Stars By O J Eggen Pp C61–70 (London H M Stationery Office, 1961) 3*s* net [158

Building Research Station Digest, No 13, (Second Series) Concrete Mix Proportioning and Control—1 Pp 4 (London H M Stationery Office, 1961) 4*d* [158

Control of Public Expenditure Pp 33 (Cmnd 1432) (London H M Stationery Office, 1961) 1*s* 9*d* net [258

Other Countries

Mental Health Book Review Index, Vol 6, Whole No 11, July 1961 Compiled by the Editorial Committee and Contributing Librarians (Sponsored by the World Federation for Mental Health, The International Council of Psychologists, The American Foundation for Mental Hygiene, and The Research Center for Mental Health, New York University) Pp xvii+62 (Flushing, N Y Miss Lois Afflerbach, Paul Klapper Library, Queens University, 1961) [158

Institute for Scientific Information, Philadelphia Descriptive Brochure Pp 18 (Philadelphia, Penn Institute for Scientific Information, 1961) (158

Chicago Natural History Museum Fieldiana Anthropology Vol 52 Mineral Creek Site and Hooper Ranch Pueblo, Eastern Arizona By Paul S Martin, John B Rinaldo and William A Longacre Pp 181 (Chicago Chicago Natural History Museum, 1961) 5 50 dollars [158

New South Wales Department of Agriculture Contributions from the New South Wales National Herbarium Flora Series, No 101, Part 1 Papilionaceae By Joy Thompson Pp ii+91 (Sydney Government Printer, 1961) [158

United States Department of Commerce Coast and Geodetic Survey Publication 65–1, Part 22 Plane Coordinate Intersection Tables (2½-Minute), Mississippi Pp iii+92 50 cents Publication 65–1, Part 40 Place Coordinate Intersection Tables (2½-Minute), Tennessee Pp iii+58 40 cents (Washington, D C Government Printing Office, 1961) [158

Arctic Institute of North America Technical Paper No 6 The Medusae of the Chukchi and Beaufort Seas of the Arctic Ocean including the description of a New Species of *Eucodonium* (Hydrozoa Anthomedusae) By Cadet Hand and Lai Bing Kan Pp 23 (Montreal Arctic Institute of North America, 1961) 1 dollar [158

Canada Publications of the Dominion Astrophysical Observatory, Victoria, B C Vol 11, No 16 Curve of Growth of C$_2$ Absorption Bands Applied to the Problem of the C^{12}/C^{13} Abundance Ratio By John L Climenha a Pp 307–338 25 cents Vol 117, No 17 The Light-Ratio and Secondary Spectrum of the Eclipsing Binary Zeta Aurigae By E K Lee and K O Wright Pp 339–353 Vol 11, No 10 Some Spectroscopic Observations of the Supergiants 67 Ophiuchi, 55 Cygni and χ^2 Orionis By Anne B Underhill Pp 353–362 (Ottawa Queen's Printer, 1960 and 1961) [158

University of California Publications in Geological Sciences Vol 40, No 1 California Pleistocene Paleotemperatures By James W Valentine and Robert F Meade Pp 1–46 (Berkeley and Los Angeles University of California Press, London Cambridge University Press, 1961.) 1 dollar [158

Metropolitan Life Insurance Company Statistical Bullletin, Vol 42, (June, 1961) Voting Population at Record High Recent Variations in Pneumonia-Influenza Mortality Longevity of Industrial Policyholders in 1960 Accident Death Rates Decrease in Most States Pp 12 (New York Metropolitan Life Insurance Company, 1961) [158

Institutt for Atomenergi Kjeller Research Establishment, Norway Quarterly Progress Report, January–March, 1961 Pp iv+41+35 figures (Kjeller, Lillestrøm Institutt for Atomenergi, Kjeller Research Establishment, 1961) [158

National Research Council of Canada Radio and Electrical Engineering Division NRC No 6404 Energy Transfer in Polyacene Solid Solutions, II—a Bibliography for 1957–1960 By F R Lipsett Pp i+65 (Ottawa National Research Council, 1961) 50 cents [158

Forschungsberichte des Landes Nordrhein-Westfalen Nr 974 Nehmen Luftelektrische Faktoren Einfluss auf die Aktivitatswechsel Kleiner Insekten, insbesondere auf die Hautungs und Reproductionszahlen von Blattläusen Von Dr Else Haine. Pp 80 (Koln und Opladen Westdeutscher Verlag, 1961) 24 30 D M [158

United States Department of the Interior Geological Survey Bulletin 1032–F Petrography and Origin of Xenotime and Monazite Concentrations, Central City District, Colorado By E J Young and P K Sims Pp iii+273–299 20 cents Bulletin 1070–C Distribution of Uranium in Rocks and Minerals of Mesozoic Batholiths in Western United States By E S Larsen, Jr , and David Gottfried Pp v+63–103 Bulletin 1084–J L A Constant-Feed Direct-Current Arc By C S Annell and A W Helz Pp iv+231–251 15 cents Bulletin 1084–K Beryllium Content of American Coals By T Stadnichenko, P Zubovic and Nola B Sheffey Pp iv+295+2 plates Bulletin 1102 Stratigraphy and Refractory Clayrocks of the Dakota Group Along the Northern Front Range, Colorado By Karl M Waagé Pp v+154+8 plates Bulletin 1123–A Geology of the Dubuqye South Quadrangle, Iowa-Illinois By C Ervin Brown and Jesse W Whitlow Pp v+93+7 plates (Washington, D C Government Printing Office, 1960 and 1961) [258

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THE SCIENTIST AND THE COMMUNITY

THE address, "Power and Responsibility in Science", which Prof. M. Swann broadcast in the Third Programme on the eve of the British Association for the Advancement of Science meeting at Norwich (August 29) in no way anticipated Sir Wilfrid Le Gros Clark's presidential address, though in his concluding passages Sir Wilfrid summoned scientists to play a special part in promoting opportunities for free intercourse and the free interchange of ideas among the peoples of the world. Prof. Swann is no less convinced that scientists have something to offer the world besides their discoveries, but he did not touch on this particular question of co-operation, except to note that, in simple terms of its influence on mankind, H. B. du Pont, when addressing the Franklin Institute, once described the development of the modern industrial corporation as the greatest invention of all, exceeding even the influence of the steel plough, steam locomotive, the telegraph and telephone, and the flour mill of Oliver Evans, which was the precursor of automatic control.

Prof. Swann began indeed by referring to certain discoveries of science which had reduced drudgery, transformed communication and transport and enhanced or improved the standard of living, or relieved pain and suffering, doubled our life-span and vastly improved food production. These discoveries, apart from the impact of science on ways of thought, could undoubtedly be placed on the credit side even if there were other things such as the atom bomb or the misuse of scientific ideas to bolster up racial, economic or political theories to be placed on the other side.

Prof. Swann, however, was not attempting to strike a balance but to indicate the vast power which science has come to wield over the human race. This power, he argued, is exercised without responsibility and this is one reason for the anti-scientific feelings sometimes encountered. Moreover, because every scientific discovery depends on what has gone before, such power as science exercises is not applied by individuals but collectively by scientists as a whole and all must in some sense accept responsibility. While at the individual level the concept that power and responsibility must be linked has long been established, its acceptance at the collective level of government and society is a much more recent achievement, and Prof. Swann taxes science with having evaded this issue of the responsible exercise of power which is at the heart of modern democracy.

It was not suggested by Prof. Swann that this evasion is deliberate. It arises out of the very nature of science as, first and foremost, a search for knowledge, and it was much later—Prof. Swann puts it within the past century—that knowledge came to mean power, and still more recently that the power and the attendant responsibility of science became glaringly obvious. To-day, he remarks, even a research worker in the most esoteric corner of science can no longer feel sure that he is not laying foundations for some new advance with all sorts of practical implications. The scientist who fails to develop a social conscience can no longer be excused.

Prof. Swann does not, however, analyse this question of responsibility at all deeply or even the position of the scientist in the community in which he works. He considers the question whether the scientist has anything beside his discoveries to offer the world in which practical decisions have to be made and discoveries translated into action, for good or for ill. He recognizes, of course, that under present conditions society contrives fairly effectively to prevent scientists as such from doing much to improve the situation. Prof. Swann makes the point that there is just as much need, and possibly more immediate prospect of success, for scientists to enter public life vigorously at lower levels than those of the higher reaches of government—in the councils, committees, boards of governors, through which the life of the local community is ordered. Moreover, he suggests that foresight is only a part of the scientist's whole attitude to tradition and progress and that here the scientist has something further to offer to the national way of thought.

Considering the way change occurs in public life, Prof. Swann points out that to the scientist the past is worthy of respect and represents the best that able minds could achieve. The scientist, above all men, depends on the work of those who have gone before him, and because we have the advantage of other men's labours we should be able to go further and do better. Prof. Swann looks on the scientist as a conservative and evolutionary influence in public life rather than a revolutionary influence, seeking to add to the structure, remodel it here and there, to change but not to destroy.

Closely linked with this is the stress Swann lays on the scientist's insistence on the experimental approach. This is built so firmly into science that the scientist scarcely realizes how much less instinctive it is in the outside world, and Prof. Swann frankly advocates an extension of the experimental approach, for example, in such matters as nationalization. Here, of course, he enters on the wide issues of influence, as opposed to power, and of the place of the scientific outlook and scientific method in public affairs, and these are vital aspects of the problem of the right relation of knowledge and power. Moreover, it is necessary to distinguish between the scientist as a scientist and the scientist as a citizen, for the responsibilities are not identical, and failure to distinguish between them has led to mistaken action as well as to the opposition to science which Prof Swann notes

The scientist as such has influence rather than power It is his responsibility to exercise his foresight so far as in him lies, and to point out the consequences that must follow the adoption of particular scientific or technical measures Only to that extent does he bear political responsibility as a scientist Whether, however, he is able to exercise that advisory function at a high enough level or sufficiently effectively or early is a matter for society as a whole, and it is here, as citizen, that the scientist must recognize a responsibility to work to ensure that the structure of government allows scientific and technical advice to be tendered appropriately and effectively This civic responsibility requires also that he should seek to ensure that educational policy is such that it provides for the task of administration those competent to assess scientific and technical advice and give it due weight along with other factors in a situation

It is only when scientists as citizens prove effective in the discharge of such responsibilities that one can speak of the scientists as exercising power Even if, and when, a scientist is appointed to a position of high responsibility in the executive government, it is not as a scientist but as a citizen that he holds and exercises power It is important that this should be clearly understood, for confusion over the relations between the layman and the scientist to-day is responsible for much of the opposition to the wider use of the scientific method and to the establishment of conditions in which scientific work is most effectively done It is scarcely conceivable that there will be any reversal, either in the advanced or the backward races, at the national or at the international level, of the process of applying the fruits of scientific knowledge But the real reason why this confusion over the relations between the layman and the scientist and their respective responsibilities for the use or misuse of science is dangerous was brought out rather better by Sir Solly Zuckerman in the Stephen Paget Memorial Lecture in November 1959, and in his address at the California Institute of Technology in June of that year (see *Nature*, 184, 135, 1959)

Sir Solly pointed out that there is a sense in which science is the handmaiden of the economic or social institutions in which it functions—a framework which is indeed to some extent continually being transformed by new scientific ideas and the application or practical development of those ideas, but which nevertheless to some extent determines what ideas shall be developed A material civilization of motor-cars, or jet planes, of radio and television, of man-made fibres and nuclear power is not necessarily the only form that civilization might have taken Now that it has taken that shape, Sir Solly remarked it helps to define for us the content and the boundaries of the area within which we exercise our freedom Pursuit of some major development, for example, the development of the steam engine, means that some other path that potentially might have been followed is not followed

This is as yet only imperfectly grasped in Britai to-day, though it is being strongly emphasized b such topics as space research There are, moreover two further points to be made in this context First this choice of development is not one that is made b the scientist as such It is a political choice, only t a limited extent made on scientific or technica advice, and Sir Solly Zuckerman reminded us that i may not even be conscious, and that only partly because of the unpredictability of any new majo scientific advance or the increasing complexity o scientific knowledge in the applied field It is also partly because in the application of scientific knowledge means tend to become ends, the discovery o new ways of doing things transforming the things done and so their purpose

Here Sir Solly's vision is clear, but its implications have not as yet been widely discussed or the requisite steps taken to provide some means of guarding against the dangers to which he directed attention Rejecting as too simple the idea that to-day democracy has abrogated its rights to a bureaucracy which is determining the direction we are taking, Sir Solly maintained that, since the element of the unknown in government increases with every step we are now taking to apply the fruits of science, if sovereign power is being abrogated it is less to a governing body, however formed, and more and more to a process of applying scientific knowledge without any real possibility of determining its final consequences Neither the voice of the majority nor those through whom it is expressed can proclaim the precise lines of the future

This is a vital point which Prof Swann overlooked, it is dangerous to speak of the power and responsibility of the scientist if it is not recognized that while the scientist may be the better able to appreciate the scientific aspects of a situation, he is not responsible as such for the application of the new knowledge and cannot be held responsible for predicting some vast social transformation which might result from a seemingly trivial observation Nor is it sufficient to urge the need to regard education in science as constituting an essential element in a liberal education We need above all to appreciate, as Sir Solly put it, that in the major scientific matters which now affect human destiny, we cannot safely take decisions for to-day unless we realize that those same decisions determine the future It is at this point that the scientist's foresight ought to be exerting its decisive influence, and the survival of mankind may well turn on the urgency and vision and determination which are brought to bear on the creation of the institutions which will enable the scientist to make that contribution effectively Nor is that solely a challenge to creative thinking or inventiveness It is equally a challenge to the scientist and technologist to respond to Prof Swann's appeal and use to the full, imaginatively and constructively, such opportunities of public service that may already confront them as citizens Even when new opportunities are provided, scientists and technologists will only be able to use them adequately as they gain experience of public work Perhaps one of the most constructive steps that our major scientific professional institutions could now be taking would be to stimulate the interest of their younger members in public service and to assist them to gain the experience and the outlook which would enable them to make their particular and unique contribution most effectively

HUMAN PARASITES

Animal Parasites in Man

By Dr N H Swellengrebel and M M Sterman Pp x+652 (Princeton, N J D Van Nostrand Company, Inc , London D Van Nostrand Company, Ltd , 1961) 67*s* 6*d*

It is difficult, as one reads through this book, to decide what type of reader its authors had in mind First issued sixty years ago, it has now been re-written and enlarged, and its authors hope it will be useful to the physician and the biologist For the physician seeking an introduction to this subject and information about the epidemiology and treatment of human parasitic infections it may well seem adequate, but the biologist or medical man who requires guidance and information on difficult points of detail or procedure may prefer one of the several text-books of medical parasitology which are available at not much greater cost The biologist, moreover, may wish for more systematics than this book attempts to give and more on the features of the main groups of parasites He may wonder, too, at some statements, such as the suggestion that all trematodes are flat "like a flatfish or the leaf of a tree", and why the authors have introduced into an otherwise excellent introduction such terms as "proliferous" and "non-proliferous" parasites, "initial aggressors" and the like , and at the division of the common parasites of man into the Protozoa, Metazoa and the "Scolecida, or lower worms", one wonders, too, whether Sabin would nowadays agree with the statement attributed to him in 1953 that toxoplasmosis is an "almost perfect host–parasite relationship in which the parasite only rarely causes serious damage to its hosts, and the hosts are not too intent on destroying the parasite"

Apart, however, from points like these, the text is, as one would expect from a book bearing the highly respected name of Prof N H Swellengrebel, accurate and as up to date as any book can, in these days of very rapid advances in knowledge, hope to be Among the best sections are the new chapters on methods of examination of the excreta and blood for eggs and larvæ of parasites and on the methods for the enumeration of the eggs of parasites and on serological methods of diagnosis The bibliographies at the end of each chapter will also be useful to readers who wish to extend their knowledge The book is written in an easy style and shows the host and parasite in conflict with each other, a point of view which leads the authors to use such phrases as "the host's defense mechanism was kept constantly on the alert" (p 127), or the host applies "topical" or "numerical" restraint on the parasite, phrases which are useful only if great care is taken not to mislead the reader An odd feature is the disproportionate space given to the rare occurrence of *Babesia bovis*, a parasite of cattle, in splenectomized human subjects It is true that babesiasis may be a risk to which man, and especially splenectomized man, may rarely be exposed, and the space given to this disease would be justified in a large and comprehensive text-book , but it should be accompanied by a fuller consideration of the Babesiidae than is given here It is difficult, too, to understand the statement (p 127) that "calves are the only hosts to suffer" from *Babesia bigemina*, because they suffer less severely than adult cattle and it is explained on the same page that they acquire pre-immunity It is implied, also, that *Boophilus annulatus* is the only invertebrate host of *Babesia bigemina*, whereas at least 10 ticks belonging to 3 genera have been recorded as its invertebrate hosts The tick, moreover, is here described as the intermediate host, whereas it harbours the sexual phases of the *Babesia* and is therefore the definitive host

Most of the numerous illustrations are taken from other text-books, and it is unfortunate that many have suffered much in the process of reproduction Outstanding examples are the figures of *Echinococcus granulosus* (p 267), and the black-and-white figures of the exo-erythrocytic stages of *Plasmodium inui* and *P falciparum*, which give no idea of the clarity and draughtsmanship of the originals These examples are the more regrettable because other figures are well reproduced

The format of the book and its general make-up and print are attractive, but its price, compared with that of the bigger and more detailed text-books available, is high G Lapage

CANCER OF THE RECTUM

Cancer of the Rectum

Edited by Cuthbert E Dukes (Neoplastic Disease at Various Sites, Vol 3) Pp xiii+304 (Edinburgh and London F and S Livingstone, Ltd , 1960) 50*s* net

This is the third volume of the series *Neoplastic Disease at Various Sites* under the general editorship of Prof D W Smithers In one way, at least, the volume is unique eleven of the eighteen contributors are connected with St Mark's Hospital, London—seven as consultant surgeons, two as consultant pathologists, and two as research assistants Not only are these contributors familiar with each others' views and methods, but also for many years the work of the surgeons has been co-ordinated through a single pathology department and follow-up system In this connexion the immense and universally acknowledged contribution of Dr Dukes himself is further made evident by the fact that his accurate observations, his plan for the examination of operation specimens and dissection of lymph nodes and blood vessels, his pathological classification, and his histological grading of rectal cancer, form the basis on which the contributing surgeons analyse their operative results, and are unquestionably the framework on which many of the recent advances in surgery of rectal cancer have been made

Theoretically, at least, the fact that so many of the contributors have been working in close association with each other, carries with it the danger that the views expressed here are narrowed through inbreeding. Only in Chapter 13, written by Prof J C Goligher of Leeds, is mention made of methods practised in other countries Some may be surprised to read that combined abdomino-perineal excision by two surgeons operating simultaneously is little practised in America, or in Germany and other Continental countries The views of an American or Continental surgeon, particularly with regard to the choice of operation, might well have made an interesting additional chapter As it is, the reader is left with the knowledge that the abdomino-perineal excision is the operation most commonly practised in Britain, but with the feeling that anterior resection with sphincter preservation might be the better operation for at least a proportion of cases On the other hand, one hastens to say that the descriptions of the different surgical techniques are both remarkably clear and admirably brief Each is written by a surgeon who has immense experience of the operation in question

Some of the tremendous advances in surgical technique, especially in the resectability-rate, during the past thirty years, are attributable to improved anæsthesia antibiotics, anticoagulants, a better understanding of surgical shock and its treatment, and to the consequent possibility of undertaking more radical operations Some have arisen as the direct result of pathological observations, for example, the observation that cancer of the rectum rarely spreads downwards and therefore that sphincter preservation is feasible Finally, some are undoubtedly the direct result of the concentration of surgical and nursing experience at St Mark's Hospital itself In this latter connexion this book is a poignant answer to those who still oppose the establishment of ultra-specializing treatment centres in Britain

The section (pp 36–39) by Dr Dukes on the control of rectal cancer in polyposis families provides an unparalleled example of cancer control. By keeping detailed records of polyposis families and by going to great lengths to keep in close touch with them, it has in many cases been possible to undertake colectomy before metastasizing cancer has developed At the same time an understanding of the inheritance of the disease has enabled the surgeon to relieve the minds of unaffected siblings of the fear that they too might eventually develop the disease

Surprisingly, no special space is set aside for consideration of the pre-operative general examination or preparation of patients On p 251, in the chapter on operative complications (Chapter 22), Mr Gordon Ungley writes " . a pre-operative intravenous pyelogram is of immense advantage, should serious urinary complications arise" The desirability of conducting this and other investigations pre-operatively in certain patients should surely have been discussed somewhere earlier in the volume

As regards alternatives to surgery, cancer chemotherapy is not mentioned, but radiotherapy is well dealt with by Dr Williams in Chapter 19 Here no strong claim is made for the value of radiotherapy other than as a palliative measure On the other hand, it is pointed out that only recently have X-ray voltages in excess of 1 MeV been available, these give less severe skin and mucosal reactions than lower voltage X-rays, and better results may be expected from radiotherapy in the future

The authors of the final section entitled "Possibilities for Future" (p 277) conclude that further improvement in prognosis must depend mainly on earlier diagnosis and earlier surgical treatment, but they do not suggest how these may be achieved It seems unlikely that exfoliative cytology, which is mentioned briefly on p 132, will be found to have much value as a quick screening test

One problem which urgently needs an answer i "to what extent, if any, does surgical biopsy, and the pre-operative or operative manipulation of tumour increase the risk of distant metastasis via the blood stream?" Now that appropriate and reliable methods for the detection of circulating cancer cells are becoming available, it is to be hoped that this question will soon be answered The technique called 'Chemosurgery' developed by Mohs[1] is being increasingly practised in the United States and some European countries It is likely that it would prove of value in the diagnosis and treatment of possibly malignant polyps and of lesions of the lower rectum near the anal margin

At the end of an excellent review chapter (Chapter 5) on "Tumours of the large intestine of mammals other than man", Dr Roberts discusses the experimental induction of tumours of the large gut by chemical substances The possibility that constituents of the diet, or that anal ointments or suppositories, play an ætiological role cannot, without further research, be excluded The fact that little space is given to consideration of the ætiology of human rectal cancer is a measure of the paucity of research in this field.

None of the criticisms made here is more than minor, and they detract very little from the value of the contents of the book The contributors and editors are to be congratulated on the clarity of style and presentation, on the high quality of most of the tables and illustrations, and on the remarkable degree of continuity Moreover, the choice of paper and size of type are admirable The book will be of value to a wide variety of qualified doctors whether primarily concerned with surgery or not In addition, the figures for operative mortality and 5-yr survival will provide a useful yardstick by which surgeons can measure their own prowess when using the same techniques and by which the value of new techniques can quickly be assessed **Francis J C Roe**

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[1] Mohs, F E, *Chemosurgery in Cancer, Gangrene and Infections* (C C Thomas, Springfield, Illinois, 1956)

DEVELOPMENTS IN VETERINARY SCIENCE

Advances in Veterinary Science

Vol. 6 Edited by C A Brandly and E L Jungherr Pp xi + 382 (New York Academic Press, Inc, London Academic Press, Inc (London), Ltd, 1960) 12 dollars

THIS volume, which follows the pattern of previous ones, covers seven main subjects In the first chapter, screw-worm research and eradication are dealt with Details are given of the biology of the screw fly, its geographical distribution and the means of rearing it under laboratory conditions Chief interest in this section will probably be found in the discussion on the means of screw-worm eradication by releasing sterilized males in an infested area The genetic basis for sterilization by irradiation is discussed in a concise but thorough manner Insecticides in the form of wound dressings, sprays and dusts, including organo-phosphorus compounds, are discussed in the concluding chapters of this section

In the second chapter foot-and-mouth disease and related vesicular diseases are dealt with in considerable detail Although a number of the facts set out here are already well known, they are very wisely reiterated in order to form a correct basis for the subsequent discussion The distribution of foot-and-mouth virus types and sub-types, the ætiology, prevention, control and eradication as well as diagnosis are covered in some detail. At the present time, when the control of this disease throughout the world is receiving active attention, the information on types of vaccine and their preparation and application is of more than academic interest Vesicular stomatitis and vesicular exanthema, virus diseases which show so many clinical signs similar to those of foot-and-mouth disease, are discussed from the same angle While in Great Britain the absence of these two diseases makes the diagnosis of foot-and-mouth disease less complicated, the position in the United States is not so simple, and differentiation between the three viral diseases covered in the chapter is a practical necessity in those States in which all three could occur simultaneously

To those primarily interested in animal husbandry, the chapter dealing with the effects of nutrition on microbial disease will have a special appeal Any confusion of thought on what really constitutes disease, infection, nutrition and ecology is dispelled by the critical and clear definition of these terms with which the chapter begins The authors summarize the effects of the various food constituents on the prevention of patent disease in the animal

In summarizing their conclusions, they liken the balance between the host and micro-organism to a game of chess in which the result more commonly depends on a series of moves and counter-moves than on a single finalizing move, and point out that the micro-organism may in many cases overcome its host by the narrowest of margins

The fourth chapter deals with anaplasmosis, covering historic development, distribution and incidence, and the evolution of epizootics in the United States The epidemiology of the two main types of anaplasma and of organisms of similar type but doubtful nature, such as eperythrozoon, are given due attention Transmission of the parasite by ticks, flies and mosquitoes and transmission *in utero* and accidentally by direct contact with infected animals are discussed Investigations of the parasite, including the use of electron microscopy, problems of its reproduction, its cultivation, its properties and its pathogenesis (aided by the use of radioisotopes), occupy a considerable part of this chapter and will be found to be of fundamental importance to those interested in this protozoan blood parasite Diagnosis by clinical and laboratory means, the pathology of the disease and its prophylaxis and therapy, with a note on the future for its control, complete a full and valuable description of this widespread disease of the blood

The fifth chapter covers a great deal of ground without being unduly long It deals with the comparative pathology of arthritis and embraces the many forms of arthritis in all classes of livestock, including birds In the concluding remarks to this chapter, it is pointed out that the chemical composition of articular cartilage has not yet been studied on a comparative basis and that little is known of the character of normal synovial fluid in the various species This observation must surely suggest lines for future research

The following chapter deals with coccidioidomycosis This fungal disease, which has been found to affect both animals and man, is described from the point of view of its history, its incidence, its classification (including its saprophytic and parasitic forms) and the conversion from one form to the other With inhalation as the usual mode of infection of men and animals, the epidemiology of the disease makes an interesting study, and it is noted that certain races of humans and certain breeds of animals are more susceptible than others Pathogenesis and pathology are discussed, and diagnosis, prognosis and treatment are dealt with. The public health significance of the disease renders an investigation of it all the more important

The final chapter deals with canine distemper, a disease of which so much has been written. Here we find the more important considerations condensed into a valuable and all-embracing treatise The question is posed "Does distemper produce a long-lasting immunity because there is one serological type or, conversely, is there one serotype because the virus evokes a high-grade immunity?"

As in the case of previous volumes, this sixth volume contains a comprehensive list of references on each of the subjects discussed as well as author and subject indexes of considerable value to the reader The high standard set by previous volumes is fully maintained and another addition of outstanding value to the teacher and research worker will find its way on to their bookshelves, not to lie there but to be consulted freely and profitably.

HAROLD BURROW

THE FATTY ACIDS

Fatty Acids

Their Chemistry, Properties, Production, and Uses, Part 1 Edited by K S Markley. Second completely revised and augmented edition. (Fats and Oils a Series of Monographs on the Chemistry and Technology of Fats, Oils, and Related Subjects) Pp ix+714 (New York Interscience Publishers, Inc , London Interscience Publishers, Ltd , 1960) 169*s*

THE first edition of this work was published in 1947 as a single 668-page volume, written entirely by K S Markley The material covered has now been expanded to such an extent that this second edition occupies four parts, each with its own index Part 1 is concerned with the content of the first third of the 1947 edition and has chapters by Markley on the individual acids and isomerism (250 and 36 pages, respectively), by R T O'Connor on X-ray diffraction and polymorphism (94 pages) and spectral properties (120 pages), and by W S. Singleton on the properties of the liquid state and of solutions (111 and 70 pages, respectively)

Markley's section on the acids has more than 600 references and covers most types of aliphatic carboxylic acid—natural and synthetic, short- and long-chain, saturated and unsaturated, and oxygenated The acids are dealt with individually, the main points of discussion being occurrence, structure, preparation, applications and properties The more important acids, industrially as well as academically—such as oleic acid and the naturally occurring acetylenic acids—are treated at some length, though the approach tends to be historical and somewhat classical rather than critical The tables of physical constants and other properties are numerous and well presented Unfortunately, the literature coverage does not appear exhaustive, though it is certainly extensive

Those vital adjuncts of modern organic chemistry, the physical methods, are expertly and concisely introduced and the relevance and application of the main methods to the fatty acid field are well brought out X-ray diffraction, ultra-violet, visible, infra-red and micro-wave spectroscopy, and nuclear magnetic resonance are each discussed The estimation of *trans*-isomer content by infra-red spectroscopy and other similarly important applications are carefully documented though, again, I would have preferred a more critical appraisal of the present situation

The properties dealt with under 'the liquid state' include heats of formation and combustion, density, molar volume, viscosity, surface tension and refractivity, many of the data being economically presented in tabular form The final chapter surveys the properties of aqueous and non-aqueous solutions of the acids and their glycerides

In summary, Part 1 of this completely rewritten and enlarged edition of *Fatty Acids* provides an unrivalled survey of the field covered The complete work, which will consist of Parts 1, 2, 3 and 4, should prove a worthy successor to the first edition

GEOFFREY EGLINTON

COTTON IN INDIA

Cotton in India

A Monograph, Vol 2 By R H Dastur, R D Asana, K Sawhney, S M Sikka, R S Vasudeva, Quadiruddin Khan, V P Rao and B L Sethi Pp viii+339 (16 plates) Rs 30, 45*s*

Vol. 3 By C Nanjundayya, R L N Iyengar, W R Natu, M B Ghatge, K S Murti, C B Parikh, B L Sethi and D N Mahta Pp viii+295 (20 plates) Rs 30, 45*s*

Vol 4 By S M Sikka, Arjan Singh, Avtar Singh, P D Gadkari, R Balasubrahmanyan, N K Iyengar, K Sawhney, V K Bederker, G B Patel, P S Pandya, V N Paranjpe and N S Panigrahi Pp x+378 (20 plates) Rs 30, 45*s*

(Bombay Indian Central Cotton Committee, 1961)

THE monograph *Cotton in India*, the publication of which has been sponsored by the Indian Central Cotton Committee, is now completed by the issue of Volumes 2, 3 and 4 Volume 1 has already been reviewed in *Nature*, 189, 86 (1961)

The second volume deals with physiology, agronomy, diseases, insect and mite pests, and with seed multiplication and distribution The third volume concerns itself with technology, ginning and pressing, marketing, consumption of cotton (seed and lint), legislative measures and exports and imports The fourth and final volume treats of the special features of the six recognized cotton regions of India

As the work covers every possible aspect of cotton in India, with a large number of contributors, it is difficult to discuss any points in detail Practically all work done in India is mentioned and, though some can only be described as trivial, important findings occur sporadically throughout the work The laborious and painstaking, though somewhat unfruitful, work of Dastur on applied physiology deserves special mention He points out that no fundamental physiological research has so far been attempted as all attention has been concentrated on the physiological causes of bud and boll shedding, bad opening of bolls with immature seeds and lint, premature yellowing and reddening of leaves, low yields of seed cotton, etc The important work on cotton physiology in Trinidad by Mason and his co-workers, Maskell and Phillis, receives only scant attention In Egypt much fundamental work was done by Balls and his colleagues, much of which has some bearing on Indian problems There is a casual reference to the *Cotton Plant in Egypt* published in 1912, but later work during 1927–47 is ignored The subterranean environment, probably as important as the aerial one, does not seem to have been studied, and it is significant that 'roots' finds no place in the index

In Volume 3, several pages in the chapter on technology are devoted to an exposition of statistics at the elementary text-book level This inclusion would appear to be unnecessary The section on small-scale spinning-tests covers less than a page and is inadequate Although of great importance to India, the pioneer small-scale spinning-plant set up by Balls and Hancock in Egypt is not mentioned

The index can only be described as amateurish and calamitously inferior It is neither comprehensive nor usable, and is quite unworthy of the encyclopædic nature of the work The virtues of the monograph, however, greatly outweigh the defects, which regrettably include an undue number of printer's errors On the whole, it is a reasonably good attempt to put on record what is known about cotton in India and will undoubtedly find wide use as a standard work of reference

S C HARLAND

SOME ASPECTS OF ASIATIC RUSSIA

Physical Geography of Asiatic Russia

By S P Suslov Translated from the Russian by Noah D Gershevsky Edited by Joseph E Williams Pp xiv+594 (San Francisco and London W H Freeman and Company, 1961) 105*s*

THIS book—a large quarto, printed on good glossy paper and containing well-reproduced text, maps and diagrams as well as photographs, although these as a rule are not so well reproduced—possesses all the potentialities of a good and informative book Actually it suffers from many original and translation defects

First and foremost the book purports to be the *Physical Geography of Asiatic Russia*, but this title does not accurately describe the contents of the book Physical geography must clearly include geomorphology and structural geology In these fields the book is very weak, whereas the sections on hydrography, soils, plants and animals are adequate and well presented The names of plants and animals are translated into vernacular English and their Latin names are printed in brackets Their habit and ecology are well described—the general impression being that the writer is a specialist in bio-geography, not physical geography On the other hand, the geomorphological and geological sections are written in a casual and often inexpert manner, occupy a small proportion of the text, are often disposed throughout the text and are inadequately illustrated by geological maps and sections This is particularly annoying since great progress has been made during the past hundred years in the study of the geology and geomorphology of the Asian part of Russia, as evidenced by the work of such authorities as Obruchev, Shatsky and Nalivkin

Returning to the title, we read *Physical Geography of Asiatic Russia* Here it must be pointed out that the frontiers of Asia with Europe follow the Urals watershed, the Ural River and the watershed of the Great Caucasus Chain In the book the eastern Uralian region and the Transcaucasian region are excluded This means that in the first part of this book, entitled "Western Siberia", which comprises the greater part of the River Ob', all the headwaters of its left tributaries and all the important mining and metallurgical zone of the Uralian region are excluded The exclusion of Transcaucasia means also the exclusion of a very important part of the Alpine orogenic belt, stretching from the Carpathians to the Hindu Kush

The first three parts of this book deal with Siberia, while the fourth and last part deals with central Asia The territorial division is done in a very peculiar manner, which agrees neither with the leading geological nor with the geographical features The first part, entitled "Western Siberia", embraces the western Siberian lowlands, Altai, Salair and the Kuznetsk basin So far, so good One would expect then that Part 2 would deal with central Siberia, but its title is "Eastern Siberia", and the title of its first chapter (Chapter 4) is also "Eastern Siberia", while the title of its Chapter 6 is "Central Siberia" For a century, the plateau region situated between the Rivers Yenisey and Lena has borne the name of "Siberian Platform", as it is characterized by a unity of geological-tectonic structure and a unity of morphological features Now, according to the authors, the northern part of the "Siberian Platform" receives the name of "Eastern Siberia" and the southern part "Central Siberia" Under" Eastern Siberia" are also included Sayan, Tuva, Cis- and Trans-Baikal regions—mountainous regions most properly linked with Altai–Salair–Kuznetsk basin Again, Part 3, entitled "Far East", is made to embrace Amour and Maritime Province, which is the true Far East, as well as Sea of Okhotsk coastal region and the Kamchatka-Kurile region, the two western provinces which, in spite of their situation, are not generally called the "Far East" Part 4 deals with central Asia, which is plausibly divided into semi-desert, desert and mountain regions

The results of this peculiar regional planning are obvious Geologically and geomorphologically Altai, Salair, Kusnetsk, Sayan and Tuva represent a definite structural-tectonic unit, which, in this book, is broken up into various disjointed chapters The balance struck between the geological–morphological and other aspects is illustrated by a typical chapter on the Altai in which, out of 45 pages, 19 are devoted to bio-geography, 6 to geology and 8½ to morphology In other chapters, such as Chapter 4, dealing with part of the central Siberian Platform, there is no section on geology In Chapter 5, dealing with the Arctic, the most important geological structure of Taimyr is not described at all The book has only one proper geological map (Kuznetsk basin) in addition to a tectonic map of the Transbaikal region and a few maps of Kamchatka-Kuriles

The book would be greatly improved if it had an introductory chapter in which the main geological, tectonic and geomorphological features were discussed Permafrost, a phenomenon typical of a very large part of Siberia, would be much more effectually discussed in an introductory chapter than within the confines of Chapter 4. Because of this chopping up of Siberia into unnatural regions, a very important geological feature has been missed out—namely, the Siberian shield, and its associated plateau lava region, one of the most extensive in the world—the so-called "Siberian Traps" Not only do the numerous basalt lava flows and sills determine the landscape of central Siberia, but also these rocks are often associated with valuable ore deposits and with the newly discovered kimberlite diamond bearing pipes But diamonds are not mentioned in the general index of this book, neither are important tin deposits of the Sikhote–Alin Range, nor many ores of iron, lead, silver and gold, nor coal nor oil All these are important ingredients of physical geography

The translation has not been properly revised, and the number of glaring mistranslations or mistakes in the original text is large Here are a few examples p 4, "Paleozoic sea" for 'Paleogene sea", p 5, "Paleocene" for "Paleogene", "Neocene" for "Neogene", p 106, "Mount Shoria" for "Mountainous Shoria" (Gornaya Shoria), p 115, "Sapropel" (organic mud) for "Sapropelic coal", p 115, "Stratified intrusive vein" (of basalt) for "sill" (of basalt), p 188, "Tungus formation" for "Tunguska formation", p 208, "Mesozoic phase of Alpine folding" for "Kimmerian folding", p 233 "Peridite" for "peridotite" p 257, "Basalt deposits" for "intrusive basalt", p 269, "Tuva" (correct) in the text and "Tuvin" (wrong) in maps 8-V and 8-VI, and p 289, "Caledonian = Lower Paleozoic" for "Caledonian = Upper Paleozoic"

The general impression is that this book was rapidly thrown together by a person or persons more interested in bio-geography than in geology and that the geological–morphological part of it is very poor and contains many original and translation errors

S I TOMKEIEFF

INFINITE-DIMENSIONAL ALGEBRAS

Normed Rings

By M A Naimark Translated from the First Russian edition by Leo F Boron Pp xvi + 560 (Groningen P Noordhoff, Ltd, 1959) 45 D fl, 12 dollars, paper bound 48 75 D fl, 13 dollars, cloth bound

THE theory of finite-dimensional algebras, or hypercomplex number systems, as they used to be called, is one of long standing, going back to Hamilton's quaternions, but only in the past twenty years or so has there been created a general theory for the infinite-dimensional case As in the case of vector spaces, one has to introduce some notion of continuity to obtain a useful theory, the algebraic operations are required to be continuous with respect to an appropriate topology, which, in the simplest cases, is defined by a norm, and we then have a normed algebra

The most familiar normed algebra is that formed by the continuous functions on a closed interval of the real line, the algebraic operations being defined in the natural way and the norm being taken as the upper bound of the absolute value of the function Another important algebra is formed by the set of all functions integrable over the whole real axis, multiplication being defined as convolution, that is,

$$(f * g)(x) = \int_{-\infty}^{\infty} f(y)g(x-y)dy$$

the norm being the integral of the absolute value of the function, this is a special case of the group algebra of a locally compact topological group These algebras are commutative, important non-commutative algebras arise when we consider continuous linear operators in a Hilbert or Banach space

Naimark's book is an admirable survey of the subject up to the appearance of the Russian edition in 1956 The 154 pages of the initial chapter could scarcely be bettered as a brief introduction to the basic ideas of functional analysis The fundamental notions of normed algebras are then introduced, we next come to an account of Gelfand's theory of commutative algebras and their representation by means of continuous functions on a topological space formed by the maximal ideals of the algebra, and its extensions by Shilov and others This is followed by a discussion of the representation of symmetric algebras by means of operators in Hilbert space and descriptions of some special types of algebras The chapter on group algebras constitutes an excellent account of the theory of harmonic analysis on commutative locally compact groups, the non-commutative theory, which is still in a state of rapid development, is touched on lightly The book concludes with a discussion of von Neumann's operator algebras in Hilbert space

Throughout the book the author concentrates mainly on the analytic aspects and applications of the subject, a more algebraic point of view is to be found in Rickart's recent book on Banach algebras The translation is well executed, with only occasional slight infelicities, it is a pity, however, that Mr Boron has not followed the example of the German translation by using 'algebra' instead of 'ring', and so avoided a certain danger of confusion F SMITHIES

COMPUTING UP TO DATE

Modern Computing Methods

Department of Scientific and Industrial Research National Physical Laboratory Notes on Applied Science, No 16 Second edition, completely revised Pp vi+170 (London H M S O , 1961) 21*s* net

THIS second edition of the handbook prepared by staff of the Mathematics Division, National Physical Laboratory, is extremely welcome A complete revision of the 1957 version has been made, revealing the considerable advances of the past few years, with particular emphasis on automatic computing, and also a greater appreciation of the purpose which the book fulfils A hard cover in dark blue adds a becoming air of respectability and greater permanence

The new edition opens with five chapters on linear equations and matrices, including a new one on error analysis, followed by chapters on zeros of polynomials, finite-difference methods, Chebyshev series, two on ordinary and two on partial differential equations, and one each on evaluation of limits, evaluation of integrals, and tabulation of mathematical functions As before, there is a valuable bibliography consisting of carefully selected and annotated references

There are many interesting changes of emphasis between the first and second editions Relaxation methods are now given passing mention only, whereas nearly twelve pages are devoted to Chebyshev series Graeffe's method and the Aitken–Bernoulli process have disappeared from the chapter on solving polynomial equations, and stress is placed on the ill-determination of the roots of high-order polynomials The names of Givens and Householder now appear in connexion with the latent roots of matrices It is perhaps in the chapters on partial differential equations that there is least evidence of progress

Modern Computing Methods should not be regarded as a text-book, nor merely as a book of reference It is a valuable supplement to any good book on numerical analysis, providing a critical selection and appraisal by experts of methods which have been put to the test For this reason it must appeal both to teachers of numerical techniques and to practitioners Not everyone will agree with the choice made, but after all experts must still be allowed to disagree We should wish a great success to this second edition and hope that the hard cover does not betoken a life of more than three or four years, after which the third edition will be eagerly awaited

R A BUCKINGHAM

MASER THEORY

Elements of Maser Theory

By Arthur A Vuylsteke Pp xiii+362 (Princeton, N J D Van Nostrand Company, Inc , London D Van Nostrand Company, Ltd , 1960) 71*s* 6*d*

THE subject-matter of this book falls into two parts, each of which occupies roughly the same amount of space The first is introductory in nature, and covers quantum theory, statistical mechanics radiation and the interaction of radiation with matter, together with a treatment of the necessary mathematical techniques The second part deals with the theory of two- and three-level masers, covers the ammonia maser and the paramagnetic solid maser in detail, and ends with a short discussion of noise theory in general and maser noise in particular Mr Vuylsteke writes with admirable clarity, but one is tempted to question the value of books of this type which attempt to do too much The detailed study of masers is unlikely to be necessary for the student, who might well find much of value in the first half of the book, the professional theoretician, on the other hand, might consider that this part merely adds to the bulk of what could have been a more modest monograph

The section on masers proper is a most useful summary of the theory underlying microwave masers Practical details are mentioned only when they are necessary for appreciation of the theory, or when they themselves have points of theoretical interest The bibliography, as the author admits, is by no means exhaustive, and is complete enough only to support the text, it is a pity that this opportunity could not have been taken to provide the reader with a complete set of references The style is lucid, and the author appreciates the value of explanatory interpolations to bring out essential points Such is the rapidity of developments in this field that the optical maser now fashionable is not mentioned at all, but most of what need be said on microwave maser theory is to be found here The book will prove invaluable as an introduction for those approaching the subject for the first time, and as a summary of the theory for those already familiar with the field

J. H SANDERS

Nuclear Reactor Stability

By Dr A Hitchcock (Nuclear Engineering Monographs, No 11) Pp x+61 (London · Temple Press, Ltd , 1960) 12*s* 6*d*. net

THE neutron multiplication in a reactor is affected by changes in temperature and other physical variables, which are in turn affected by changes in power. This circular situation can cause small changes in power-level to build up, so that the reactor becomes unstable The possibility of unstable behaviour limits the available power output and must be either corrected by control action or, if possible, avoided by correct design This monograph presents concisely, but with an admirably lucid and readable style, an introduction to the theory of reactor stability and the present methods used in establishing conditions for the stability of solid- and liquid-moderated power reactors, a theory which over the past few years has led, ahead of experimental confirmation, to important advances in power reactor control The treatment, avoiding the use of specialized techniques and the jargon of control system analysis, assumes only an elementary knowledge of linear differential equations and Fourier series

The first two chapters show how the physical mechanism of several kinds of instability may be related to the partial differential equations of reactor dynamics In the third and fourth chapters the modal expansion method is applied to yield conditions for instability, first due to temperature effects and then due to the relatively slow effect of fission product (xenon) poisoning The last two chapters deal with voidage instability in boiling reactors and the principles of spatial control

Proceedings of the International Conference on Nuclear Structure

Kingston, Canada, August 29–September 3, 1960 Edited by D A. Bromley and E W Vogt Pp 990 (Toronto University of Toronto Press , Amsterdam North-Holland Publishing Company, 1960) 125*s*

A FREQUENT criticism of conference proceedings is that they tend to present a heterogeneous collection of papers which might more suitably and more speedily have been published in the appropriate technical journals This criticism does not apply to the volume under review Here we have a well-edited handbook of contemporary ideas on nuclear structure It leads in logical sequence from a consideration of the properties of nuclear forces and infinite nuclear matter to a series of contributions on the detailed properties of levels in actual nuclei The book is interfused throughout with contributions on the various reaction mechanisms by which our knowledge of nuclear structure is obtained It is excellently produced with good, clear type and is well illustrated with diagrams reproduced from the contributors' slides This is particularly praiseworthy because this book was available to the delegates within five weeks of the closing date of the conference

The editors have succeeded in effecting a pleasing compromise between the linguistic correctness of a submitted manuscript and the informality of expression in the conference hall This happy blend has been much aided by the inclusion of verbatim reports of the lively discussion which followed the main contributions This book has been made what a good conference report should be It provides those who were there with an accurate record of what was said, while at the same time it preserves something of the atmosphere of the conference hall for their less fortunate colleagues who were unable to be present

D. L Allan

Progress in Dielectrics

Vol 3 Edited by Dr J. B. Birks and Prof J Hart Pp vii+292 (London : Heywood and Co , Ltd , 1961) 63*s*. net.

THE third volume of this series is lively and well written Three of the articles are concerned with electrical properties and molecular structure R H Cole's review "Theories of dielectric relaxation and polarization" is helpful by its lucidity even when it covers old ground, and it contains some illuminating remarks, for example, on the dynamics of the Onsager equation J B Hasted's article "The dielectric properties of water" (and ice) fills a long-felt want This subject might deserve a book, particularly since it is relevant to biology Hasted also gives a useful review of microwave techniques R J Meakins systematically reviews new work on long-chain crystals, hydrogen-bonded compounds, occlusion compounds, and other types of solids the structure of which implies distinctive features of dielectric relaxation

R Stratton's review of theories of electrical breakdown is well presented, but this subject is perhaps being reviewed too frequently. The article by D G Kiely, "Dielectric waveguides and aerials", presents a mathematically difficult subject in a manner comprehensible to the experimentalist The readable article by C W Hamilton on "Recent developments in cable insulation in the United States" is interesting in that it illustrates the gap which still exists between the questions asked by the engineer and the answers provided by the physicist and chemist

V Daniel

Electrical Activity of Single Cells

Edited by Yasuji Katsuki Pp viii+312 (Tokyo Igaku Shoin, Ltd , 1960) n p.

THIS book consists of 18 separate articles by different workers in the field of electrophysiology Their common feature, given by the editor as reason for grouping these articles together, is that they all represent work done in Japan with the intracellular capillary micro-electrode The subjects of investigation include sensory receptors, peripheral and central nerve cells, the neuromuscular junction and cardiac muscle Each article has the strictly limited range of interest of a paper in a scientific journal Furthermore, apparently because the numerous authors were intent on presenting as much of their experimental results as space allowed, very little attention is given to describing the method or to justifying its application in different experimental situations This is particularly unfortunate here, as it is the method which is the unifying feature of the book A welcome exception to this is an article by Takeuchi and Takeuchi, who give a careful account of the application of the voltage-clamp technique to the localized response of the neuromuscular junction

Much of the material in this book has already been published in readily accessible journals (in English), and it is clear that the remainder will soon follow The book does not therefore fulfil any general need

P. Fatt

BIOSYNTHESIS OF PORPHYRINS AND CHLOROPHYLLS*

By DR. K. D. GIBSON, MARGARET MATTHEW, PROF. A. NEUBERGER, F.R.S., and G. H. TAIT
Department of Chemical Pathology, St. Mary's Hospital Medical School, London, W.2

IT is now generally accepted that all the carbon and nitrogen atoms of the hæm of hæmoglobin, myoglobin, catalase, peroxidase and the various cytochromes, and probably also those of the dihydroporphyrin of the chlorophyll of green plants and algae and those of the tetrahydroporphyrin of bacteriochlorophyll are derived from the one nitrogen atom and the five carbon atoms of δ-aminolævulic acid. The enzyme which is responsible for the formation of this acid, δ-aminolævulic synthetase, has been found in a great variety of cells and must be presumed to be present in all cells containing either hæm proteins or chlorophylls. This synthetase catalyses the condensation of glycine with succinyl-coenzyme A to give δ-aminolævulic acid, carbon dioxide and free coenzyme A. The enzyme has not been prepared in a high state of purity, but so far it appears that the only co-factor required for this reaction is pyridoxal phosphate.

The importance of this substance in the synthesis of δ-aminolævulic acid was indicated in the work of Lascelles[1] on porphyrin synthesis in *Tetrahymena vorax* and that of Schulman and Richert[2] on hæm synthesis in bird erythrocytes. The participation of pyridoxal phosphate in the enzymic reaction was later directly demonstrated independently by two groups of workers[3,4]. On the basis of the work of Braunstein and Snell, it seems reasonable to assume that pyridoxal phosphate (combined with a specific enzyme protein, possibly through its phosphate group) can react with the amino group of glycine to form an aldimine or Schiff's base, and this then reacts with succinyl-coenzyme A to form a new C—C bond. This reaction has been discussed in detail by Shemin *et al.*[5] and by one of us[6]; moreover, mechanisms of reactions of this type have recently been considered by Perault *et al.*[7], who have applied the molecular orbital method (L.C.A.O. method) to this problem. These workers have confirmed the belief that in the anionic form of the glycine–pyridoxal phosphate complex the carbon atom which is derived from the α-carbon atom of the amino-acid has a high electron density, and thus will react with the electrophilic carbonyl carbon atom of succinyl–coenzyme A.

Inhibition of δ-Aminolævulic Synthetase by Aminomalonate

The requirement for pyridoxal phosphate in the enzymic formation of δ-aminolævulic acid fully explains the inhibitory effects of substances like cysteine, penicillamine or cyanide, which are known to react with the aldehydic group of the coenzyme and thus prevent the formation of the aldimine. We have recently found that the formation of δ-aminolævulic acid can also be inhibited by aminomalonate. The effect of this substance is very marked, even at a low concentration. Kinetic investigation showed that the inhibition is of the competitive type with respect to glycine, and so far it appears that aminomalonate has no marked effect on other enzymes requiring pyridoxal phosphate. Monoethyl aminomalonate is a somewhat weaker inhibitor than aminomalonate, and the diethyl ester has only a relatively slight effect which is of the same order of magnitude as shown by many other amino-acids such as threonine, α-alanine or β-alanine. While kinetic investigations with crude enzyme preparations cannot be considered to give results of exact quantitative significance, the following values give some information of the inhibitory power of aminomalonate: K_m for glycine, $1·4 \times 10^{-4}$ *M*; K_i for aminomalonate, $2·2 \times 10^{-5}$ *M*; K_i for monoethyl aminomalonate, $3·1 \times 10^{-5}$ *M*. It seems likely that aminomalonate combines with the pyridoxal phosphate – enzyme complex, the amino group forming an aldimine with the aldehyde group of pyridoxal. One or two carboxyl groups probably combine with part of the enzyme protein and the resulting complex which cannot react further with succinyl–coenzyme A may be sterically more favoured than the corresponding complex with glycine, which is the intermediate in the enzymic synthesis of δ-aminolævulic acid.

Transamination of δ-Aminolævulic Acid

Shemin and Russell[8] found that with rats given δ-aminolævulic acid labelled with carbon-14 in the carbon atom bearing the amino group, the isotopic carbon appeared in substantial amounts in respiratory carbon dioxide, urinary formate and in the ureido groups of uric acid. They proposed that δ-aminolævulic acid was metabolized by a cyclic pathway, the succinate–glycine cycle, involving the formation of a one-carbon intermediate derived from the δ-carbon of δ-aminolævulic acid. The general metabolic importance of this pathway cannot be assessed at present and the enzymic steps involved have not as yet been properly defined with the exception of the first reaction, the conversion of δ-aminolævulic acid to γ,δ-dioxovaleric acid. A specific transaminase catalysing this reaction has been found in mammalian tissues and in *Corynebacterium diphtheriae*[9,10], we have further examined this enzymic reaction as it might provide a possible control mechanism for the synthesis of hæm and chlorophyll. The earlier workers investigating this reaction have incubated δ-aminolævulic acid and α-oxoglutarate and measured the glutamic acid produced. However, using extracts from *Rhodopseudomonas spheroides*, we have found that the equilibrium appears to be in favour of the production of δ-aminolævulic acid, and we have therefore incubated γ,δ-dioxovaleric acid (supplied by Dr. A. T. Carpenter) with the amino-acid, and estimated δ-aminolævulic acid, for which a sensitive method is available. Moreover, we have found that L-alanine and β-alanine are from five to seven times more effective than glutamic acid in this reaction. Other amino-acids tested showed very little activity. The *p*H optimum is 7·0–7·2, and the reaction is strongly inhibited by pyruvic acid and methylglyoxal, whereas oxoglutarate has little effect. So far no evidence has been obtained which would indicate that the transaminase has any controlling effect on the synthesis of porphyrin or chlorophyll in *Rps. spheroides*.

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* Substance of two lectures given by Prof. A. Neuberger at University College, London, on May 8 and 11, 1961.

Role of Biotin in the Synthesis of Porphyrin and Bacteriochlorophyll

In an earlier account by one of us[6], the effect of biotin in these reactions was considered, and the possibility was discussed that it acts by catalysing the decarboxylation of α-amino-β-oxoadipic acid present as an intermediate in the pyridoxal phosphate – δ-aminolævulic acid synthetase complex. The evidence in favour of such an assumption was the finding (since confirmed by Lascelles[11]) that δ-aminolævulic acid synthetase activity in extracts of organisms grown in media containing very low concentrations of biotin was apparently specifically reduced if compared with those of δ-aminolævulic acid dehydrase, threonine dehydrogenase, β-hydroxybutyric dehydrogenase or alanine – δ-aminolævulic acid transaminase. The finding, however, that illumination of biotin-deficient organisms with addition of biotin to the medium produced growth and synthesis of porphyrin and bacteriocholorophyll without any increase in the synthetase activity as measured in extracts indicates that any effect of biotin is not exercised directly through a control of the level of δ-aminolævulic acid synthetase activity. Moreover, it was found that other conditions which are unfavourable to formation of bacteriochlorophyll by *Rps. spheroides*, such as a deficiency in nicotinic acid or thiamine, or replacement of malate in the growth medium by acetate, are associated with a depression of δ-aminolævulic acid synthetase activity. It therefore appears that the effect of biotin on the level of this enzyme is unspecific, and that the depression of the synthesis of porphyrin and bacteriochlorophyll by biotin deficiency is caused by some other mechanism, possibly through a reduction in the concentration of pyridoxal phosphate in the cells.

It was also reported earlier[6] that both a commercial preparation of avidin and preparations made in our laboratory by Dr. N. M. Green, containing 2·5 and 4·9 units of avidin/mgm. protein inhibited δ-aminolævulic acid synthetase activity in crude extracts of *Rps. spheroides*. This inhibition appeared to be specific; but when this 'avidin' was pre-incubated with biotin, inhibition was still observed. This seemed surprising, as other biotin-containing enzymes are not inhibited by avidin which has been incubated with biotin before being added to the enzyme under investigation. However, when Dr. Green achieved a further purification of the avidin by column chromatography and made a preparation available to us which had an activity of 9·6 units/mgm., and an estimated purity of 80 per cent, no inhibition of δ-aminolævulic and synthetase activity was obtained. No inhibition was observed with other egg-white proteins such as ovalbumin, lysozyme and ovomucoid, and it must be concluded that some protein not yet identified and which contaminates crude avidin, has a specific inhibitory effect on δ-aminolævulic acid synthetase.

Chemical Conversion of Porphobilinogen to Uroporphyrins

The next step in porphyrin biosynthesis is the conversion of δ-aminolævulic acid to porphobilinogen. This reaction is catalysed by the enzyme δ-aminolævulic acid dehydrase and is fully discussed in a recent review of Bogorad[12]. Porphobilinogen is then transformed to uroporphyrinogen by enzymes to be discussed later, but first it is worth while to consider briefly the non-enzymic reaction which occurs fairly readily under relatively mild conditions. It has been known for some time[13] that on heating porphobilinogen in acid solution, there is obtained a mixture of uroporphyrins in good overall yield and that this mixture contains an appreciable amount of uroporphyrin III. These findings were confirmed by Westall, who isolated porphobilinogen in pure form[14], and by Cookson and Rimington[15], who determined its structure and showed it to be an intermediate in porphyrin biosynthesis. Recently, Mauzerall[16] improved the analysis of individual isomers by decarboxylating the uroporphyrin and estimating the coproporphyrins after chromatographic separation by a modification of the method of Falk *et al.*[17]. In this way it was possible to determine the amounts of isomers I and II and the combined amounts of III and IV in a mixture. Mauzerall was thus able to study the formation of uroporphyrin semi-quantitatively and to determine the distribution of isomers, although the sensitivity of the method is still not very high and the fact that isomers III and IV cannot be separated makes interpretation somewhat difficult. The results obtained by Mauzerall and the earlier workers can be summarized as follows:

(1) The products of the reaction are not uroporphyrins, but uroporphyrinogens, that is, hexahydroporphyrins in which the pyrrole rings are linked through methylene bridges.

(2) If porphobilinogen or any of the four uroporphyrinogen isomers is heated in acid solution, the distribution of the resulting uroporphyrinogen isomers is as follows: 3/4 III + IV, 1/8 I and 1/8 II. In neutral or alkaline solution, no isomer II is formed from porphobilinogen and about half the total porphyrinogen was found to be isomer I.

(3) If the isomerization of uroporphyrinogen in acid is carried out in the presence of [^{14}C]formaldehyde, radioactivity is found in the final mixture of uroporphyrinogens; however, no such incorporation of formaldehyde takes place when uroporphyrins are heated in acid or when uroporphyrinogens are heated at neutral or alkaline *p*H. Radioactivity is found in uroporphyrinogens when these are formed by heating solutions of porphobilinogens in the presence of [^{14}C]formaldehyde and this occurs at acid, neutral and alkaline *p*H values.

(4) In the isomerization of uroporphyrinogen in acid solution and in the formation of uroporphyrinogen from porphobilinogen in acid or neutral solution, yields of up to 80–87 per cent of total porphyrinogen are obtained. Although some material behaving like dipyrrylmethanes was formed, no evidence was obtained for the presence of higher linear polypyrrylmethanes, and it must be assumed that once a linear tetrapyrrylmethane with one aminomethyl or hydroxymethyl group is formed, cyclization to the macrocycle, uroporphyrinogen, is very fast.

Enzymic Conversion of Porphobilinogen to Uroporphyrinogen

The enzymic conversion of porphobilinogen to uroporphyrinogen has been studied in extracts of *Chlorella*, spinach leaf and wheat germ, and in fowl erythrocytes, by Bogorad, Mauzerall, Granick and Rimington (for review see Bogorad[12]) and more recently by Lockwood and Benson[18], and in *Rps.*

spheroides by Heath and Hoare[19]. The main findings were as follows: the product of the reaction catalysed by the crude enzyme system is uroporphyrinogen III and not uroporphyrin III. When the crude enzyme system is heated to 55° or 60° (depending on *p*H) the product is not uroporphyrinogen III, but isomer I. From the heated extracts an apparently single enzyme called porphobilinogen deaminase can be obtained which can be further purified and which converts porphobilinogen quantitatively into uroporphyrinogen I. The second and more heat-labile enzyme, called uroporphyrinogen isomerase, has no detectable action on uroporphyrinogen I or on porphobilinogen alone. Its substrate is either a linear tetrapyrrole or, what seems less likely, a dipyrrolic or tripyrrolic substance which is converted by the isomerase to uroporphyrinogen III through further condensation with one or two porphobilinogen molecules.

Although, as pointed out above, isomer analysis of porphyrins is neither so sensitive nor so specific as might be desired, it appears from evidence at present available that enzymic condensation does not give rise to uroporphyrinogens II and IV. It also appears that formation of uroporphyrinogen by the two enzymes is quantitatively paralleled by consumption of porphobilinogen. Moreover, neither the intact nor the heat-altered enzyme preparation incorporates radioactive formaldehyde into porphyrin. This finding is in accordance with the observations of Bogorad[20] and of Carpenter and Scott[21,22], that opsopyrroledicarboxylic acid and isoporphobilinogen are not substrates for the deaminase or isomerase; in fact, the two substances act as competitive inhibitors.

Many suggestions have been put forward to explain both the chemical and enzymic condensation of porphobilinogen to uroporphyrinogen and particularly the proportions of isomers formed. Most of the schemes are not readily compatible with the experimental findings; particularly those obtained in the enzymic reaction. Porphobilinogen deaminase might perhaps best be visualized as catalysing the addition of a porphobilinogen molecule to the free α-position of another porphobilinogen molecule or a dipyrrole or tripyrrole of the structures *PA PA*—$CH_2NH_3^+$, *PA PA PA*—$CH_2NH_3^+$ (*A* stands for acetyl and *P* for propionyl side-chain). It is postulated that the action of this enzyme stops at the linear tetramer stage and that the deaminase is highly specific with regard to the nature of the β-substituents, both in the electrophilic and the nucleophilic partner of each step of the reaction. Alternatively, the condensation of the four porphobilinogen units on the enzyme surface may be simultaneous. In the absence of the isomerase the linear tetramer will cyclize spontaneously to give uroporphyrinogen I. The findings of Mauzerall[16] have clearly shown that at the tetrameric stage cyclization is greatly favoured over further linear addition, and this author has also adduced convincing reasons for such preference.

In an important paper, Mathewson and Corwin[23] suggested that the substrate of the isomerase is the linear tetramer and that this enzyme catalyses an attack by the $CH_2NH_3^+$ group, not on the free α-position of the pyrrole at the other end of the tetramer chain, but on the 'occupied' α-position of that pyrrole. The cyclic structure thus produced will then become re-arranged to give uroporphyrinogen III. Mathewson and Corwin have also proposed that three of the rings in the linear tetramer are in the pyrrolenine form, thus giving even greater flexibility to the structure. In the cell, co-operation between the two enzymes is almost perfect and thus only minute traces of uroporphyrin I are normally formed. In congenital porphyria this co-operation breaks down, with the result that the linear tetramer accumulates and this spontaneously changes into uroporphyrinogen I.

The mechanisms operating in the non-enzymic formation of uroporphyrinogens from porphobilinogen are more obscure than those involved in the enzymic reaction. The scheme proposed by Mauzerall[16] explains the incorporation of formaldehyde and the isomer ratios, but the suggested mechanism of Mathewson and Corwin, though satisfactory for explaining the enzymic condensation, does not readily accommodate the exchange with formaldehyde so convincingly demonstrated by Mauzerall.

Conversion of Uroporphyrinogen III to Protoporphyrin

The decarboxylation of uroporphyrinogen III to coproporphyrinogen is a straightforward reaction and requires little comment in this discussion. The next step, however, is more complex and has been investigated recently.

Sano and Granick[24] have described an enzyme which is present in a great variety of tissues, but is found in particularly high concentration in the liver, bone marrow and small intestine, and which converts coproporphyrinogen III to protoporphyrin IX. This enzyme, which has been purified approximately twenty times, is localized primarily in the mitochondria. The enzyme responsible for the condensation of succinyl coenzyme A and glycine to give δ-aminolævulic acid is also localized in particulate structures of the cell, whereas δ-aminolævulic acid dehydrase is present in the cytoplasm. It would therefore appear that δ-aminolævulic acid is synthesized in mitochondria, then leaks out into the cytoplasm, to be converted ultimately into coproporphyrinogen, which migrates back again into the mitochondria, to be transformed into protoporphyrin. Sano and Granick suggest that this localization might permit a control of porphyrin synthesis by the cell through permeability factors.

The enzyme which acts on coproporphyrinogen removes two hydrogen atoms and a carboxyl group from each of the two propionic acid side-chains in rings *A* and *B*, and at the same time porphyrinogen is converted to porphyrin. The latter reaction occurs fairly readily in the presence of oxygen and is not necessarily catalysed by an enzyme. The first two reactions, however, appear to be part of a concerted mechanism as the appropriate diacrylic acid is not an intermediate. Oxygen acts in this somewhat impure system as a hydrogen acceptor and cannot be replaced by any other co-factor tested. This is of considerable general importance, since it might indicate that oxygen was present in the atmosphere of the Earth at a time in evolution when protoporphyrin first appeared.

Biosynthesis of Chlorophyll and Bacteriochlorophyll

The work of Granick[25] has produced strong presumptive evidence that chlorophyll and bacteriochlorophyll are synthesized by green plants and certain bacteria from protoporphyrin Although the enzymic steps involved cannot as yet be clearly defined, Granick has proposed, mainly on the basis of work with mutants of *Chlorella*, that the following pathway operates

protoporphyrin → magnesium protoporphyrin or
protoporphyrin monomethyl ester →
magnesium protoporphyrin monomethyl ester → → →
magnesium vinyl phæoporphyrin a_5 →
magnesium vinyl phæoporphyrin a_5 phytyl ester →
chlorophyll a → bacteriochlorophyll

Although this is a rational scheme, it does not necessarily follow that compounds which accumulate in mutants, in which the enzyme for a particular step in a biosynthetic pathway is absent, are true intermediates It is now known, for example, that intermediates in hæm synthesis are porphyrinogens and not uro- and coproporphyrins, although these latter substances may be accumulated or excreted under certain conditions In a similar manner, protoporphyrinogen or one of its derivatives might be a precursor of chlorophyll

We have recently been able to define at least one step in Granick's pathway more clearly In work aimed at elucidating the effect of biotin, it was observed that ethionine at a concentration of about 10^{-4} *M* inhibited bacteriochlorophyll synthesis by *Rps. spheroides* almost completely, and at the same time produced a markedly increased excretion of copro- and uro-porphyrin into the medium in which the organism was illuminated At that concentration very little inhibition of protein synthesis or growth occurred Many other substances, such as methionine, other amino-acids, keto-acids and related compounds, amino-acid antagonists and other inhibitors of protein or nucleic acid synthesis, were tested to see whether they produced similar effects The results were negative with the exception of threonine, the addition of which to the medium at a concentration of 10^{-3} *M* inhibited bacteriochlorophyll synthesis and produced a considerable increase in porphyrin excretion The effects of ethionine and of threonine were reversed by methionine and by homocysteine, but not by cysteine or serine The effect of threonine was also reversed by homoserine This suggested that ethionine interfered either with the synthesis of methionine or its utilization and further that methionine had a specific function in the synthesis of chlorophyll from protoporphyrin involving the methyl-group donating capacity of this amino-acid The effect of threonine was baffling at first, until it was realized that methionine and threonine share a biosynthetic pathway leading from aspartic acid through aspartyl β-phosphate and aspartyl β-semialdehyde to homoserine The latter can be converted either to homocysteine and methionine or to threonine. Thus threonine could inhibit methionine synthesis from aspartic acid by a feed-back mechanism involving either repression or inhibition of one of the enzymes catalysing the various steps leading to homoserine (Recent results have shown that in *Rps spheroides* threonine is a competitive inhibitor of homoserine dehydrogenase, although it does not repress the formation of this or any of the other enzymes involved in homoserine synthesis)

The most likely step in the biosynthesis of chlorophyll involving the methyl group of methionine is the methylation of protoporphyrin or of its magnesium complex to give magnesium protoporphyrin monomethyl ester Organisms were illuminated in a suitable medium to which methionine labelled with carbon-14 in the methyl group was added At the end of the experiment, bacteriocholorophyll was isolated, purified and counted The chlorophyll had high radioactivity, which was shown on degradation to be entirely confined to the methyl ester group (Table 1) With appropriate amounts of [^{14}C]methionine the dilution was only about three times, and this indicated that the methyl group of methionine was almost certainly the immediate and specific precursor of the porphyrin ester carbon atom The less specific utilization of formate for the methyl ester group of chlorophyll had already been shown some time earlier[26] More recently two of us, using chromatophore preparations of *Rps spheroides*, have been able to demonstrate directly the methylation of magnesium protoporphyrin to its methyl ester by *S*-adenosylmethionine[27]

Table 1 LABELLING OF BACTERIOCHLOROPHYLL SYNTHESIZED IN THE PRESENCE OF [METHYL-^{14}C]METHIONINE

Rps spheroides were illuminated at 2 mgm dry wt /ml in Mixture I of Lascelles (ref 31) for 18 hr at 32–34° [Methyl-^{14}C]methionine (10^{-4} *M*) was also present The bacteriochlorophyll was extracted and purified A portion of it was degraded in methanolic potassium hydroxide The methanol, bacteriochlorin and phytol were isolated and estimated for radioactivity by liquid scintillation

Fraction	Specific radioactivity (counts/min /μmole)
Bacteriochlorophyll	9,800
Methanol	9,900
Phytol	64
Bacteriochlorin	6

Control of Synthesis of Bacteriochlorophyll

Micro-organisms such as *Rps spheroides* or *Rhodospirillum rubrum* can grow either anaerobically in the light or aerobically in the dark, using as source of energy a number of aliphatic acids or alcohols Elsden and Ormerod[28] showed that *R rubrum*, under aerobic conditions in the dark, metabolized all substrates by the tricarboxylic acid cycle Anaerobically in the light, acetate and butyrate are metabolized by this cycle, but propionate, malate and succinate are utilized by another pathway which is not inhibited by fluoroacetate The question therefore arises how the type of metabolism is regulated Following van Niel's pioneer work, this problem was first systematically investigated by Cohen-Bazire, Sistrom and Stanier[29] They showed that organisms grown aerobically in the dark contained little pigment, whereas those grown anaerobically in the light contained relatively large amounts of bacteriochlorophyll, that transference of organisms from the dark to light resulted, after a lag phase, in synthesis of pigment, that exposure to oxygen of organisms growing in the light inhibited synthesis of pigment immediately, that is, without a lag phase, and removal of oxygen resulted in immediate resumption of synthesis of pigment and that increasing light intensity beyond a certain value had the same effect as an increase of oxygen tension On the basis of these results, they suggested that the synthesis of both bacteriochlorophyll and carotenoids is controlled by the state of oxidation or reduction of a carrier of the electron-transport chain They did not specify the identity of the substance postulated or the mechanism by which this control operates

Lascelles[11,30] examined changes in the activities of various enzymes involved in porphyrin synthesis in extracts from cells grown under conditions under which the rate of bacteriochlorophyll synthesis is either high or low When synthesis of pigment was inhibited or when levels of pigment were low, the activities of δ-aminolævulic acid synthetase and dehydrase were also reduced, and synthesis of these enzymes was apparently inhibited She also found that the transference of organisms from highly aerobic conditions in the dark to conditions of low oxygen tension in the dark, or to anaerobic conditions in the light, not only caused pigment synthesis but also resulted in an increase in the activities of δ-aminolævulic acid synthetase and dehydrase This adaptation could be inhibited by inhibitors of protein synthesis Lascelles[11] observed that hæmin represses formation of the synthetase and dehydrase in whole cells, and there was some evidence that formation of these two enzymes could be regulated by the concentration of iron porphyrins present in the cells We have found that hæmin, in addition to its repressive effect on the formation of these enzymes, inhibits the synthetase activity in cell extracts and that protoporphyrin inhibits the dehydrase activity Accumulation of hæmin and of protoporphyrin under certain conditions of growth may thus reduce synthesis of pigment both by repression of enzyme formation and by inhibition of the activities of enzymes already present in the cells

There is, however, good evidence that levels of porphyrins and hæm are not the only factors controlling synthesis of pigment The experiments on the effects of ethionine and threonine demonstrate the importance of the methylation step, and the excessive amounts of porphyrins excreted in the cells in the presence of moderate quantities of ethionine and threonine in the medium suggest that porphyrin formation is normally controlled by a substance X, possibly a phæoporphyrin derivative, which occurs after magnesium protoporphyrin in the biosynthetic pathway of bacteriochlorophyll If this substance is not synthesized, for example, owing to inhibition of the methylation of magnesium protoporphyrin or to inhibition of methionine formation, porphyrin synthesis 'runs wild' and excessive amounts are formed which are released into the medium In addition to these direct mechanisms of control, it appears that in general the rates of formation of chlorophyll and of carotenoids are closely parallel, and we know little about the factors regulating the formation of the latter Similarly, phytol is a component of the bacteriochlorophyll molecule and its mode of synthesis is still obscure, although it would appear likely that it is derived from a carotenoid or a carotenoid precursor Mechanisms which increase or depress terpene synthesis might therefore also control synthesis of bacteriochlorophyll through the availability of the phytol side-chain.

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ANIMAL BEHAVIOUR

A SYMPOSIUM on animal behaviour was held on September 4 by Section J (Psychology) of the British Association for the Advancement of Science, during the recent meeting at Norwich As was pointed out by the chairman, Mr D E Broadbent (Medical Research Council Applied Psychology Research Unit, Cambridge), the Section has usually considered primarily human behaviour, but in this it is ceasing to be in step with university departments of psychology, which are devoting more effort than they once did to research on animals Animal behaviour is a subject of great interest in itself It also has relevance to human beings, because it reveals simple general principles about behaviour that may also apply to men, and it makes possible experiments on causal factors in behaviour which cannot, for moral and practical reasons, be performed on men Thus the influence on behaviour of rewards, of the structure of the brain, of restricted infantile experience, and of drugs and instincts, can be studied in animals, and papers on each of these topics were included in the symposium

Mr H M B Hurwitz and Dr. J R Millenson (Birkbeck College, University of London), proceeded to discuss and demonstrate the control of behaviour by reward Their emphasis was on the possibility of building up valid empirical statements about observed relationships without appeal to theoretical concepts Thus, if a response R was followed by a reinforcing agent S^r, such as food, the frequency of R would increase If S^r ceased to follow R, the latter would decrease in frequency Such statements could be made without any discussion of events within the animal In a similar way, but with more complex experimental manipulations, behaviour such as problem solving or delayed reaction could be studied

The authors then demonstrated a pigeon and a rat the behaviour of which had been shaped in a complicated fashion The pigeon would look at three keys, peck the middle one, and observe a coloured light on that key which was sometimes red and sometimes green One of the other two keys was red and one green, and the bird would then peck the key which was the same colour as the middle key This sequence occurred continuously at a rate rather faster than most humans could make the comparison The method of training had been, first, to teach the bird that food would appear in a certain place when a light was on, then to teach that pecking either of the two end keys would produce the food, this training was followed by withholding the food unless a peck at the centre key was made before the peck at the end key, and so on until the full sequence was learned

The rat had been taught in a similar way to press one lever five successive times, and then press another lever to obtain food Both animals behaved as they had been taught despite the strange surroundings and the audience and provided a most convincing demonstration that suitable manipulation of reward could indeed control behaviour so as to produce exceedingly complex learned actions

Dr L Weiskrantz (University of Cambridge) emphasized the importance of such behavioural techniques, but he was concerned with the brain and with its relation to behaviour rather than with purely external observations of animals Historically, the cortex of the brain had been regarded as composed of projection areas through which messages entered and left, and association areas which were more general in function Indeed, according to Lashley's principle of mass action, the amount of deterioration, in performance in mazes, produced by removal of association cortex from a rat's brain depended on the weight of cortex removed and not the location More recent research had shown both that the projection areas were less specific and that the association areas were more so than had been thought Removal of the projection area which was fed by the ear had little effect on the ability of an animal to detect sounds On the other hand, damage to the frontal association areas would produce an impairment in the ability to remember, over a short period, which of several possible hiding places contained food The latter impairment did not occur with damage elsewhere in the brain Similarly, damage to the temporal lobes would affect the ability to discriminate between two stimuli whereas damage to the frontal lobes would not Thus different parts of the brain do have different functions

Experiment in this area is made difficult by the irreversible nature of a lesion in the brain once the frontal lobe has gone the animal cannot be tested again in its normal state Recent work at Cambridge had, however, explored the possibility of temporary interference with particular regions by electrical stimulation, and it seemed that the ability to make delayed responses to hiding-places could indeed be removed temporarily while stimulation was being applied in the frontal lobes The animals were afterwards normal in their performance Once again different parts of the brain showed different effects

Whereas the classical view of the nervous system was of a series of levels, in which the lower mechanisms dealt with isolated actions and higher ones with combined and co-ordinated ones, a different interpretation was now gaining support On this view the nervous system might rather be compared with a candle in which the wick dealt with gross and undifferentiated action of the whole organism, and the outer skin of the candle dealt with finely discriminated functions To take another analogy, on the old view, lower levels might be compared with isolated instruments and the cortex to the whole orchestra, on the new view, the contrast was rather between the orchestra tuning up, all simultaneously, and the orchestra playing in complex harmony

Dr Weiskrantz ended with a clear and well-reasoned argument for a physiological approach to behaviour as well as the approach in which actions alone are studied without reference to events within the animal, and as well as the approach in which mechanical models are constructed without physiological reference He regarded these approaches as healthy in their scepticism, but negative, and firmly supported the importance of the brain in behaviour It may be added that his paper was both witty and modest, and that it produced a deep impression on the audience

Dr R Stretch (Queen's University, Belfast) gave a general review of experiments on the early experience of animals, including a few which show effects of ante-natal experience It appears that exposure of pregnant rats to frightening situations may produce offspring which are unduly susceptible to fear possibly through some ill-understood hormonal mechanism Most research has, however, concentrated on early post-natal experience, and in this area the relationships seem to be complex Electric shock administered to rats during infancy seems, for example, to produce adults which are more resistant to emotional disturbance On the other hand, if animals are reared in a very restricted environment they seem as adults to be poor in ability to learn There is some evidence that exposure to a complex environment in infancy produces adults with a lower concentration of cholinesterase in the brain, and this may be related to the differences in ability There are also genetic differences as well as those produced by environment, and in this connexion it is interesting that wild rats differ from laboratory rats in the extent to which they will show curiosity or exploration Wild rats avoid novel objects, while laboratory animals will investigate them and even feed in their presence There may therefore be some interaction between anxiety and curiosity, and the effects of early experience on curiosity are of interest

Dr Stretch then turned to some experiments of his own on this latter problem One group of rats was kept in ordinary laboratory wire-mesh cages for the period just after weaning, while another group of rats was kept in restricted cages through the walls of which they could not see In the latter case there was little background noise, and the environment was in general a rather unstimulating one As adults the rats were tested for curiosity in two ways One of these was a maze shaped like an *H*, the measure of exploration being derived from the number of parts of the maze entered On this test the animals which had had a restricted environment explored more Another test could, however, be applied For this a box was used, in an alcove of which novel cards with patterns on them were placed, and a record was kept of the extent to which the rats entered the alcove rather than the rest of the box On this test the restricted rats gave a lower score As Dr Stretch pointed out, these results suggest that rats with a restricted early experience were more fearful They therefore avoided particular novel objects, but if placed in a completely novel situation ran around looking for a means of escape There are therefore implications both for the methods to be used in studying curiosity and also for the possible mechanism by which variety of early experience has its effect

The last speaker, Dr J M Cullen (University of Oxford), was a zoologist rather than a psychologist Accordingly he gave more attention to the natural behaviour of animals than did the other members of the symposium His particular interest was the possibility of separating different classes of behaviour as affected by one drug rather than another By this means one might ultimately disentangle the mechanism by which drugs affect behaviour The animal which he had studied was the stickleback, and the drug benactyzine

The stickleback lives in schools in the winter, but in the breeding season each male defends a territory in which it builds a nest It courts an approaching female, she lays her eggs in the nest, departs, and the male then looks after the eggs When defending the territory, the male will attack other males close to his own nest, but flee from them at greater distances The border-line between these two behaviours moves nearer to the nest under benactyzine, which suggests at first sight that fear is increased

When the female appears, drugged males less frequently court her, and are more likely to attack her than undrugged males are This is not altogether consistent with the effect on size of territory, but might perhaps be explained by supposing that sexual behaviour is even more sharply reduced than fear is On the other hand, kicking the tank in which the fish live causes them to cluster into a school, which suggests that schooling is a fear response and the drug reduces schooling rather than increases it

Feeding is reduced by frightening stimulation and also by the drug but closer examination shows that the effects are rather different In feeding, the stickleback orients towards the food, possibly several times, before each grasp at it When frightened there are fewer grasps but also fewer orientations per grasp, whereas under benactyzine there are fewer grasps with a normal number of orientations per grasp The latter type of behaviour also results simply from reduced hunger, so that the drug is in this respect apparently similar to a reduction in hunger

The common factor in all these effects might perhaps be a reduced responsiveness to visual stimulation, and in fact sticklebacks placed in a situation where a striped wall rotates around them are less responsive to this stimulation At the moment the most promising hypothesis is therefore that the drug reduces responsiveness rather than the strength of one category of behaviour, but the experiments remain an illustration of the way in which a knowledge of instinctive patterns may be used to analyse the operation of drugs

It was clear from all four papers that work on animal behaviour is developing rapidly, and that important principles are beginning to emerge

D E Broadbent

NEWS and VIEWS

Pharmacology at St. Bartholomew's Hospital Medical College: Prof. J P. Quilliam

Dr J P. Quilliam, at present reader in pharmacology in the University of London at St Bartholomew's Hospital Medical College, has been appointed to the newly created chair of pharmacology at that Medical College as from October 1. Dr Quilliam graduated with honours (physiology) in the University of London, and took his M Sc in 1938, and his M B . B S (London) in 1941. After a period of war service, in the Royal Air Force, he was appointed lecturer in pharmacology at King's College, London, and in 1946 he was appointed part-time lecturer in pharmacology at St Bartholomew's Hospital Medical College to assist in the early development and expansion of that Department In that early development he played a very active part and was appointed senior lecturer in charge of the Department and later reader in pharmacology in 1958. Dr Quilliam has built up at St Bartholomew's a very active Pharmacology Department with wide research interests and comprehensive teaching courses for medical, dental and science students He has developed a department which is particularly well equipped for the use of electro-pharmacological techniques for research in pharmacology and for the teaching of that subject He has built up a very strong and enthusiastic research team, with many postgraduate workers in his group, and he has attracted for his work important and valuable research grants from the United States His research interests are manifold and his particular interests have centred on neuromuscular and ganglionic transmission and its modification by centrally-acting drugs He has had wide experience of teaching methods, and he has spent a year as a Fellow in residence at Johns Hopkins University, Baltimore He has had much experience as an examiner in the University of London and other universities in Britain, and is the chairman of the Board of Studies in Pharmacology in the University of London He is a member of the General Optical Council He has carried out important editorial duties as an editor of the *British Journal of Pharmacology and Therapeutics*, and his own publications involve investigations on the pharmacological action of hypnotic drugs, fluorophosphonates and many other drugs

New Zealand Department of Scientific and Industrial Research: Senior Research Fellowships

The New Zealand Department of Scientific and Industrial Research has announced the award of the 1961 Senior Research Fellowships to Dr B H Howard, principal scientific officer, Rowett Research Institute, Bucksburn, Aberdeen, Scotland, and to Dr R Melville, principal scientific officer, Royal Botanic Gardens, Kew, Richmond, Surrey, England. Dr Howard's work has been concerned since 1954 with the study of the biochemistry of micro-organisms of the rumen, under Dr D P Cuthbertson, director of the Rowett Research Institute Prior to that he was studying biochemistry of micro-fungi, under Prof. H Raistrick at the London School of Hygiene and Tropical Medicine He has published several papers, and in his work under the Fellowship in New Zealand he hopes to apply techniques used in the United Kingdom to the different ruminal flora and fauna found in New Zealand ruminants Dr Melville is in charge of the Australasian Section of the Herbarium at Kew and is working mainly on Australian plants and to some extent on New Zealand plants, in particular Ranunculaceae, Epacridaceae and Rutaceae He has published several papers, in particular on *Ulmus*, and is very interested in the application of biometrical methods to taxonomy and the evolution of the angiosperm flower He will be working in the Botany Division of the New Zealand Department of Scientific and Industrial Research

Zoological Society of London : Senior Research Fellowships

Dr I W Rowlands has been appointed by the Zoological Society of London as Senior Research Fellow in reproductive physiology He will take up his appointment on January 1, 1962, and will be in charge of the Wellcome Institute of Comparative Physiology which is to be built in the Society's grounds next year For the past twelve years Dr. Rowlands has been on the staff of the Agricultural Research Council and has worked at the Institute of Animal Physiology, Babraham, Cambridge. on problems concerned with the ovarian cycle of mammals Before this, he worked at the Wellcome Veterinary Research Station, Frant, Sussex Dr Rowlands began his research career at the University College of North Wales with Prof F W Rogers Brambell and was afterwards with Dr A S Parkes at the National Institute for Medical Research in London

Research Fellowships and Studentships

Dr G H Manley, who is at present on the staff of the Department of Zoology, University of Nottingham, has been appointed to a research fellowship, and will take up his appointment on January 1, 1962, working initially with Prof A St G Huggett at St Mary's Hospital Medical School, on reproductive behaviour of the lower primates Mrs A D Woodhead, who was been appointed to a research fellowship, has already taken up her appointment, and will work at the Fisheries Laboratory, Ministry of Agriculture, Fisheries and Food, Lowestoft, on the physiology and reproduction of fish Mr D B A Symons and Mr G Glough have been appointed to research studentships

Science at the British Council ·

Mr E Bolton King

Mr E Bolton King will retire from the posts of assistant controller, Arts and Science Division (Science Group), and director, Science Department of the British Council, at the end of this year He will immediately thereafter take up the post of British Council science officer in Chile Mr Bolton King was educated at Warwick School and Balliol College, Oxford, where he was a mathematical exhibitioner and obtained first-class honours in physics finals He was elected to a research studentship at Christ Church, Oxford, and carried out research at the Clarendon Laboratory, largely on photo-electricity Later he formed the Oxford Instrument Co Early in the war he joined the Ministry of Supply Rocket Team under Sir Alwyn Crow and was engaged on rocket development, with special emphasis on proximity fuses, investigation of the performance of British and enemy rocket weapons in the Mediterranean theatre, and liaison with rocket development in the United States, where he was an assistant director of the British Commonwealth Scientific Office At the end of the War he went to Germany in connexion with the British firings of the V-2 rocket and became superintendent of the Ministry of Supply Establishment, Cuxhaven

Dr. P. A. I. Tahourdin, O B.E.

Dr P A I Tahourdin will succeed Mr Bolton King as an assistant controller, Arts and Science Division, with responsibility for various departments, including the Science Department Dr Tahourdin graduated in physics at Oxford in 1943 and afterwards obtained a Ph D after nuclear work under the late Sir Francis Simon He joined the British Council in 1946 and has served as science officer in Italy, assistant representative in Yugoslavia, and representative in Israel He will assume his new duties early in 1962

Mr F. M Beatty, M B E

Mr F M Beatty will become director of the Science Department in April 1962 He was trained as a physicist at Cambridge, taking his degree in 1940, and served during the War in the Royal Navy He joined the British Council in 1946 and has served in the Science Department at headquarters, as science officer in Poland and in Austria, assistant representative/science officer in Brazil and, since 1959, as deputy representative in Pakistan

Delegation from Academia Sinica in Britain

On the invitation of the President and Council of the Royal Society, a delegation from the Academia Sinica in Peking is visiting Britain during October16–November 2 The members of the delegation are: Dr Chu Ko-chen, vice-president and department member of Academia Sinica (meteorologist), Dr. Pei Shih-chang, department member and director of the Institute of Biophysics, Academia Sinica, Dr Wang Shou-wu, deputy director of the Institute of Semiconductors, Academia Sinica; Dr Chang P'ei-lin, deputy director of the Institute of Metals, Academia Sinica Mrs Chu is accompanying the delegation Short visits are being made to Cambridge, Edinburgh and Oxford, and Dr Chang is also visiting Sheffield. Visits will be made to university scientific departments, the National Physical Laboratory, the National Chemical Laboratory and other scientific institutes On October 19 a special presentation and lecture on "Astronomy in Classical China" was arranged at the London Planetarium

The Wellcome Trust: Grants for Research on Tropical Anæmias

The Wellcome Trustees are to provide up to £100,000 to assist an integrated programme of research by several groups of workers into the severe anæmias prevalent in tropical countries, with special reference to the malabsorption syndrome and tropical sprue The Trustees already support work in this field by Dr Henry Foy and Dr Athena Kondi at the Medical Research Laboratories, Nairobi, Kenya, and Dr Selwyn Baker and others at the Wellcome Sprue Research Unit at the Christian Medical College, Vellore, South India Under the new arrangements the work of both teams will be associated with that of a new Wellcome Laboratory of Tropical Hæmatology which is to be established in Prof J V. Dacie's department at the Postgraduate Medical School of London, Hammersmith The total allocation of £100,000 for the work on tropical anæmias and kindred disorders is made up as follows £45,000 to build and equip the Wellcome Laboratory of Tropical Hæmatology at Hammersmith, £14,000 to organize and maintain a scheme of close co-operation between the groups led by Dr Foy, Dr Baker, Dr D L Mollin of the Hammersmith Laboratory, and a Royal Army Medical Corps team in Singapore, £25,000 for the continued support of Dr Baker's unit in Vellore, £15,000 for new X-ray equipment at Vellore

Awards in the United Kingdom and the United States

OTHER major grants in aid of research out of the total of £248.00 distributed by the Trust during the past half-year included £40,000 to Prof H O Schild and others for the extension to the Pharmacology Building at University College, London, £15,000 to Prof J D Lever to purchase an electron microscope for the Department of Anatomy, University College of South Wales and Monmouthshire, Cardiff, £13.500 to Prof D H R Barton for the purchase of a nuclear magnetic resonance spectrometer for the Department of Organic Chemistry, Imperial College of Science and Technology, £10,500 to Dr D P Cuthbertson for the purchase of new radiographic equipment for the Rowett Research Institute, Aberdeen, £10,000 to Prof H E de Wardener, Department of Medicine at the Fulham branch of Charing Cross Hospital, £9,850 to Prof F W Rogers Brambell to increase the accommodation for post-graduate research workers in the Department of Zoology, University College of North Wales, Bangor. £4,000 to Dr A H Harthoorn, to provide a mobile laboratory for field research in comparative physiology, University College of East Africa, Nairobi A five-year senior Wellcome research fellowship has been provided for Dr Karlis Adamsons in the Columbia-Presbyterian Medical Center, New York City, at a cost of 50,000 dollars His work will be concerned chiefly with the physiology of the new-born

New Member of Trust

THE Wellcome Trust announces that Mr Lancelot Claude Bullock, who was appointed a trustee under the will of the late Sir Henry S Wellcome, has retired. For many years he was senior partner in the London firm of solicitors, Markby, Stewart and Wadesons, where his associations with Sir Henry Wellcome go back to the time he was asked to advise and draft the will under which the Trust was created He is succeeded by Mr Robert Malleson Nesbitt, also of Markby, Stewart and Wadesons

The State and the Freedom of the University

PROF M BOUCHARD, writing in *Science and Freedom* (No 19, June 1961) on "The Universities in France Freedom and Autonomy", expresses some concern at the effect on university autonomy of the growing dependence of the universities on State support While the maintenance of university freedom depends in the last resort on the moderation and liberal outlook of the State, Prof Bouchard is no less concerned that the individual members of the universities must be allowed full scope for their inquiries and research in whatever field they please, without let or hindrance They must be free to form their own conclusions and say what they believe to be true In France, university teachers have the status of Civil servants and are protected from all arbitrary measures by a special legal clause which offers security so long as the spirit of independence is kept alive in the universities, and Prof Bouchard pleads that care should be given to the preservation of this vital principle To the same issue, Prof L Massart contributes a note on the problems and prospects of the Belgian universities Pointing out that the appointments of teaching staff are not immune from political influence, he stresses, however, that the real problems of the universities in Belgium arise out of their growing dependence on State support and the increasing number of students He is disturbed at the persistence of obsolete methods of teaching, the failure to increase the number of students trained for research commensurately with the national needs and the effects of inbreeding from which the university system suffers. He believes that a thorough reappraisal of the university structure in Belgium is required While the number of students has increased from 10,307 in 1938–39 to 26,057 in 1958–59, and the number of graduates from 1,995 to 4,930 over the same period, the number of doctorates has only risen from 79 to 120, a 40 per cent drop in the rate per 100 graduates

The Berkshire, Buckinghamshire and Oxfordshire Naturalists' Trust, Ltd

THE first annual report of the Berkshire, Buckinghamshire and Oxfordshire Naturalists' Trust, Ltd, which was incorporated as a limited company on January 9, 1961, covers the year 1960 (Pp 7 Kennington, Oxford Berkshire, Buckinghamshire and Oxfordshire Trust, Ltd, 146*a* Poplar Grove, 1961) At its incorporation the Trust had 328 members, and the major task of the Provisional Executive Committee during the first year was the preparation of a draft constitution which was approved at a meeting of members on November 12, 1960 The objects of the Trust, as set out in its memorandum of association, are to record and investigate places and objects of botanical, zoological, geological, archæological or scientific interest, or of natural beauty, and to protect these from injury, ill-treatment or destruction, to establish, form, own, maintain and manage nature reserves or bird sanctuaries, to encourage the breeding of wild-birds, insects and plants which are harmless, useful, beautiful or rare, and to promote, organize, carry on and encourage study and research for advancing knowledge in the natural sciences The Trust has already been concerned with threats to several important sites In December 1959 it joined with the Nature Conservancy and other bodies in successfully resisting the proposal to build a 250-ft radio tower in Little Lenham Wood, near Beacon Hill It has also joined with the Banbury Ornithological Society in asking for the preservation of certain woods in that neighbourhood if further large-scale ironstone working is authorized, and has also sought to preserve the heronry at Chievsley Furze, the famous crocus field at Inkpen, Berkshire, and the sites on which the military and monkey orchids, the red helleborine and the wild fritillarias grow

Museums Journal

THE second issue of the new format of the quarterly *Museums Journal* (61, No 2, September 1961) maintains the high standard and deals with a variety of topics Mr F I. G Rawlins emphasizes the value of the scientific outlook in conservation, Mr Philip Rawson describes the new Gulbenkian Museum in the University of Durham and Mr Robert Hill deals the various types of electronic lecture guide systems which are now proving of such absorbing interest to museum curators Much of this issue is devoted to a record of the annual conference of the Museums Association held at Plymouth in June 1961 A report on the symposium held at the British Museum (Natural History) on "The Museum and the Naturalist", and book reviews, complete an informative issue

The Bernice P. Bishop Museum, Honolulu

Under the title of "Work Half Done" the Bernice P Bishop Museum at Honolulu issues its report for 1960 The title refers to the fact that at the end of 1960 the Museum was in the middle of a particularly important project, the new Planetarium-Observatory. This ambitious building is being constructed without the necessary total of capital funds but with the conviction that these will be forthcoming when the value of the venture is demonstrated An interesting experiment was involved in the trapping of air-borne insects to determine the role of air currents in insect dispersal This was carried out both on board ship and by a special device in aeroplanes

The Stanley Clifford Milk Industry Award

The Central Milk Distributive Committee, in recognition of the valuable services rendered in the dairy industry by Stanley Clifford, who was for many years chairman of the Committee, has established a fund for the purpose of providing awards to enable a suitable applicant to make a short study tour of the dairying industry in the United Kingdom or abroad Employees in the dairy industry or in dairy engineering in the United Kingdom who are below the age of thirty are eligible The Award, which will be made annually, will be administered by the Society of Dairy Technology. It has been decided that the first award shall be granted for a study tour in Denmark immediately following the International Dairy Congress which is to be held in Copenhagen during September 3–7, 1962 Applications for this Award should be made to the Secretary, Society of Dairy Technology, 17 Devonshire Street, London, W 1, not later than January 31, 1962

Royal Institute of Chemistry Lecture Series

The first three numbers of the Royal Institute of Chemistry 1961 series of lectures have just been published (The Royal Institute of Chemistry Lecture Series 1961 London Royal Institute of Chemistry, 1961 5*s* each) The first, entitled *Atmospheric Photochemistry*, is by Sir Harrie Massey and Dr A E Potter, the second, *A Chemist's Introduction to Statistics, Theory of Error and Design of Experiment*, by Dr D A Pantony, and the third, *Recent Developments in Polarography*, is by Dr G W C Milner The booklets also contain a list of other recent publications of the Institute

The Night Sky in November

New moon occurs on Nov 8d 09h 59m U T and full moon on Nov 22d 09h 44m The following conjunctions with the Moon take place Nov 2d 05h, Regulus 0 01° N, Nov 13d 13h, Saturn 3° S, Nov 14d 00h, Jupiter 3° S, Nov 23d 03h, Aldebaran 0 7° S, Nov 29d 13h, Regulus 0 3° S In addition to these conjunctions with the Moon, Venus is in conjunction with Spica on Nov 4d 17h, Venus being 4° N Mercury is a morning star, visible low in the south-east before sunrise, on November 7 it rises almost two hours before the Sun Venus is a morning star, rising at 4h 50m, 5h 30m and 6h 20m on November 1, 15 and 30, respectively It is rather too close to the Sun for convenient observation Mars is too close to the Sun for observation Jupiter and Saturn are evening stars, setting about four hours after the Sun They may be seen low in the south-west after sunset, they are not very favourably situated for observation Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich Nov. 20d 16h 56·9m, ξ^2 Cet (*D*); Nov 21d 2h 22 9m, μ Cet (*D*), Nov 23d 2h 52 8m, α Tau (*D*), Nov 23d 3h 44 3m, α Tau (*R*), Nov 24d 4h 23 5m, 119 Tau (*R*) Nov 24d 5h. 07 4m, 120 Tau (*R*), Nov. 24d 21h 14 4m, 71 Ori (*R*); Nov 27d 3h 56 7m, ζ Cnc (*R*), *D* and *R* refer to disappearance and reappearance, respectively The Taurid meteors are active during the first half of the month, maximum activity being on November 7 The radiant is at R A 3h 36m, Dec + 14°, and conditions for observation are favourable The Leonid meteors are active on November 16, the radiant being at R A 10h 08m, Dec + 22°, and conditions for observation are favourable

Announcements

Prof W T Williams, professor of botany at the University of Southampton, has been appointed a member of the Agricultural Research Council in succession to Prof S J Watson, who has retired after ten years service

The one-hundred-and-twenty-eighth meeting of the American Association for the Advancement of Science will be held in Denver, Colorado, during December 26–30 Further information can be obtained from the director of public information, Sidney S Negus, Medical College of Virginia, Richmond, Virginia

An international conference on "Magnetic and Electric Resonance and Relaxation" is to be held at Eindhoven, The Netherlands, in July 1962, under the auspices of the Netherlands Physical Society and the *Groupement Ampère* Further information can be obtained from Dr D J Kroon, Philips Research Laboratories, Eindhoven, Netherlands

The second International Symposium on Continuous Culture of Micro-organisms will be held in Prague during June 18–23, 1962. It is being sponsored by the Czechoslovak Academy of Science Further information can be obtained from Academician Ivan Málek, Czechoslovak Academy of Science, Institute of Biology, Na cvičišti 2, Prague 6, Czechoslovakia

Messrs Macmillan regret that an incorrect price for Volume II of Partington's "History of Chemistry" was given in *Nature* of October 14, p lxxxix, the volume is being published at £5 5*s*. net

Errata Dr D H Trollope has written stating that some omissions and misprints have occurred in the communication entitled "Effective Contact Stresses and Friction" published in *Nature* of July 22, p. 376 Equation 7 should read $R = \frac{W}{N} - ua + \rho a$, equation 8 should read $F_j = \mu\left(\frac{W}{N} - ua + \rho a\right) = \mu\left(\frac{W}{N} - u\frac{A}{N} + \rho\frac{A}{N}\right)$, equation 9 should read $S = \mu(\sigma - u + \rho)$ On p 376, col 2, line 41, for 'macro-fraction' read 'micro friction', line 56, for 'invert' read 'inert'; line 57, for $\mu = 0$ read $u = 0$

SCIENTISTS IN THE PUBLIC SERVICE

SPECIAL PROMOTIONS

As in previous years a number of special posts has been created in the Scientific Civil Service under the provisions included in the White Paper on the Scientific Civil Service (Cmd 6679, 1945) to provide for the promotion of individual research workers of exceptional merit The promotions were effective from July 1 and include the following ·

Deputy Chief Scientific Officers

Dr L J Bellamy, who is superintendent of Analytical Services at the Ministry of Aviation's Explosives Research and Development Establishment, Waltham Abbey, entered Government service in 1939 and undertook various analytical jobs until he was posted to the Directorate of Chemical Inspection, Woolwich, in 1945 Here he became responsible for the development of new methods of analysis, particularly physical methods, and became a member of the World Health Organization Expert Advisory Committee on Insecticides In 1955 he was transferred to his present post, where he has been responsible for the development of new analytical methods, research and assessment on the stability and compatibility of explosives, X-ray and electron microscopy techniques and polymer synthesis and properties Dr Bellamy's major personal contributions have been in the fields of the interpretation of the spectra of large molecules, solvent effects, and more recently on the difference in the associative properties of protons and deuterons. On the interpretation of spectra of large molecules, Dr Bellamy has developed a broad theory, whereby the behaviour of the more important group frequencies can be predicted and understood This has revealed new relationships between frequency shifts and other physical properties On solvent effects, his work has led to a better understanding of inter-molecular forces in solution

Dr. A F Gibson is leader of the Semiconductor Physics Research Division at the Royal Radar Establishment of the Ministry of Aviation at Malvern, and has been associated with that establishment since 1944 Dr. Gibson's work has included luminescence, infra-red photo conductors, transistor physics and research of a fundamental character on the properties of semiconductors He has applied his research to practical needs and has been responsible for the development of a new form of far infra-red and millimetre wave detector, based on cyclotron resonance and an avalanche injection diode of value in fast-switching circuits He has carried out investigations of the microwave properties of germanium and the effect of carrier injection and extraction on the absorption. Dr Gibson's most recent work has been concerned with 'hot' electrons in germanium, which has shown the complex nature of the processes whereby energy given to electrons in a semiconductor by an electric field is exchanged with the lattice to give ohmic heating and how, when this cannot be done sufficiently rapidly, the energy is spread unequally among the various 'valleys' in momentum space Similar investigations carried out with 'holes' have led to a better understanding of the band structure of the complex valency band of germanium

Senior Principal Scientific Officers

Dr J M Burch, of the National Physical Laboratory, specializes in metrological optics, particularly in the production and application of photographic gratings These gratings are of two kinds large photographs of very closely spaced interference fringes for spectroscopic purposes, and gratings for linear and radial measurement based on the improvement of conventional rulings by methods of 'photographic averaging' Gratings, read by optical and electronic methods to which Burch has also made important contributions, are now being extensively used in the control of machine tools and in metrology Dr Burch has recently taken charge of a research project concerned with the development and application of optical masers

Prof J C Cooke, one-time professor of applied mathematics in the University of Malaya, is now working on fluid dynamic theory in the Aerodynamics Department at the Royal Aircraft Establishment, Farnborough

Dr. D A L Davies, attached to the Immuno-Chemistry Section of the War Department's Microbiological Research Establishment at Porton, is concerned with work on the isolation and structure of bacterial antigens. His work has laid the basis for a chemical and immunological approach to tissue-cell antigens

Mr D Firth is now in charge of the work of the Fluid Power Section of the National Engineering Laboratory concerned with research into oil hydraulic and pneumatic devices for general industrial use Earlier this year Mr Firth won the Wolfe Award for his work on hydrostatic transmissions.

Mr J F Gittins, of the Services Electronics Research Laboratory Extension, run by the Admiralty at Harlow, has undertaken research in the field of high-power microwave valves, particularly of the travelling-wave variety This has led to the use of the travelling-wave tube as an effective high-power amplifier at microwave frequencies Mr Gittins is also chairman of the Power Valve Research Advisory Panel of the Co-ordination of Valve Development Committee

Mr B E J Pagel is in charge of the Astrophysics Department at the Royal Greenwich Observatory In addition to carrying out theoretical and observational research Mr Pagel has also assisted in the design of new apparatus and introduced improved techniques

Dr G B Preston, of the Royal Military College of Science, Shrivenham, has been engaged in research in the field of pure mathematics, particularly in the theory of semi-groups

Dr J T Stuart, of the Aerodynamics Division, National Physical Laboratory, has made contributions to the study of the stability of fluid flow Of particular significance is his research work into the non-linear theory of hydrodynamic instability, the growth of wave disturbances and the effects of Reynolds stresses on transition from laminar to turbulent flow

Dr R H Wood is in charge of that part of the Building Research Station research programme that deals with structural engineering design problems; his particular interest is the structural interaction between the various elements of construction in multi-storey framed buildings. This interaction, or mutual stiffening of the parts of a building so as to strengthen the whole, has an important influence on building design, and Dr. Wood's work has contributed greatly to the understanding of the structural behaviour of complete buildings and to the more efficient allowance for composite action in structural design.

Similar promotions have been made by:

(1) United Kingdom Atomic Energy Authority: Senior Principal Scientific Officers

Mr. J. C. Martin has worked at the Atomic Weapons Research Establishment, Aldermaston, since its foundation in 1953. His early work was concerned first with small-scale explosives research, investigating the motion of fluid surfaces and later with the development and use of scintillation counters. More recently he has worked on neutron sources and on the development and application of sub-microsecond radiographic techniques for the experimental work on explosions. Starting with an early prototype 35-MeV. linear accelerator for which he developed a double focusing system, he has brought it to a state of output and reliability which has made it a valuable tool in the solution of certain hydrodynamic problems. He is now attempting to extend the flash X-ray technique from its present limit in the region of 1 MV. up to about 6 MV. He has contributed a number of original ideas in the field of advanced nuclear and thermo-nuclear weapons.

Dr. J. B. Taylor has worked at the Atomic Weapons Research Establishment, Aldermaston, for six years. He contributed first to the improvement of neutronic calculations used in the theory of explosions and in the computation of the stochastic behaviour of small populations in a supercritical system. He then carried out a number of investigations, ranging over several fields and at all levels of detail, to illuminate and help to direct the astrophysical engineering behind the megaton test series of 1957–58. He later worked on problems connected with fast reactors and on electromagnetic phenomena from explosions, before turning to problems of the thetatron and other controlled thermonuclear reactors.

(2) Agricultural Research Council: Deputy Chief Scientific Officer

Dr. R. Markham joined the staff of the Plant Virus Research Station, Cambridge, now the Agricultural Research Council Virus Research Unit, in 1940 and became director of the Unit in 1960. His research has been concerned mainly with the biochemistry of the smaller plant viruses, and in 1949 he was able to demonstrate that the ribonucleic acid of the turnip yellow mosaic virus, which he and Dr. Kenneth Smith first isolated and purified, was necessary for the infectivity of the virus and was enclosed in a protective shell of protein. This work led to an intensive investigation of nucleic acids in general and resulted in the development of a large number of delicate methods for the analysis of nucleic acids and their constituents. It also led to an understanding of the way in which some of the enzymes which degrade nucleic acids, particularly pancreatic ribonuclease, act. Dr. Markham has also been concerned with synthetic mechanisms by which small polynucleotides can be made. He was elected a Fellow of the Royal Society in 1956.

Senior Principal Scientific Officers

Dr. C. H. Cadman is head of the Virology Department, Scottish Horticultural Research Institute, Mylnefield, Dundee, and is at present particularly concerned with the transmission of plant viruses by nematodes.

Dr. B. **Kassanis**, of the Plant Pathology Department, Rothamsted Experimental Station, has discovered and described a number of viruses, and recently discovered that the multiplication of the virus depends on the simultaneous multiplication of another.

Dr. A. **Kleczkowski**, of the Plant Pathology Department, Rothamsted Experimental Station, is primarily a serologist, but has made substantial contributions to a range of subjects, and is now studying the surface structure of the protein units which make up tobacco mosaic virus.

THE ROYAL SCOTTISH MUSEUM

By Dr. D. A. ALLAN, C.B.E.

Formerly Director

THE foundation stone of the Royal Scottish Museum was laid by the Prince Consort in October 1861, and thereafter there arose in instalments the Venetian Renaissance building which now occupies more than half of Chambers Street, west of the Old College, University of Edinburgh. It is a grey stone structure consisting of a central block elongated east and west, with two wings stretching southwards. The area within was afterwards built on to yield a series of rectangular halls, most of them with a main ground-floor surmounted by two balconies, the earlier ones supported on cast-iron pillars, the later either cantilevered or upheld by ferro-concrete structures. A 'grand staircase' was added at the rear in 1931, with arches at its mezzanine landings to lead to a prospective future block. A lecture hall had been included in the earliest part of the building but was later sacrificed to yield much-needed exhibition space, yet the necessity for a hall for lectures and demonstrations and later to provide educative film shows was ever present, and just before the Second World War foundations were dug beyond the 'grand staircase' for a rectangular lecture hall . . . and were left to be obliterated by ashes from the boilers. In May 1959 work was commenced on a theatre block of an entirely novel design, and its completion was achieved early in September 1961, the opening ceremony being performed by the Right Hon. the Earl of Rosse, chairman of the Standing Commission on Museums and Galleries, with the Right Hon. John Scott Maclay, H.M. Secretary of State for Scotland, in the chair.

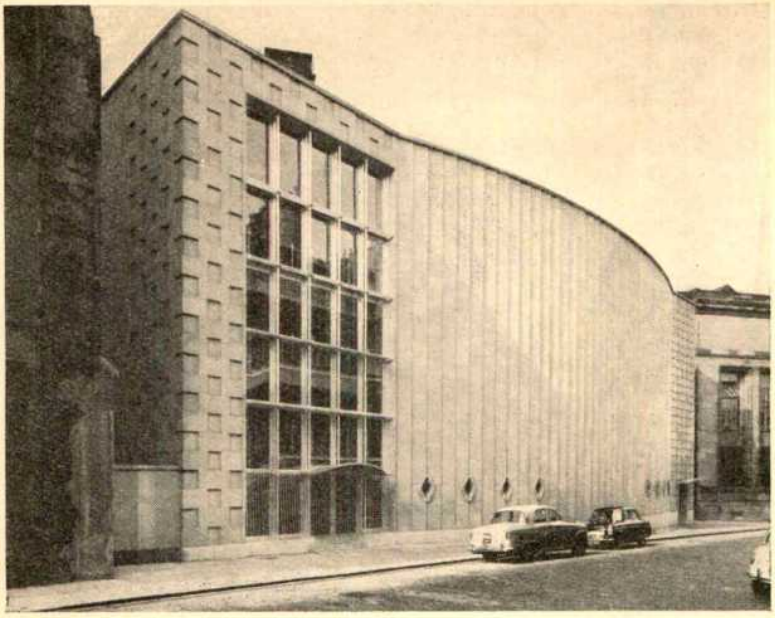

Fig. 1. The Royal Scottish Museum, New Theatre Block

The site was a difficult one, being a triangle hemmed in by part of the back of the old building, a busy street and a little cul-de-sac. Again, whatever occupied it had to marry with a 1914 red sandstone somewhat classical façade of the Museum zoological galleries beyond and the grey sandstone of the Robert Adam Old College building further on.

The principal contribution being a lecture theatre, it had to be given the lion's share of the space, and the angle of the seating floor was determined by the number of persons to be accommodated. As the Museum's opening ceremonies attract around 200 guests—and the early 1900 public lectures, organized by the Museum but housed in the adjacent University examination hall, as many as 250—it was determined that the new theatre should hold 300. The plans allowed for this number of seats in ten curving rows of thirty, rising steeply on steps, with three aisles. The platform is low, long and narrow, so that everyone can have an uninterrupted view of speaker, demonstrations or of the screen occupying the central portion of the wall beyond. The back of the auditorium is against a solid bow-shaped, windowless street wall, impervious to light and sound. At the narrowing end of the triangle is an emergency staircase, with a side door at street-level. The wider end of the triangle is a three-storeyed block, each floor consisting of a curving chamber running from the street back to the older part of the building. The ground-floor is a foyer with a door to the street, another to the main museum building and the double sound-proof doors to the front of the auditorium. Above are two more floors, reached by an open staircase of steps cantilevered on a single supporting beam, with landings against an immense window rising the height of the building and facing the street. Because of differences in level, the foyer is broken across the middle and a short flight of curving steps leads from the new end to the old. The top-floor gallery opens on to an extensive exhibition hall over the lecture theatre, top lit and with an additional exit to the top of the emergency staircase. The proportions of the building can be put briefly and roughly—two-thirds width and two-thirds height for the lecture theatre, with the remaining third at the top exhibition hall and the remaining third at the side foyer with two overlying exhibition rooms. Underneath is a large cellar space, which will later be developed to advantage for much-needed storage. In contrast to the prevalence of right angles throughout the many halls of the old building, those in the new are limited to the contacts between floors and walls and walls and ceilings.

The lecture hall, which will be used for opening ceremonies connected with special exhibitions in the adjacent halls, conferences, lectures, school-class talks and cinema shows, presents a novel, modern and entirely charming interior, with its major sweep across its width, and the steeply tiered rows of tip-up chairs, upholstered in grey plasticized fabric, flanked and backed by walls lined to the full height with *Abura* wooden strips of a warm brown colour. The ceiling, which is also acoustically designed, presents a pattern of shallow rectangles in relief to the audience but projects a battery of lights from the unseen forward and downward projecting ends on to the platform. The lighting can be controlled either from the stage or from the projection room in the rear of

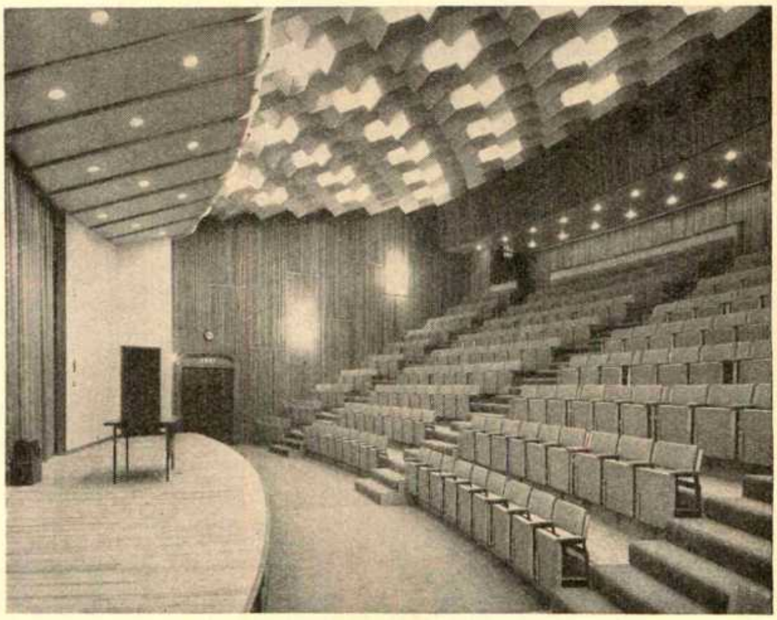

Fig. 2. New Lecture Theatre, Royal Scottish Museum

the ceiling. The tiers and access gallery at the back are covered in grey marbled plastic flooring, with gold-coloured carpet on the three access stairways and blue carpet fronting the stage, the curtains of which are in gold. Hardwood strip has been used in the floors of the exhibition halls and travertine for the foyer. The 'flying' staircase is finished with hardwood treads with rubber inserts and aluminium; plate glass and plastic have been used for the balustrade. The architect has designed an appropriately carved teak door for the emergency exit. The designs begin at the base with early forms of life and fossils, pass up to various animals and man, show locomotives, telephones and television sets, and lead to a top row of aeroplanes, sputniks and an atomic explosion. The 'finale' is a row of question marks.

The exhibition halls are heated by low-pressure water coils contained within the floor. Background heating for the theatre is provided by coils behind the stage front and this will be supplemented when the hall is in use by warmed air introduced through the slats on the walls near the stage and extracted at the ceiling below the projection room. The 16-mm. projector is sited in a special room reached by a passage above and behind the auditorium. The building could well have taken a full-size projector, but in view of the difficulty of getting documentary films suitable for educational purposes, the smaller size had to be chosen.

The façade of the new extension is spectacular and modern, yet it provides a pleasing gradation with its neighbours. The bastion-like central mass consists of plain ribs of cream-coloured sandstone, reminiscent of a clinker-built boat, and standing on a simple polished granite base. In the lower portion of the sandstone are a few ovoid windows.

The curved central mass is flanked by two unequal flat walls, that to the east being smaller and having its square sandstone blocks split into two facets angled diagonally, so as to yield an attractive shadow pattern. The greater flank to the west is framed in square sandstone blocks with alternate ones set back to provide a different pattern, but the main face is a rectangular aluminium frame with a slightly arched doorway in the centre surmounted by five tiers of plain glass panes, the horizontal crossings marked by narrow bars which combine decoration with utility in supporting window cleaners. Through the glass, the stair landings add a decorative motif. The architect was Stewart Sim, a senior architect of H.M. Ministry of Works, and the contract was carried out by Thaw and Campbell, Ltd., Glasgow, at a cost of approximately £120,000. Thus the Royal Scottish Museum displays the march of time in its architecture and its furnishings—in its exhibits as well—over a full century.

CANCER RESEARCH IN THE UNIVERSITY OF PERUGIA MEDICAL SCHOOL

By Prof. LUCIO SEVERI
Director

On June 26, the occasion of the centenary celebrations of the chair of morbid anatomy of the University of Perugia, Dr. Thelma B. Dunn, head of the Cancer Induction and Pathogenesis Section, National Cancer Institute (U.S.A.), opened at Monteluce an extension to the Division of Cancer Research, University of Perugia Medical School.

The ceremony included a visit to the new animal-house and the remodelled older one, on the main floor, in which normal work was in progress. The guests consisted essentially of the participants of the international conference on the "Morphological Precursors of Cancer". At the opening of the extension, all the doctors *honoris causa* of the University of Perugia Medical School were present, including Prof. A. Haddow, director of the Chester Beatty Research Institute, London; Prof. J. W. Orr, director of the Department of Pathology and Cancer Research Laboratories, University of Birmingham; Prof. J. J. Bittner, Division of Cancer Biology, University of Minnesota, Minneapolis; Dr. L. C. Strong, director of the Biological Section, Roswell Park Memorial Institute, Springville; Dr. J. R. Heller, president of the Memorial Sloan–Kettering Cancer Center, New York; Prof. C. Krauspe, director of the Institute of Morbid Anatomy, University of Hamburg; Prof. V. R. Khanolkar, vice-chancellor of the University of Bombay; and Prof. T. Yoshida, dean of the Faculty of Medicine, University of Tokyo.

The extension was designed by Prof. G. Nicolosi, Rome, and the work was carried out under the direction of Dr. G. Castori, Perugia, being erected by Messrs. Fioroni, Perugia, at a cost of 50,000,000 lire. The new accommodation doubles the previous floor area and makes the Division the largest and best equipped organization of its kind in Italy.

The extension consists of four storeys of concrete-cased steel-framed structure, faced with grey-coloured plaster. The various floors are formed with pre-cast concrete flooring units. The various services to the laboratories, which include hot and cold water, gas, electricity, compressed air, vacuum and steam, are visible, not concealed. In this way easy access is possible to the various services and pipe-lines when it is desired. Each floor is served by an automatic electrically operated passenger lift. The new building is linked with the older Division of Cancer Research by an underground passage, and contains extensive animal-house accommodation and laboratories for virology, tissue culture and cytogenetics.

The main financial responsibility of the Division of Cancer Research has always been borne by the University of Perugia. Additional funds are provided by the National Cancer Institute of the U.S. National Institutes of Health, and to a lesser, but valued, extent from the Lega Italiana per la Lotta contro i Tumori, Consiglio Nazionale delle Ricerche, Farmitalia Milano, and from other private Italian benefactions.

It is only since the Second World War that the idea of a Division of Cancer Research in the University of Perugia was conceived. But it was several years before the plan could be carried out. On November 30, 1952, the Minister of Public Education, on the occasion of the opening of the academic year, announced the establishment of a University Division of Cancer Research at Perugia. In the first building were established an animal

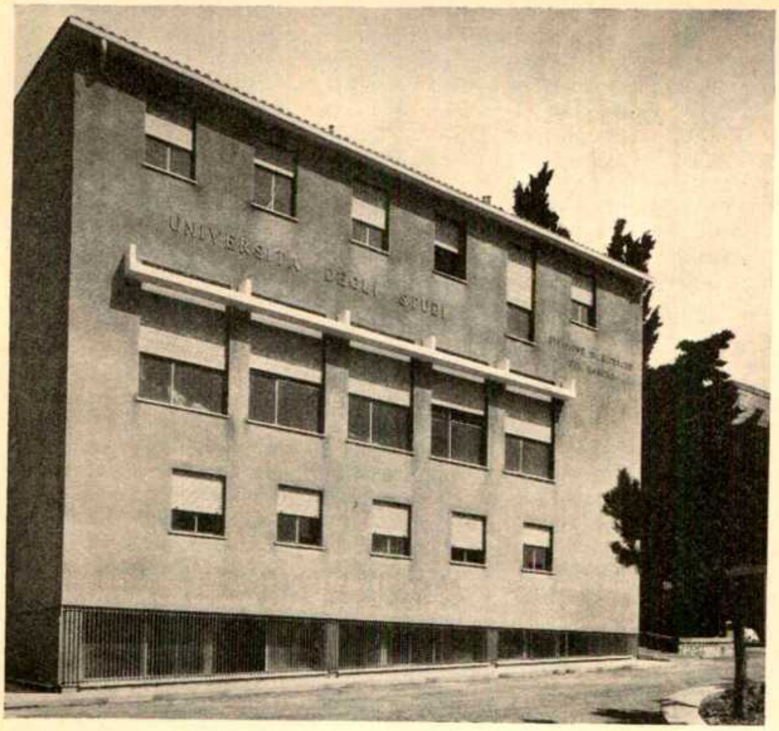

Fig. 1. New Extension of the Division of Cancer Research, University of Perugia Medical School

colony and laboratories for research in biology and pathology. Later, in the years 1956 and 1957, a programme of improvement was completed with the aid of grants and contributions.

During recent years, investigations have been carried out in the Division by various groups of scientists on different aspects of cancer research. Work on the spreading of metastases in human and experimental tumours has been described in a series of papers dealing with the various facets of the problem. A valuable contribution to the conflicting problems of the morphological precursors of human breast cancer was made by the staff of the Division of Cancer Research working as a unit. In the initial phase, different types of mammary cancer, arising from different lesions, and characterized by a different age at onset, ætiological pattern and morphology were detected and classified. It was then shown that the frequency and the latency period were also different for each of these types of cancer. On this basis, an attempt was made to determine the order of importance of the respective morphological precursors; this is still regarded as a useful factor for prognosis.

In recent years, much effort has been transferred to the study of causation, behaviour and evolution of mammary tumours in inbred mice. The origins, nature, fate and hormonal control of the hyperplastic alveolar nodules in the mammary glands of virgin and breeding animals carrying the milk factor have been analysed in a series of papers. After the discovery of a new kind of pre-malignant change, the plaque, in hybrid animals, various inbred strains of mice normally bred were investigated to determine the occurrence of plaques. These have been found in two of the three strains investigated. In connexion with these experiments, an analysis of responsiveness and progression of mammary tumours of inbred mice was carried out, which led to the detection of the existence of strain differences in the biological and morphological characters of neoplasms. Finally, a recent extension of these investigations has shown that the strain differences found might be due to differences in the properties of the respective milk factors.

A new field of investigation, cytology, opened at the Division of Cancer Research with the conversion of a spontaneous mammary tumour into an ascites tumour. Work on the mitotic rate and chromosomal pattern of this ascites tumour is now in progress.

Recently, it was demonstrated that isoniazid, a drug very widely used in the management of tuberculosis, is carcinogenic for the lungs of *BALB/c* mice. Due to its practical importance, this field of investigation has been rapidly extended in order to see whether isoniazid is carcinogenic to other species of animals, whether it is carcinogenic in itself or through some of its metabolites, and how this carcinogenic action may be prevented.

Since its foundation, the Division of Cancer Research has encouraged, within the limits of its facilities, visits of qualified scientists from foreign countries, in order to exchange information and ideas on the work in progress. A large number of such visitors have come during recent years. Several of them delivered lectures. For others, small seminars were organized with the staff. Other visiting scientists have spent several months working at the Division of Cancer Research with members of the staff, in fields of common interest.

THE INTERNATIONAL FEDERATION FOR DOCUMENTATION

THE twenty-seventh conference of the International Federation for Documentation was held at Caxton Hall, London, during September 6–16. The Federation has its headquarters at The Hague, and its work on many different problems of documentation is carried on mainly through correspondence, committees and meetings of the Executive Bureau and a General Assembly held each year in a different country. The large, unrestricted, conference gatherings take place only every three or four years and this was the first to be held in England since 1938. It was attended by more than 500 delegates coming from 38 countries and from all five continents. Aslib, as the British representative body in the Federation, was responsible for the arrangements

The earlier part of the conference, that is, during September 6–11, was devoted to meetings of committees, the Executive Bureau, and the General Assembly with a special session on September 9 to consider present-day systems and trends in automatic documentation such as the use of electronic computers and research into the possibilities of mechanical translation Excursions to Oxford and Cambridge were arranged for September 10

During the second week of the conference, an exhibition of new processes and experimental work in documentation was open, and included a device for the automatic identification of printed numerals demonstrated by Dr M B Clowes and Mr J R Parks of the National Physical Laboratory

The official opening of the full conference took place on September 12 when the Baroness Wootton of Abinger, deputizing for Lord Hailsham, Minister for Science, gave the opening address The chairman, Dr Burton W Adkinson, head of the Office of Science Information, U S National Science Foundation, referred to the great loss to the Federation through the recent death of Fritz Donker Duyvis His passing had been "the quiet closing of a door on an age", the age of the pioneers of documentation such as Otlet and La Fontaine, and of the devoted amateurs who followed them such as "D D " who, for more than two decades, was the embodiment of the International Federation for Documentation After this tribute to the late secretary-general of the Federation, the meeting heard four papers describing recent developments in documentation These were presented by Dr K Schneider (Germany), who described the use of electric typewriters operated by punched cards, Mr A C van Schaik (Netherlands), who showed that advantages could come through bibliographical co-operation within a country, Mr C W Cleverdon (United Kingdom), who outlined the work of the Aslib Cranfield investigation into the efficiency of indexing systems, and Mr Karl F Heumann (United States), who presented Verner W Clapp's summary of recent important progress in documentation in the United States In the evening delegates attended a reception given by the Vice-Chancellor of the University of London and Mrs Noble at the Senate House

The morning sessions of September 13 dealt with scientific and technical information under the chairmanship of Dr M A T Rogers, research controller, Imperial Chemical Industries, Ltd , deputizing for Dr T E Allibone, who was indisposed Papers were contributed by Mr R G l'Hermite (France), Dr A Moore (United States), who dealt particularly with the problem of information in the pharmaceutical industry, and Dr Y Niwa, who described the history and growth of the Japan Information Centre of Science and Technology and similar services in his country

The afternoon session was presided over by Mr G Deurinck, of the Fondation Industrie-Université pour le Perfectionnement des Dirigeants d'Enterprises, Belgium The papers covered the documentation of management (Prof R W Revans, United Kingdom, and Prof J L Mey, Netherlands), and the use of information for economic planning (Mr K Havlíček, Czechoslovakia) In the evening delegates were invited to a reception given by Imperial Chemical Industries, Ltd , at Imperial Chemical House, Millbank

The efficient use of information was the theme of the morning session for September 14 when the chair was again taken by Dr Burton W Adkinson Mr R. E McBurney described the work of the Canadian Technical Information Service of the National Research Council Mr K Klintoe, director of the Danish Technical Information Service, spoke on productivity in information service, Mr William T Knox gave an interesting talk on the use by some United States companies of senior scientists or engineers as information research workers, and Mr J de Laclémandière (France) set out his views on information and the 'law of least effort' The afternoon was left free for visits and other activities and in the evening a reception was held by H M Government in the unique setting of the King's Library, British Museum, when Lord and Lady Hailsham received the guests

The final public session and the official closure of the conference took place on the afternoon of September 15, with Dr Alexander King, president of the Federation. in the chair The General Assembly this year had been attended by representatives of twenty-four member countries and by observers from several other regions Dr King welcomed the presence of Miss Hilda S Lindstedt, formerly librarian of the Royal Technical University, Stockholm, and now an honorary member of the Federation Dr King went on to say that the decision to break away from tradition and to hold the 1960 meetings at Rio de Janeiro had had important consequences for the International Federation for Documentation, including some changes in constitutional procedure In future, presidents would be elected a year in advance of the date on which they would take office and retiring presidents would serve an additional year as members of the Executive Bureau, all this made for continuity

Dr King announced that Dr Burton W Adkinson would succeed him as president in 1962 Vice-presidents were working appointments chosen on a regional basis The vice-presidents for Western Europe were now Miss Barbara Kyle (United Kingdom), Dr Z Paulo (Portugal), Dr W Schurmeyer (Germany) and Prof E Wegelius (Finland). East European vice-presidents were Dr L Janzky (Hungary) and Prof A I Mikhailov (U S S R) Dr S R Ranganathan (India) had retired and his place had not yet been filled, but Far Eastern representation was in the capable hands of Prof H. Ootuka (Japan) For the Americas, Mr K. F Heumann (United States) would replace Dr Burton Adkinson, now president-elect, and Prof Lydia Sambaquy (Brazil) would continue in office

Turning to future conferences, Dr King announced that the meeting for 1962 would be at The Hague It was hoped that a meeting in one of the Asian countries could be arranged for 1963 or 1964, and the United States had issued an invitation to hold the next unrestricted conference in Washington in 1965

Dr King then dealt with the committees, the secretariats of which were now each located in different member countries Mr E Ohman (Sweden) had been appointed the first full-time officer of the Universal Decimal Classification at The Hague The training of personnel for carrying out documentation was being actively investigated under Prof Majewski at Warsaw, a newly constituted committee to consider the theory of classification would be based in Denmark, responsibility for linguistic problems had been accepted by the Netherlands, and mechanized documentation was the special concern of the German section. A new *ad hoc* Committee had been set up to give an impartial study to the future of the Universal Decimal Classification.

For the closing moments of the conference Dr. Burton Adkinson took over the chair and voiced the thanks of the delegates to the representatives of the host country, and in particular to Aslib, for the organization of the conference and for the accompanying programme of visits and entertainment.

E. M. R. Ditmas

X-RAY MICRO-ANALYSIS

List 177/2, recently issued by the Cambridge Instrument Co., Ltd., is an illustrated booklet* giving a comprehensive description of the Company's 'Microscan' X-ray analyser which is now in production. The technique of X-ray micro-analysis consists of focusing an electron beam, normally accelerated from 0 to 50 kV., on to a region of the surface of the specimen, approximately 1μ in diameter and rather less in depth, and analysing spectroscopically the resulting X-rays excited from the specimen. R. Castaing, in 1951, published details of the first practical instrument that could be described as an 'emission' micro-analyser, but Castaing's instrument, in common with similar instruments developed independently by others, used a static electron probe; it was thus limited to carrying out only point-by-point micro-analyses on parts of the sample surface previously selected under an optical microscope. The Cambridge instrument incorporates a deflexion system to control the electron probe, and in addition to static point-to-point micro-analyses it can also be used for scanning a maximum area of about $\frac{1}{2} \times \frac{1}{2}$ mm.^{2} on the sample surface. It is based on the instrument originally designed by Dr. D. A. Melford and colleagues at the Tube Investments Research Laboratories in collaboration with Dr. V. E. Cosslett and Dr. P. Duncumb, who were responsible for the prototype developed at the Cavendish Laboratory, University of Cambridge.

In the booklet the arrangement, constructional details and functioning of the analyser are clearly set out, together with photographs of parts of the instrument and of results obtained with the instrument. The analyser consists of four separately standing sections—the electrical supply unit, the main console, and three racks occupied mainly by scanning, counting and display electronic equipment, respectively. In the main console are housed the vacuum system consisting principally of a 2-in. oil diffusion pump backed by a rotary pump, the electron-optical system, the specimen chamber on which the electron-optical system is mounted and which is designed to allow from outside the maximum possible manipulation of the specimen which is itself under high vacuum. Sixteen positions are provided on the table and the table can be rotated to bring any one of the positions under either the electron beam or the optical microscope, the binocular optical microscope for conventional optical examination of the specimen for comparison with the electron image, and the semi-focusing X-ray spectrometer of a special and simplified design, containing three curved, rotatable, lithium fluoride crystals and two interchangeable detectors, one a sealed-off proportional counter for the detection of hard radiations and the other a flow proportional counter for soft radiations.

The technique of X-ray micro-analysis has so far been successfully applied to investigating the micro-structure of both metals and minerals, and although the 'Microscan' was designed primarily for metallurgical work it can be used for substances other than metals. By providing both X-ray and electron pictures of small surface regions, the analyser is invaluable as a metallurgical tool, and the examples of its application to the examination of various alloys and inclusions in steel which are discussed and illustrated in the booklet show clearly its advantages. Its main limitation, in common with other similar instruments, is its inability at present to detect the soft X-rays from specimens consisting of elements of atomic number less than twelve.

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* *The Microscan X-ray Analyser.* Pp. 32. (London: Cambridge Instrument Co., Ltd., 1961.)

DISTRIBUTION OF INTENSITY AMONG VARIOUS ULTRASONIC DIFFRACTION ORDERS

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The generalized theory of Raman and Nath gives very complicated expressions which, with certain restrictions, have been modified by Nath[1] to get closed expressions for intensities of ultrasonic diffraction orders. The intensity of the supersonic field is assumed to be not strong enough to excite the second and higher orders and solving the difference-differential equation obtained in the generalized theory, the following expressions for the intensities of the first and central zero orders have been obtained for normal incidence:

$$I_1 = \frac{4}{\rho^2 + 8} \sin^2 \left\{ \frac{\sqrt{\rho^2 + 8}}{4} \xi \right\}$$

$$I_0 = 1 - \frac{8}{\rho^2 + 8} \sin^2 \left\{ \frac{\sqrt{\rho^2 + 8}}{4} \xi \right\} \qquad (1)$$

where $\rho = \frac{\lambda^2}{\omega\omega_0\lambda^{*2}}$ and $\xi = \frac{2\pi\omega L}{\lambda}$

This expression clearly reveals that the intensity of the zero order I_0 on diffraction is less than unity by a value of $2 \times I_1$ where I_1 is the intensity of the first order of diffraction. The intensity of the zero order is shared by the two first orders of diffraction equally on either side of the central order. If the individual intensities of various orders, I_0, I_{+1} and I_{-1} could be measured accurately, then it can be expected, according to expression (1), that the sum of the three intensities, that is $(I_0 + I_{+1} + I_{-1})$, should be equal to the original slit intensity I of the zero order without diffraction. With the view of verifying the above statement, intensities of the various diffraction orders were measured with a sensitive photomultiplier tube and the results obtained are presented here.

It is very important to note that only the first orders of diffraction are excited, since expression (1) ignores the intensity of higher orders. Further, when second and higher orders are excited, multiple diffraction effects set in and these are carefully avoided in the present experiments using very low acoustical power.

Table 1 INTENSITY DISTRIBUTION AMONG VARIOUS ORDERS (f = 3·8 Mc/s—normal incidence)

Liquid	I_0/I (per cent)	I_{+1}/I (per cent)	I_{-1}/I (per cent)	Aggregate intensity as percentage of incident intensity
Water	93·9	1·1	1·1	96·1
Acetone	96·6	1·6	1·6	99·8
Methyl alcohol	98·0	0·6	0·6	99·2
Toluene	97·2	0·7	0·7	98·6

A progressive wave in water at 3·8 Mc/s is set up and the intensities of I_0, I_{+1} and I_{-1} on diffraction and the intensity I of zero order without diffraction are measured with the photo-tube. The intensity of the diffraction order expressed as a percentage of the original slit intensity of the zero order is tabulated in Table 1 using different liquids. Table 1 clearly shows that the aggregate intensity of various diffraction orders compares very well with the intensity of the original incident light in the zero order. These experimental results are in good agreement with expression (1).

The second aspect of interest is to study the distribution of intensity among various ultrasonic diffraction orders with obliquity of the sound wave-front. Raman and Nath[2], starting from very simple elementary considerations, have shown that diffraction effects disappear periodically, whenever the obliquity Φ of the sound wave-front from the position of normal incidence is equal to $\tan^{-1}\frac{n\lambda^*}{L}$, where $n \neq 0$ is an integer, λ^* is the sound wave-length in the medium and L is the length of the ultrasonic path traversed by the light beam. The expression for the relative intensities is given by $\frac{J_m{}^2(V)}{J_{n^2}(V)}$ where

$$V = V_0 \sec\Phi \frac{\sin\left(\frac{\pi L}{\lambda^*}\tan\Phi\right)}{\pi L/\lambda^* \tan\Phi} \qquad (2)$$

$$\text{and} \qquad V_0 = \frac{2\pi\omega L}{\lambda}$$

The behaviour of the above expression explains very well the variation of intensity with Φ among various diffraction orders and it is interesting to study this phenomenon with the photo-multiplier.

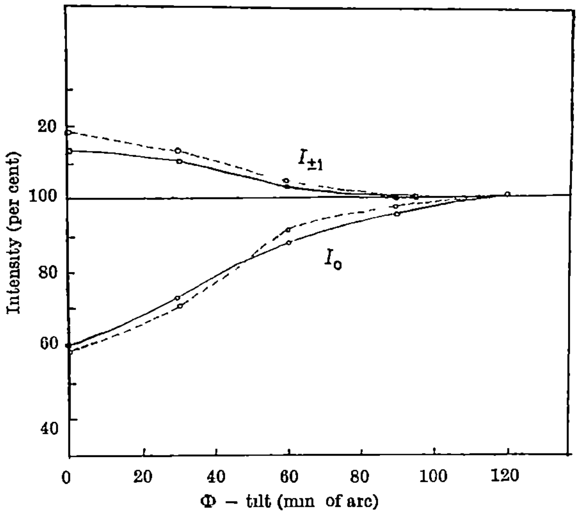

Fig. 1. Variation of intensity of the diffraction orders with obliquity of the sound wave-front

——, Experiment, - - -, theory, f = 3·8 Mc/s, L = 1·5 cm

As before, a progressive wave in water at 3·8 Mc/s is employed and, using moderate power, only the first orders of diffraction on either side of the zero order are excited. The intensities of I_0, I_{+1} and I_{-1} are recorded with the photo-tube for various angles of obliquity Φ in steps of 30′. The value of the parameter V is calculated for the angles of tilt Φ chosen using the expression (2) and the corresponding intensities are evaluated using the Bessel functions $\frac{J_m{}^2(V)}{J_n{}^2(V)}$. The value of L used here is 1·5 cm. The theoretical results evaluated in this way and the experimental ones obtained by the photo-tube for I_0 and $I_{\pm1}$ are plotted in Fig. 1. It can be seen that the agreement between the two curves is very satisfactory, showing the validity of expression (2). The intensity I_{+1} becomes zero at 95′, as can be seen in Fig. 1, and this value compares very well with the angle of 92′ computed by $\tan^{-1}\frac{\lambda^*}{L}$ for the disappearance of the first order.

Expression (2) is very important in that it can predict the poor intensities of the very high frequency diffraction orders observed by several investigators[3,4]. It is well known that at moderately high frequencies, the sound wave-front is to be tilted with respect to the light beam to make the first-order diffraction line appear and therefore the tilt angle Φ is to be taken into account in estimating the intensity of the high frequency diffraction orders. The expression (2) can be written as $V = V_0 \sec\Phi \frac{\sin t}{t}$ where $t = \frac{\pi L \tan\Phi}{\lambda^*}$.

The parameter V_0 in the case of normal incidence is given by $V_0 = \frac{2\pi\omega L}{\lambda}$ and does not include λ^*. But the moment the light beam is tilted, as in the case of high frequencies, the parameter V includes not only V_0 but also λ^*, the sound wave-length in the medium. It is important to see how Φ affects V. The value of Φ involved experimentally is generally small, namely,

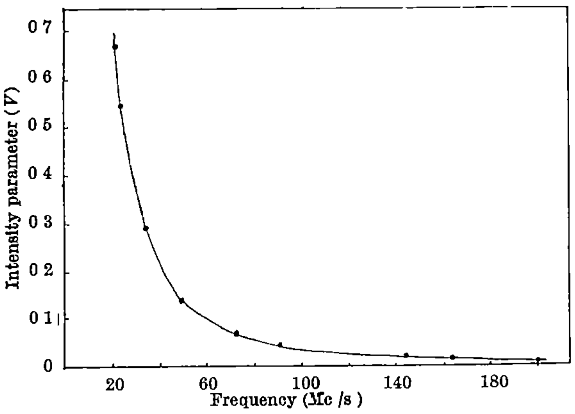

Fig 2 Behaviour of the intensity parameter with increasing frequencies $L = 1\ 5$ cm

< 2°, and therefore one can approximate tan Φ and sin Φ to Φ and write $t = \frac{\pi L \Phi}{\lambda^*}$ But sin t is never equal to t under the experimental conditions since t involves $\frac{\pi L}{\lambda^*}$, which is very large At high frequencies $\frac{L}{\lambda^*}$ tends to increase as λ^* decreases with increasing frequencies, t therefore assumes large values making sin t approach its maximum value unity at very high frequencies Under these valid approximations, the parameter V can be written as $V = V_0/\pi L \left(\frac{\lambda^*}{\Phi}\right)$ and is directly proportional to $\left(\frac{\lambda^*}{\Phi}\right)$ At high frequencies, Φ approaches the Bragg angle[5] to bring the diffraction order into the field of view and λ^* decreases, with the result the ratio $\frac{\lambda^*}{\Phi}$ tends always to decrease with increasing frequencies Therefore the intensity parameter V goes on diminishing with high frequencies as shown in Fig 2, thus giving a natural explanation to the poor intensities observed in high-frequency ultrasonic diffraction phenomena

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EIGHT-CO-ORDINATE DIARSINE COMPLEXES OF QUADRIVALENT METAL HALIDES

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As part of a systematic study of the ditertiary arsine complexes of transition metal halides, we have been investigating the behaviour of titanium, zirconium and hafnium tetrachloride with the chelate group *o*-phenylene*bis*dimethylarsine[1] (diarsine or *D* in the following account)[1] The ditertiary arsine complexes of metals in the first half of the transition series have been little investigated One reason for this is undoubtedly the great interest in transition metals which have potential double-bonding capacity such as nickel, palladium and platinum In addition, the metals at the beginning of each transition series were thought to co-ordinate with the more electronegative donor atoms such as oxygen rather than with arsenic Provided that one uses a non-hydroxylic solvent as the reaction medium, however, these transition metals will co-ordinate with the diarsine

We have prepared a mono-chelate derivative of titanium tetrachloride with the formula $TiCl_4$ diarsine by the reaction of titanium tetrachloride with the diarsine in 1 1 molar ratio in carbon tetrachloride solution[2] The composition of this compound has been confirmed by analysis and some of its properties are given in Table 1 It is a non-conductor in nitrobenzene solution , it is considered to be a typical octahedral non-electrolyte but it is not iso-structural with $SnCl_4$ diarsine originally described by Allison and Mann[3]

From the reactions between the diarsine and zirconium tetrachloride and hafnium tetrachloride in acetone, we have isolated only the *bis*-diarsine compounds $ZrCl_4$.2diarsine and $HfCl_4$ 2diarsine The corresponding *bis*-diarsine titanium tetrachloride complex $TiCl_4$ 2diarsine can be prepared by treating $TiCl_4$ diarsine with excess diarsine or by a curious reaction between titanium trichloride and the diarsine in glacial acetic acid[2] The reaction of vanadium tetrachloride with the diarsine in carbon tetrachloride solution gives VCl_4 2diarsine This compound is paramagnetic, with one unpaired electron, as expected Some properties of these four *bis*-diarsine complexes are shown in Table 1 They are iso-structural as shown by X-ray powder photography and have nearly identical infra-red spectra

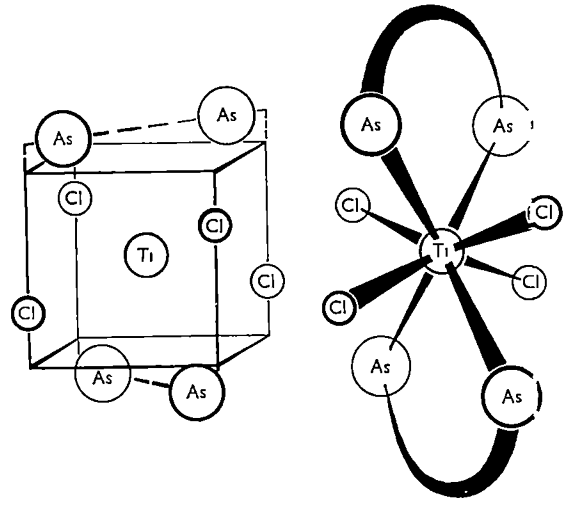
The *bis*-diarsine titanium complex, which is slightly soluble in nitrobenzene, has been more fully investigated than the other derivatives because they have much lower solubilities in all solvents tried In nitrobenzene, $TiCl_4$ 2diarsine forms a non-conducting solution , this implies that in solution either (1) the complex involves an eight-co-ordinated titanium atom, the diarsine behaving as a chelate group , or

Table 1 Properties of Ditertiary Arsine Complexes

Compound	Colour	Melting point in deg (decomp)	Conductivity in nitrobenzene		
			Concentration (mol cm $^{-3}$)	Λ_m (cm ^{2}ohm^{-1} mol^{-1})	μ_{eff} (B M)
$TiCl_4$ *D*	yellow-orange	210	$5\ 0 \times 10^{-3}$	0 5	0 0
$TiCl_4$ 2*D**	orange-red	174	$2\ 3 \times 10^{-3}$	0 9	0 0
VCl_4 2*D**	orange	134	—	—	1 8
$ZrCl_4$ 2*D**	white	290	—	—	0 0
$HfCl_4$ 2*D**	very pale pink	284	—	—	0 0

* Isostructural *D* = diarsine

Satisfactory analytical results for carbon, hydrogen, chloride, arsenic and metal have been obtained for these compounds



(a) (b)

Fig. 1. (a) Drawing showing the relationship between the co-ordination polyhedron and a cube; (b) a perspective view of $TiCl_4$ 2diarsine in which the phenyl rings are represented diagrammatically

(2) the complex dissociates, forming, for example, one mole of $TiCl_4$ diarsine and one mole of free diarsine. (We consider that the possibility that the titanium atom is six-co-ordinate with the diarsine behaving as a monodentate group is excluded by the infra-red spectrum.)

The diffuse ultra-violet and visible spectra of the solid $TiCl_4$ diarsine and $TiCl_4$ 2diarsine have been recorded. The charge transfer band of the former occurs at 4650 Å and of the latter at 5100 Å. Both compounds, however, show the same spectrum in polar solvents ($\lambda_{max} \sim 4600$ Å) and in benzene ($\lambda_{max} = 4480$ Å). This suggests that $TiCl_4$ 2diarsine dissolves by dissociation into $TiCl_4$ diarsine and free diarsine. Both compounds can be recrystallized from benzene and nitrobenzene solutions, but the *cis*-diarsine complex is less soluble, presumably because of the mass action effect of the liberated diarsine.

From the infra-red spectrum and an X-ray diffraction investigation of $TiCl_4$ 2diarsine, we have shown that in the solid state both diarsine molecules are co-ordinated to the titanium atom. The intense infra-red bands at 884, 846 and 741 cm^{-1} of the free diarsine are not present in the compound, but corresponding bands some 20 cm^{-1} higher in frequency are present. This shift is characteristic of co-ordinated diarsine.

The structure of the solid $TiCl_4$ 2diarsine has been studied by single-crystal X-ray diffraction methods. The point symmetry of the titanium atoms is $\bar{4}2m$, with all the atoms in the molecule except the methyl groups lying on mirror planes. The co-ordination polyhedron around the titanium atom is a dodecahedron with eight vertices consisting of four equivalent arsenic atoms and four equivalent chlorine atoms. The arsenic atoms are 2.71 ± 0.02 Å from the titanium atom and are at an angle of 36° to the tetragonal axis. The chlorine atoms are 2.46 ± 0.02 Å from the titanium atom and are at an angle of 73° to the tetragonal axis. So far as we know, these are the first examples of eight-co-ordination of atoms in the first long period of the Periodic Table.

The molecular symmetry of these compounds is the same as that reported for the complex ion $[Mo(CN)_8]^{4-}$ (ref. 4). For this symmetry a set of hybrid bond orbitals determined by Racah[5] from d^4sp^3 orbitals has one group of four with maximum strength 2.968 at an angle of 72° 47′ to the tetragonal axis and another group of four with maximum strength 2.995 at an angle of 34° 33′ to the tetragonal axis.

The direction of the titanium–chlorine bond coincides with that of the first group of these hybrid bond orbitals. The angle between the direction of the titanium–arsenic bond and the tetragonal axis (36°), however, is 2° larger than that of the corresponding maximum strength hybrid orbital, while the neighbouring carbon′–carbon–arsenic angle (117°) is 3° smaller than the ideal trigonal angle of 120°. While barely significant, this suggests that the stability of the molecule is sensitive to the titanium–arsenic distance, and that the angular distortion necessary to obtain this distance is correspondingly distributed between these two angles.

An investigation of the reactions of diarsine with other tetrachlorides and with other halides is in progress. Further details of this work will be published elsewhere in full.

We are indebted to British Titan Products, Ltd. (R. J. H. C.), and to the United States Department of the Army through its European Research Office (G. B. R.) for financial support, and to O. S. Mills and J. S. Rollett for the use of their computer programmes.

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UNSTABLE FLOW OF MOLTEN POLYMERS

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When molten polymers are extruded at low stresses through dies of circular cross-section, the extrudates are smooth and cylindrical. At higher stresses the surface may be marred by two defects. The first is known as mattness or in its more severe form as 'shark-skin', which consists of ridges at a separation usually small compared with the diameter of the extrudate. It tends to occur at a constant value of the average velocity and is attributed to a stick-slip motion at the die exit[1]. The second is on a larger scale, and the extrudates may take a helical or an irregular, distorted form. This second defect, with which this article is concerned, has been attributed[2] to 'melt fracture' within the polymer. The term fracture implies that the ultimate cause of the distorted extrudates lies in the failure of inter- or intra-molecular bonds within the melt. The evidence to be presented suggests the origin of the defect as being a stick-slip phenomenon at the die wall.

In extrudates of polytetrafluoroethylene and polyvinyl chloride there is clear evidence of macroscopic fracture, with the formation of free surfaces, but it has not been demonstrated that these arise before the material enters the die, they could be produced at the exit In less viscous polymers such as 'Diakon', polythene and polypropylene, fracture is not obvious in the extrudate, but is sometimes inferred from the 'breakage' during extrusion of a central coloured filament embedded in colourless material, in experiments with transparent apparatus the apparent breakage is seen to occur before the material enters the die

As part of an investigation into the causes of distortion in extrudates, we have carried out experiments to examine the possibility of slipping at the die wall Clegg[3] considered the possibility of a stick-slip mechanism being responsible for unstable flow, but his experiments showed that changes in the roughness of the die surface had little effect on the flow of polythene However, it seems likely that the polymer properties rather than the roughness of the surface are usually predominant in determining the onset of slippage, since on a molecular scale all surfaces are rough. In our experiments we measured the distribution of velocity in the die at extrusion-rates above and below the point of instability The movement of a coloured disk of poly(dimethyl siloxane) gum was determined from ciné films of its motion in the die after the sudden application of the appropriate shear rate. Poly(dimethyl siloxane) gums were used because their flow behaviour at room temperature resembles that of molten polymers at much higher temperatures[1] Working at room temperature avoids elaborate temperature control equipment and makes possible the use of easily fabricated transparent 'Perspex' dies

The results are shown in Fig 1, where the relative displacements are shown at equal time-intervals after an arbitrary zero chosen when the velocity of the extrudate reached a constant value It is evident that slipping is indicated in the two high stress measurements (*a*), (*b*), where at low stresses the profile (*c*) is very similar to that predicted (*d*) from the measured shear stress–shear rate relation

The displacement profiles in (*a*) and (*b*) suggest that 'plug' flow is occurring, but similar experiments, using a less viscous silicone gum, show that when slipping takes place there is still, as would be expected for such material, a wide distribution of velocities within the melt This probably explains why it is difficult to detect slipping in low-viscosity polymers since the large range of velocities in the melt mask the relatively small difference in velocity between the die wall and the material in contact with it Conversely, it is easier to detect slippage in highly viscous liquids

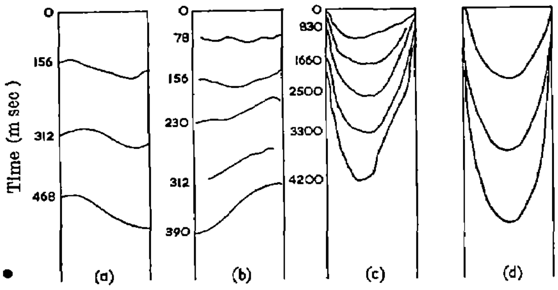

Fig 1 Displacement profiles of poly(dimethyl siloxane) at high and low stresses *a*, stress = 1 2 × 10^6 dynes/cm ^{2}, *b*, stress = 1 2 × 10^6 dynes/cm ^{2}, *c*, stress = 6 × 10^5 dynes/cm ^{2}, *d*, profile calculated from measured stress–shear-rate relationship

Because the stress was applied suddenly and the measurements necessarily ended when the disk was extruded, it may be argued that the experiments are too transient in character and do not correspond to steady-state extrusion However, this criticism applies equally to the experiments above and below the point of instability, and the difference in behaviour remains valid To establish this point more convincingly, additional qualitative experiments were carried out in which coloured material was injected at the die wall under steady-state conditions and these again showed no slip at a low stress, but appreciable slip when the flow was unstable. Similar experiments with highly viscous molten polythene extruded through a glass die also showed that slipping was taking place when the stress was above that at which instability began. Our results show that when molten polymers are extruded at sufficiently high stresses the velocity at the die wall is not zero Since the stress at which slipping occurs is near the stress at which the results of unstable flow in the die can be detected in the extrudate, this suggests that the two effects are connected and that the unstable flow may be due to a stick-slip mechanism

The typical forms which non-cylindrical distorted extrudates take in molten polymers can also be qualitatively explained in terms of a stick-slip mechanism, helical extrudates found with many polymers could be caused by a precession of the point of slipping or the point of maximum slip around the circumference of the die, and the defect known as 'kneeing' in polypropylene could be caused by slippage taking place alternately at opposite ends of a diameter

There is much indirect evidence which supports the stick-slip hypothesis Experiments in which a metal plate is pulled across the surface of a pool of low-viscosity (10^4 poises) siloxane fluid show that at high speeds even a weighted slider will slip When polythene is extruded it has been shown[4] that the 'swelling ratio' passes through a maximum at or below the point of instability, the decline from the maximum when instability sets in could be due to the storage of less elastic energy in the extrudate when slipping occurs Since the stress is a maximum at the die wall and the onset of instability occurs at a critical stress, it is reasonable to expect the cause of the instability to be at the wall rather than in the polymer The effects of slipping are more easily observed with highly viscous polymers, so that any differences in the amount of slip which takes place when using dies of different composition will also be more easily detected with highly viscous polymers Experiments with a very viscous polythene melt suggest that, at high stresses, the stress for a given output-rate is slightly less through a polytetrafluoroethylene than through a steel die of the same dimensions

The facts presented here seem to us strong evidence that unstable flow of molten polymers is associated with slipping at the die wall, and we further suggest that instability may be the result of a stick-slip mechanism

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SOMATIC CHROMOSOMES OF THE GORILLA

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ACCURATE knowledge of chromosome number, morphology and behaviour in mitosis and meiosis has often assisted genetical and evolutionary analysis at the species-level

Recent technical advances have made possible a more accurate study of chromosomes in several species of mammals, including man[1]. By using *in vitro* cultures of biopsy specimens of skin[2,3] and peripheral blood[4], precise delineation of somatic chromosomes may be obtained This involves little discomfort and no danger to the animals As a direct result of these developments, the chromosome number of some twenty-five species and sub-species of primates has been recently determined[5] The anthropoid apes, however, have thus far been relatively neglected, mainly because of the difficulties of obtaining suitable material [See also p 285 of this issue]

The diploid chromosome number of the chimpanzee was determined as 48 by Yeager *et al* [6], who used testis material. This was recently confirmed by Young *et al* [7] in preparations from the bone-marrow of nine animals, but a detailed analysis of the karyotype is not as yet available

We describe here the chromosomes of the gorilla and attempt a comparison with the human chromosome complement, of which several analyses have been published[8]

Two gorillas, one of each sex, kept at the Basle Zoological Gardens, were made available for examination while some of us were on the teaching staff of the World Health Organization international laboratory course in methods of human cell culture and cytology at the Anatomical Institute of the University of Basle Both gorillas are of the Lowland sub-species (*Gorilla gorilla gorilla*, Savage and Wyeman) The male, which was about nine months old at the time of biopsy, was captured in the Cameroons in March 1960 The female was born in the Basle Zoological Gardens on the night of September 22–23, 1959 8 ml of blood were drawn from an arm vein of the male and cultures were prepared by a technique similar to that described for human leucocytes[4]

A skin biopsy was taken from the female and cultured by a plasma clot technique in the presence of a fluid medium Cells from both animals were pretreated with 'Colcemid' (Ciba, desacetylmethylcolchicine) and hypotonic solution, fixed and afterwards stained with acetic orcein or unna blue. Chromosome counts were performed directly on preparations from both primary and (with the skin) transferred cultures Karyotype delineations and measurements of the relative lengths of individual chromosomes were obtained from enlarged photomicrographs Absolute measurements were made with a micrometric ocular

Sexual dimorphism of interphase nuclei Smears of oral mucosa cells were obtained from both animals and stained with thionin[9] 42 per cent of the nuclei in the female possessed a characteristic chromatin mass lying adjacent to the nuclear membrane (Fig 1

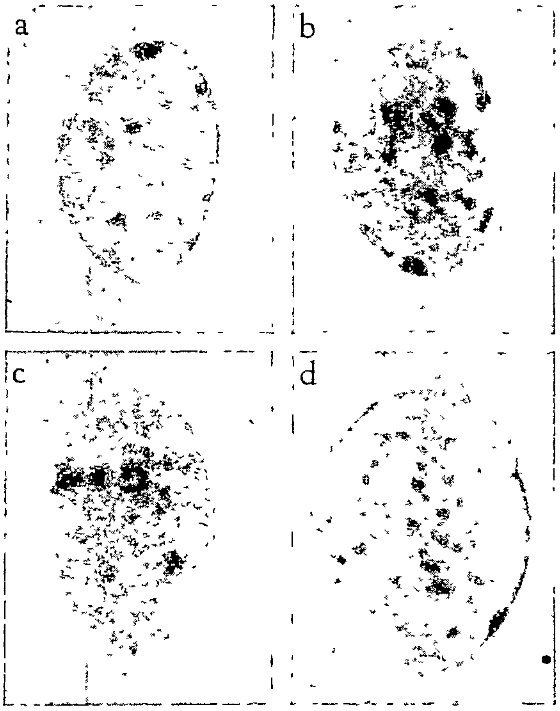

Fig 1 Nuclei of buccal mucosa of the female gorilla (*a*, *b*, *c*) and of the human female (*d*) for comparison

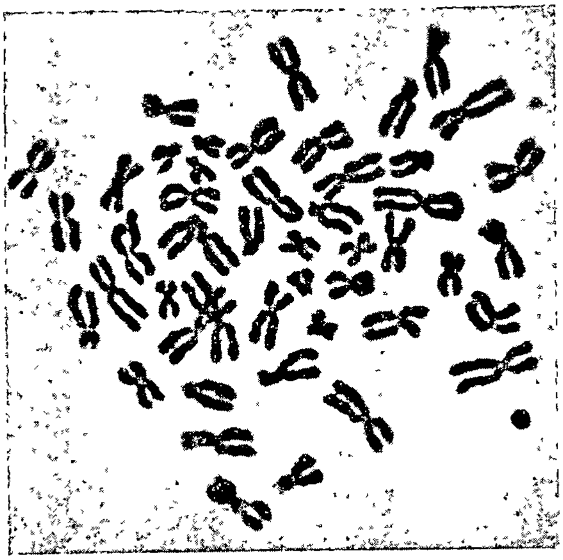

Fig 2 Mitotic metaphase in cultured cell from peripheral blood of the male gorilla Acetic orcein Phase contrast Photographic enlargement × 1,600

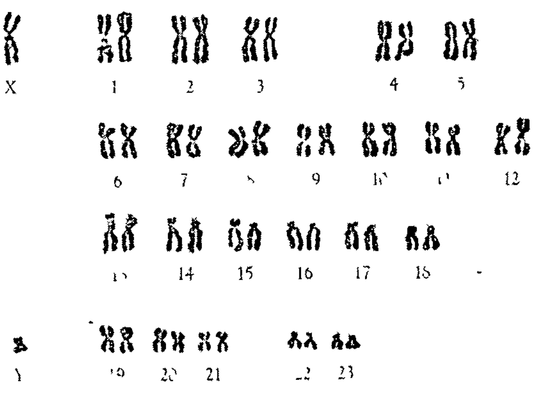

Fig 3 Karyotype of the male gorilla Constructed with the chromosomes of Fig 2

a, b, c) This usually appears larger and more rectangular in shape than in man (Fig 1 d) 4 per cent of the nuclei in the male contained a similar peripheral chromatin mass A variable number of smaller, centrally placed chromocentres were frequently observed in the nuclei of both sexes These are seen less frequently in man The fine, particulate, nucleolar-associated chromatin was observed in about the same amount as usually seen in human nuclei[10] The majority of interphase nuclei of cultured cells derived from the skin of the female also showed a characteristic sex-chromatin body

Chromosome counts The distribution of chromosome counts independently obtained by four of us is set out in Table 1

Table 1 CHROMOSOME COUNTS IN THE GORILLA

Individual	Type of culture	Observer	Chromosome No 45	46	47	48	>48	Total
Male	Blood	J L H	2		2	28	2*	34
Female	Skin	L DC F N	1	2	3	22	—	28
		M F	1	3	1	46	2*	53
	Total		4	5	6	96	4*	115

* Polyploid cells

Table 2 SOMATIC CHROMOSOMES OF THE GORILLA

Group 1–3	Long chromosomes with approximately median centromeres It is difficult to distinguish them from each other The *X*-chromosome is similar to these but easily separated by its length It is the longest chromosome of the complement
Group 4–12	Medium-sized chromosomes with median or sub-median centromeres There is little difference in length between individual pairs in this group, and the chromosomes are difficult, if not impossible, to distinguish one from another by comparative measurements Chromosome 12 can be identified by the presence of a prominent secondary constriction in its long arm, close to the centromere
Group 13–18	Medium-sized acrocentric chromosomes Chromosomes 13, 14, 15 bear satellites, chromosome 16 apparently does not Chromosomes 17 and 18 are shorter than the other chromosomes in this group Their short arms bear prominent satellites
Group 19–21	Medium to short chromosomes with median centromeres These can usually be distinguished from each other and from chromosomes of other groups by their length and centromere position
Group 22–23	Short acrocentric chromosomes Chromosome 22 bears prominent satellites, chromosome 23 has slightly smaller ones The *Y*-chromosome is similar to these chromosomes, but generally appears more metacentric

The diploid chromosome number is 48 The counts of less than 48 chromosomes are probably due to loss of chromosomes during the technical procedures A few of these cells were analysed and no consistent pattern of chromosome loss was found The four cells with chromosome counts greater than 48 were probably polyploid, and two of them had exact counts of 96 chromosomes

Karyotype analysis A summary of the chromosome morphology of the gorilla is given in Table 2,

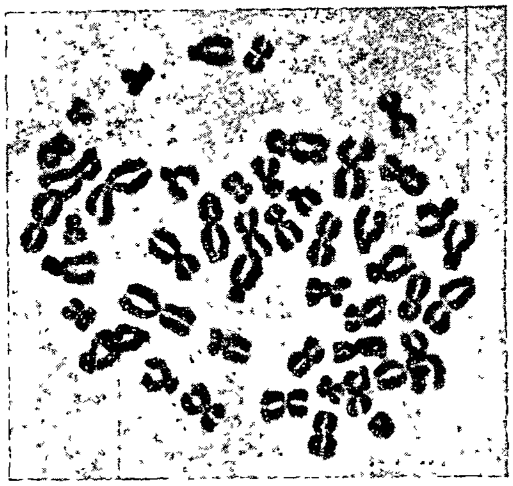

Fig 4 Mitotic metaphase in cultured skin cell of the female gorilla Acetic orcein Phase contrast Photographic enlargement × 1,600

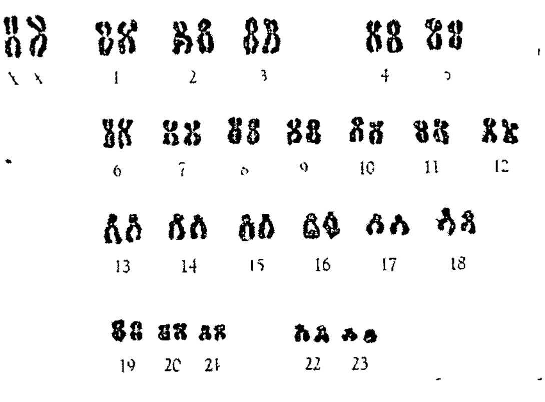

Fig 5 Karyotype of the female gorilla Constructed with the chromosomes of Fig 4

Table 3 MEANS AND RANGE OF VARIATION (IN BRACKETS) OF THE ABSOLUTE LENGTH (IN μ) OF THE LONGEST AND SHORTEST CHROMOSOMES OF THE GORILLA AND HUMAN COMPLEMENT

The estimates are based on measurements performed on 10 cells (treated with colchicine) for each type of tissue

Chromosome measured	Blood		Skin	
	Gorilla	Man	Gorilla	Man
Longest	5.3 (3.6–6.3)	5.6 (4.5–8.0)	6.3 (4.5–8.1)	6.4 (5.9–7.2)
Shortest	1.6 (1.0–1.9)	1.6 (0.9–1.8)	2.2 (1.4–2.7)	1.6 (1.4–1.8)

and representative metaphase plates and karyotypes in Figs 2–5. The complement has been divided into five groups and the chromosomes numbered so far as possible in order of descending length The presence of secondary constrictions or, in the case of acrocentric chromosomes, of satellites, has aided the identification of certain chromosomes This system is based on the same criteria as the Denver standard system of nomenclature proposed for the human chromosome complement

Idiograms of the chromosome sets of the gorilla and man, based on average relative measurements of the chromosomes of seven cells from the two gorillas and on the measurements of human chromosomes given in the Denver statement[11], are compared in Fig 6 The major points of difference are

(1) The X-chromosome of gorilla is metacentric and is the largest chromosome in the complement

(2) Chromosome 12 has a prominent secondary constriction in its long arm, no counterpart of this chromosome is found in the human karyotype

(3) Six pairs of medium-sized acrocentric chromosomes (group 13–18) are present in gorilla and only five pairs in man (groups 13–15, 17–18)

(4) The acrocentric chromosomes of group 13–18 have more definite short arms and more prominent satellites than the acrocentric chromosomes (groups 13–15, 17–18) in man Satellites of chromosome 13 (gorilla) are particularly prominent

While several of the longest autosomes of gorilla are relatively shorter than those of man, absolute measurements of comparable cells (Table 3) show that there is no significant difference between the absolute lengths of the longest and of the shortest chromosomes in the two species

Discussion The similarities between the karyotypes of man and gorilla provide further evidence of the close evolutionary relationship of the two species Man has one acrocentric chromosome less than gorilla, although the number of metacentric chromosomes is the same (Fig 6)

Differences in chromosome number may originate in three ways by polyploidy, polysomy or structural change Polyploidy is a source of karyotype evolution in plants[12], but whether it has played any part in the evolution of mammals remains uncertain[13,14] Polysomy is now known to be a component of pathological chromosome variation in man[15] and the mouse[16] Changes of the type first recorded by Robertson[17] in the Acrididae have been found in two species of mammals, and cause inter- and intra-specific variation in chromosome number with little or no loss of chromosome material[18]

One of the metacentric chromosomes of the gorilla (No 12 in our system) has a prominent secondary constriction in its long arm A similar characteristic autosome has been reported in the chromosome complements of all species of primates which have been examined[5], but not in man The size and position of the secondary constriction are morphologically similar throughout the group, although the autosome which contains it may differ in length and arm ratio from species to species

The significance of this chromosome region is uncertain and its absence in man may be explained in several ways A single reciprocal translocation with one break near the centromere of one of the long acrocentric chromosomes, followed by loss of the centric fragment, is one way in which the change in number may have occurred If the other break were to have been in or near the secondary constriction of chromosome 12, it would also conceivably explain the apparent disappearance of this region

The X-chromosome of the gorilla is relatively longer than the human X-chromosome This might be correlated with the apparent larger size of the sex-chromatin mass in the nuclei of the female gorilla The increased number of centrally placed chromocentres observed in the nuclei of the buccal mucosa cells of the gorilla may be similarly related to the increased number of negatively heteropyknotic regions observed in the metaphase chromosomes Further speculation about the mechanism of karyotype evolution in man and

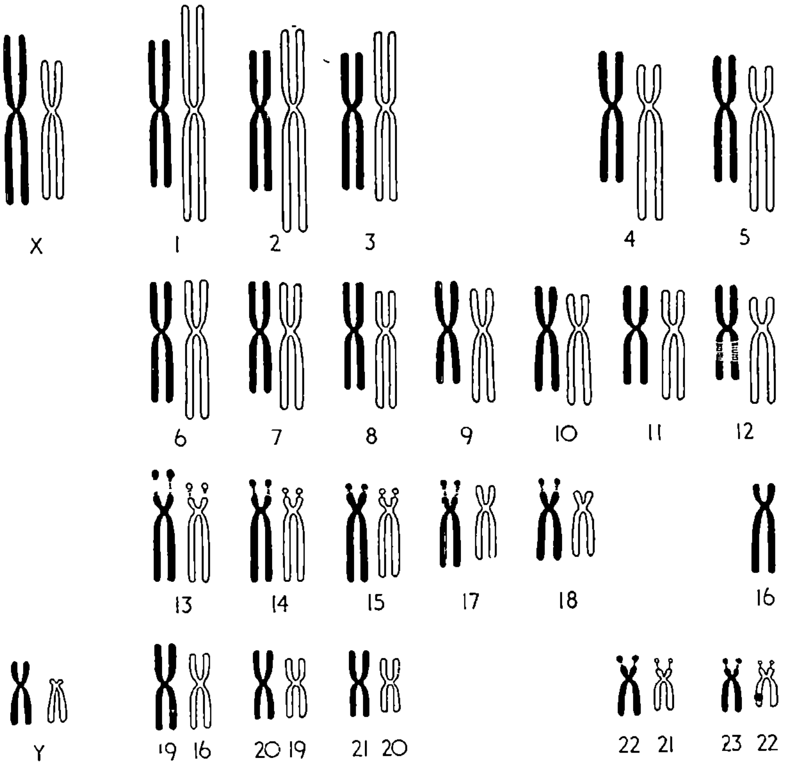

Fig 6 Tentative comparison of the idiograms of gorilla (black) and man (outlined) The human chromosomes are numbered according to the Denver system (ref 11)

the anthropoid apes must wait additional data Now that simple biopsy techniques are available, institutions with collections of anthropoids will perhaps allow examination of the chromosomes of their charges To quote the words of Darlington[14] "Great institutions have been devoted to collecting, and preserving, describing and naming, the bony fragments of our ancestors and relatives, and great bodies of literature have been brought forth by their labours Rightly so Yet at the cost of a few hundred cells, and a few drops of blood, a few moments of discomfort to the animals, and a few hours of work to a cytologist, the genetic record of human evolution could be laid bare It would, perhaps, be worth while"

We wish to thank Drs R Schenkel and E Undritz for their help in collecting the material, and Mr G Clarke for his work with some of the tissue cultures We are indebted to Dr C E Ford for constructive criticism of the manuscript

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EFFECT OF GAMMA-RAYS ON THYMUS NUCLEOPROTEIN IN SOLUTION

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THERE have been many investigations into the action of ionizing radiations on deoxyribonucleic acid (DNA) and, from the point of view of macromolecular structure, the effects may be broadly classified as degradation into smaller fragments and denaturation, that is, loss of the double helical conformation Since these substances occur *in vivo* only in combination with protein, earlier work[1–3] has been extended to an examination of the effects of γ-irradiation on the molecular weight and shape of the soluble deoxyribonucleohistone which may be obtained from calf thymus glands by extraction with water after a preliminary washing of the glands with versene and saline[4] The method afforded dilute solutions of the nucleoprotein in 0 7 m*M* phosphate buffer (*p*H 6 8) The viscosities of these solutions, as measured in Ubbelohde viscometers, changed by less than 5 per cent during five days at 25° C

The molecular weights of nucleoprotein were determined by the method of light-scattering (details in ref 2) and had an average value of 22 × 10^6, with a maximum variation of 10 per cent from one independent extraction to another Better reproducibility was frequently obtained, as in the particular preparations used in this work (Table 1) The stability and reproducibility of this material are thus confirmed Analysis of these preparations (Mr P M Bayley) showed that the N/P ratio was 3 70 in agreement with Doty and Zubay[4] Nucleoprotein solutions were clarified from dust by centrifugation at 29,000 r p m for 30 min (Spinco Model *L*), no fractionation occurred during this centrifugation

The solutions of nucleoprotein (about 0 02–0 09 per cent) in 0 7 m*M* phosphate buffer were irradiated by γ-rays from a cobalt-60 source, without exclusion of oxygen. Dosages (*R*) are stated as eV /phosphorus atom, where 1 eV /phosphorus atom is equivalent to 28 1 or 13 2 kilorads given to a 1 mgm /ml solution of nucleic acid or nucleoprotein, respectively After irradiation, the solutions were stored at 3° C for at least 24 hr and then used for light-scattering work (This precaution proved to be unnecessary since no decrease in viscosity occurred during this period) Aggregation of the nucleoprotein was observed at all dosages greater than 60 eV /phosphorus atom (up to 430 eV /phosphorus atom) Irradiation caused a decrease in viscosity, with no 'after-effect', and no increase in ultra-violet absorption at 259 mμ This suggests that a decrease in molecular size occurred without denaturation of the nucleic acid of the complex

The decrease in weight-average molecular weight (M_L) and in radius of gyration (ρ_g), also derived from the light-scattering measurements, are shown in Fig 1 and Table 1 At dosages of less than 18 eV / phosphorus atom, the relative decrease in molecular weight was a linear function of *R*, in contrast[2] to DNA, where it is dependent on R^2 This relation to *R* appeared to take a new direction above about 15 eV /phosphorus atom in the case of the most extensively studied preparation (*D*, conc = 4 2 × 10^{-4} gm /ml) Moreover, the slope of the relation depended on the concentration of the nucleoprotein during the irradiation, even though *R* is expressed in eV /phosphorus atom, which should allow for the usual variation of an indirect effect with concentration In all these respects, irradiation of the nucleoprotein differed from that of nucleic acid[1–3] Another important difference was that, whereas ρ_g of irradiated nucleic acid was[2] proportional to $M_L^{0\ 5}$, ρ_g of irradiated nucleoprotein declined only very slowly as the molecular weight decreased for example, to 70 per cent of its original value when the molecular weight

Table 1 EFFECT OF γ-IRRADIATION ON CALF THYMUS DEOXYRIBONUCLEOPROTEIN

	$10^{-6} \times M_L$					ρ_g in Å				
Preparation	*B*	*C*	*D*	*E*	*F*	*B*	*C*	*D*	*E*	*F*
Dosage *R* in eV /phosphorus atom										
0	22 5	22 7	22 2	21 8	22 0	2000	2100	2050	2200	2100
1 53			16 7					2100		
3 37		15 6					1800			
4 22			11 1					1700		
5 66		12 8					1950			
5 78			9 5					1800		
6 15				5 3					1600	
6 30	14 7					2000				
7 90			7 1					1600		
9 96			5 9					1600		
12 0			3 8					1300		
12 6	10 0					1800				
13 0			4 0					1300		
15 6			4 2					1300		
17 7				2 2					1750	
19 0	6 9					1800				
24 3			2 1					1300		
36 2			1 5					1300		

B, C, D, E, F, independent extractions of nucleoprotein Concentrations of solutions as extracted and irradiated *B*, 2 3 × 10^{-4} gm /ml , *C*, 3 77 × 10^{-4} gm /ml , *D*, 4 2 × 10^{-4} gm /ml , *E*, 8 77 × 10^{-4} gm /ml Solvent 0 0007 *M* phosphate (*p*H, 6 8) M_L = mol wt , ρ_g = radius of gyration, both from light-scattering measurements

had been reduced more than ten-fold, at R = 24 3 and 36 2 eV /phosphorus atom (In terms of a power relationship ρ_g is approximately proportional to $M_L{}^{0\ 2}$) Clearly a process must be occurring whereby the molecular weight can be reduced without a comparable change in the extension of the molecular system primarily contributing to the observed ρ_g The most likely explanation of this is that, on irradiation, the smaller protein units dissociate from the DNA, the molecular weight of which is unchanged and the extension of which is modified only relatively little If this view were correct, the dissociation process should continue until the protein is fully dissociated when M_L should be approximately 4 × 10^6, taking the molecular weight of intact nucleic acid as 8 × 10^6 A value of 4 5 × 10^6 for the mean molecular weight of the fully dissociated nucleoprotein system was observed when the solvent was 2 0 *M* sodium chloride (This process of dissociation by salt has been the subject of separate work[5]) For this dissociated nucleoprotein, for non-irradiated and γ-irradiated deoxyribonucleic acid[2], and, in the present experiments, for γ-irradiated nucleoprotein of M_L < 4 × 10^6 the values of the ratio $M_L^{1/2}/\rho_g$ were all approximately unity, in distinction from the value of 2 3 obtained with the original nucleoprotein This suggests that when the mean molecular weight has fallen to less than 4 × 10^6, the shape is determined by nucleic acid free from protein Beyond this point in the irradiation, the changes previously reported for nucleic acid[1-3] should be observed and denaturation should begin Further experiments are needed to elucidate this point

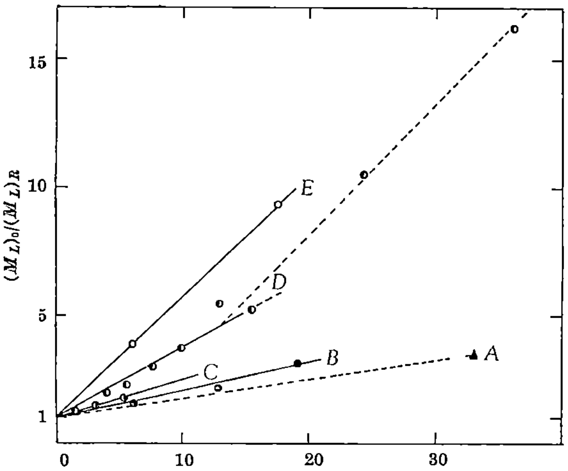

Fig 1 Relative change in the molecular weight of thymus nucleoprotein caused by γ-irradiation

Ordinate = (M_L before irradiation)/(M_L after dosage R) Concentration of nucleoprotein (independent preparations) *A* (▲), 1 15 × 10^{-4} gm /ml , *B* (●), 2 3 × 10^{-4} gm /ml , *C* (◑), 3 77 × 10^{-4} gm /ml , *D* (◐), 4 2 × 10^{-4} gm /ml , *E* (○), 8 77 × 10^{-4} gm /ml

The first major effect of irradiation on the nucleoprotein complex therefore appears to be dissociation of the small protein molecules from the nucleic acid, which remains relatively intact until most of the protein is dissociated This proposal is consistent with the earlier observation of Kaufmann *et al* [6] that the decrease in viscosity of nucleoprotein solutions caused by X-rays did not involve depolymerization of the nucleic acid Hems[7] has obtained evidence for rupture of the linkage between DNA and an acidic protein which is present in nucleoprotein prepared by the method of Kay *et al* [8] At what point in the dissociation nucleic acid itself begins to degrade and denature needs to be determined The changes in M_L and ρ_g show that the nucleic acid must form a central core the extension of which is somewhat modified as the protein is removed, but not in proportion to the decline in its molecular weight This is entirely in accordance with a structure for deoxyribonucleohistone (the protein is almost all histone in this preparation) which consists of a central core of double-helical deoxyribonucleic acid around which the histone chains are wound[9] However, the possible role of any protein bridges must not be overlooked in more detailed work The effect of changes in concentration (Fig 1) indicate that the dissociation process does not have the usual characteristics of an indirect effect Whether or not the protein is degraded before it dissociates is not known

Although nucleic acid by itself is very sensitive to denaturation by γ-rays (1 6 eV /phosphorus atom causes 70 per cent denaturation[3]), there was no evidence for denaturation of the nucleic acid in the irradiated nucleoprotein even up to 40 eV /phosphorus atom This agrees with the chemical evidence of Emmerson *et al* [10] that up to dosages of 1 3 × 10^5 rads ($\simeq$ 50 eV /phosphorus atom with their 0 02 per cent solutions) practically no radicals actually penetrate to the nucleic acid and attack either the bases or the phospho-ester bonds It is also consistent with investigations on electron spin resonance made by Kirby-Smith[11], these detect radical attack and suggest that ionizing radiation should at first damage the protein of a nucleoprotein complex Thus it seems unlikely that γ-irradiation would readily cause denaturation of the nucleic acid in the nucleoprotein, for this is now known to be effected by the free radicals[12]

We thank the Medical Research Council for financial assistance and Dr F Ellis and Mr R Oliver for their co-operation in the use of the cobalt-60 source of the Radiotherapy Department, Churchill Hospital, Oxford

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TRANSMISSION OF MOUSE LEUKÆMIA WITH PURIFIED NUCLEIC ACID PREPARATIONS

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LEUKÆMIA in the mouse has been demonstrated to be transmitted by hereditary factors There exist certain inbred strains where the spontaneous incidence of this disease is high Because of known genetic transmission of leukæmia I have attempted to transmit the disease with purified nucleic acid preparations from both normal and leukæmic tissues of mice of one of these inbred strains with a high spontaneous incidence of the disease

Gross[1], in 1951, demonstrated that leukæmia of *AKR* mice could be transmitted to $C3H_f/Bi$ mice if they were inoculated with cell-free filtrates of *AKR* leukæmic tissues when new-born Law[2] was able to increase the incidence of spontaneous leukæmia in $(C3H_f \times AKR)F_1$ hybrid mice from 27 7 per cent in control animals to 60 9 per cent in experimental animals by implanting in them fragments of normal thymic tissue from young *AKR* mice Stewart[3] increased the incidence of leukæmia from 23 per cent to 60 per cent in this same hybrid cross by inoculating cell-free filtrates of *AKR* leukæmic tissues into new-born animals Because of the work of these investigators indicating strain susceptibility of the $(C3H_f \times AKR)F_1$ hybrid and $C3H_f/Bi$ mice to transmission of *AKR* leukæmia, new-born hybrid mice and $C3H_f/Bi$ mice were utilized as recipient animals for nucleic acids prepared from tissues of normal and leukæmic *AKR* mice

Preliminary experiments[4] carried out in this Laboratory showed that when nucleic acids were injected subcutaneously into suckling mice less than 24 hr of age of an F_1 hybrid of a $C3H_f \times AKR$ cross, there was an increased incidence of leukæmia in the injected animals when compared with the controls Nucleic acids injected into new-born $C3H_f/Bi$ mice did not result in leukæmia

In the work to be reported here it has been possible to demonstrate, with larger numbers of animals than previously reported, that there is a significant increase in the incidence of leukæmia in $(C3H_f \times AKR)$ F_1 hybrid mice when injected with purified nucleic acid preparations from lymphoid tissues of *AKR* mice These nucleic acids have not been associated with leukæmia development in $C3H_f/Bi$ mice, but other tumours have been seen to develop in one small group of these mice injected with nucleic acid preparations into the thymus

The *AKR/JAX* mice used as a source of spontaneous leukæmia were from an inbred colony that has been maintained in this Laboratory for four years The original litter came from the Jackson Memorial Laboratory, Bar Harbor, Maine

The $C3H_f/Bi$ mice have been maintained as an inbred strain for five years, the original litter being a gift from Dr L Gross, Veterans Administration Hospital, Bronx, New York.

The F_1 hybrid mice were a cross between *AKR* males and *C3H* females of these two strains

The animals were housed in wire-mesh cages containing stainless-steel-sponge basins with wood shavings for nests They were supplied with Rockland rat diet and water *ad libitum* and the pregnant females were given lettuce and oatmeal once weekly The new-born mice used for inoculation were 12–14 hr of age in the subcutaneously injected group and 24–72 hr of age in the intravenously injected group The subcutaneous injections were made on the back with a 30-gauge needle and sealed with collodion in most cases The intravenous injections were made with a 33-gauge needle into the orbital vein The animals were observed until natural death Autopsies were performed in all cases and appropriate organs taken for microscopic investigation when indicated

The nucleic acids were prepared by the method of Simmons as previously described[4], preparations used for intravenous injection substituted xylene sulphonate for sodium dodecyl sulphate as a detergent The final nucleic acid yield from 5 gm of tissue was dissolved in physiological saline and stored as a 1 per cent solution in 1 ml aliquots at −10° until used The preparations contained both deoxyribonucleic acid and ribonucleic acid No attempt was made to separate the nucleic acids in these experiments These preparations will be referred to as DNA–RNA

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The leukæmias in both experimental and control animals were grossly and microscopically similar to the disease which was seen to occur spontaneously in the *AKR* mice The morphology of the tumour was similar to that described by Dunn[5] as a lymphocytic neoplasm or lymphocytic leukæmia

Experiments with $(C3H_f \times AKR)F_1$ *Hybrid Mice* The first group of animals was injected subcutaneously with DNA–RNA $(C3H_f \times AKR)F_1$ hybrid mice of 12–48 hr of age were used The results are summarized in Table 1 Forty-five animals were injected with 0 1 ml of DNA–RNA Seventeen (37 7 per cent) of these animals developed leukæmia Nineteen had received nucleic acids prepared from pooled spleen and lymph nodes of non-leukæmic *AKR* mice with eight developing leukæmia Twenty-six animals had received DNA–RNA from leukæmic *AKR* tissues (spleen, thymus and lymph node) Nine of these developed leukæmia 175 animals were from non-injected litters of the same hybrid cross These animals were raised concomitantly with the experimental animals and under the same conditions Thirty-five animals in this group developed leukæmia, an incidence of 20 per cent The mean age of development of leukæmia was approximately 12 months in both groups The onset of the disease was 6–22 months in the injected group and 8–20 months in the controls These results give a *P* value of less than 0 01 and confirm the results obtained in the preliminary experiments reported elsewhere[4]

Table 1 RESULTS OF INJECTION OF PURIFIED NUCLEIC ACID PREPARATIONS FROM *AKR* MICE INTO $(C3H_f \times AKR)F_1$ HYBRID MICE AT 12–72 HR OF AGE

Amount and route of injection	No of animals	No of leukæmias observed	Percentage
0 1 ml DNA–RNA subcutaneously	45	17	37 7
No injection	175	35	20 0
0 05 ml DNA–RNA intravenously	54	20	37 0
No injection	108	25	23 0

A second group of $(C3H_f \times AKR)F_1$ hybrid animals was injected intravenously with 0 05 ml of DNA–RNA from leukæmic *AKR* tissues The injections were made in the orbital vein at 24–72 hr of age The controls again were alternate non-injected litters The results of these experiments are included in Table 1 Fifty-four animals were injected with 0 05 ml of DNA–RNA intravenously Twenty of these animals (37 per cent) developed leukæmia at an average age of 10 5 months 108 control mice were observed and 25 mice (23 per cent) developed leukæmia at an average age of 10 7 months The *P* value for these results was less than 0 05

Tumour cells from ten of the leukæmias developing in mice injected with DNA–RNA were implanted intraperitoneally into 2-month-old $C3H_f/Bi$, *AKR*, and hybrid mice None of 20 *C3H* mice developed leukæmia Four of 19 hybrid mice and four of 23 *AKR* mice developed leukæmia In one case leukæmias occurred in *AKR* and hybrid mice receiving the same cell suspension The leukæmias developed within 4 wk of injection of cells and were assumed to be due to progressive growth of injected cells and not to have developed spontaneously

Tumour transfers were carried out from cells of three leukæmias developing in control hybrid mice No leukæmias developed in the 5 injected $C3H_f/Bi$ and 4 hybrid mice, but 2 out of 4 injected *AKR* mice developed the disease within 4 wk of transfer of cells This would indicate that the leukæmias developing in the control and DNA–RNA injected mice had essentially the same transfer characteristics However, the lack of consistent growth of these tumours in hybrid mice is difficult to explain

Experiments with $C3H_f/Bi$ *Mice* A group of similar experiments was carried out utilizing the $C3H_f/Bi$ mice Ninety mice were injected intravenously with DNA–RNA at 12–72 hr of age Two leukæmias developed at 11 months

Three litters were injected with 0 05 ml of a DNA–RNA preparation to which had been added an equal volume of 50 per cent sucrose These animals were injected into the thymus at 2–5 days of age The majority of the animals died during the first 24 hr after injection However, five animals in this group survived Three of these animals developed parotid gland carcinomas and one developed an adrenal gland neoplasm The parotid tumours occurred at 7, 9 and 10 months of age and were local bilateral growths The adrenal tumour was found at 10 months of age It was metastasized to the liver and thoracic lymph nodes The remaining mouse died of pneumonia at four months of age

Four litters were injected with 0 05 ml of cell-free 'Selas'-filtered extract of *AKR* leukæmic tissues into the thymus at 2–5 days of age Eight of these animals survived Their litter mates died during the first 24 hr after injection The cell-free extract was prepared as that described previously[6] None of the eight animals that had received cell-free extract developed neoplasms or leukæmia

One leukæmia was found in 114 non-injected control $C3H_f/Bi$ mice raised at the same time and under the same conditions as the experimental animals

Other work[4] had shown that the subcutaneous injection of DNA–RNA at 12–24 hr of age in seventeen $C3H_f/Bi$ animals did not result in leukæmia development However, cell-free ('Selas'-filtered) extracts when injected into 36 mice of this strain resulted in the development of ten leukæmias No leukæmias were seen in non-injected control animals

A summary of the results of these experiments with $C3H_f/Bi$ mice is given in Table 2 The tumours that developed in the DNA–RNA injected mice are grossly and histologically similar to those described by Stewart[7] as occurring in $(C3H_f \times AKR)F_1$ hybrid mice after injection with cell-free extracts and later shown by Stewart and Eddy to be due to the polyoma virus[8] Neoplasms of the parotid and adrenal gland have not been noted to occur spontaneously in the $C3H_f/Bi$, *AKR* or hybrid colonies

These experiments demonstrate that the injection of nucleic acid preparations from normal and leukæmic *AKR* mice into new-born $(C3H_f \times AKR)F_1$ hybrid mice is associated with a statistically significant increase in the incidence of leukæmia in the injected mice when compared with non-injected controls

Table 2 RESULTS OF INJECTION OF PURIFIED NUCLEIC ACID PREPARATIONS COMPARED WITH THOSE OF CELL-FREE EXTRACTS AND NON-INJECTED CONTROLS IN $C3H_f/Bi$ MICE

Material	Age injected	Route of injection	No of animals	No of leukæmias	Other tumours
Cell-free extract	12–24 hr	Subcutaneous*	36	10	0
Cell-free extract	2–5 days	In thymus	8	0	0
DNA–RNA	12–24 hr	Subcutaneous*	17	0	0
DNA–RNA	12–72 hr	Intravenous	90	2	0
DNA–RNA	2–5 days	In thymus	5	0	4†
No injection			114	1	0

*These results are taken from previously published experiments (ref 4)

†3 Parotid tumours, 1 adrenal tumour

However, nucleic acids from leukæmic *AKR* mice prepared and injected in the same manner do not increase the incidence of leukæmia in $C3H_f/Bi$ mice

These two observations would seem to indicate that an inherited tendency to develop leukæmia in the hybrid mouse can be accentuated by the addition of nucleic acids from lymphoid tissues of a high-incidence leukæmia strain in the new-born period This is possibly an observation of the effects of addition of more *AKR* genetic material with leukæmogenic properties to that of a mouse with an inherited leukæmic tendency from its *AKR* parent

The $C3H_f/Bi$ mouse is not susceptible to leukæmia induction with nucleic acids but is susceptible to induction of the disease with cell-free extracts of *AKR* tissues, presumably containing a virus[1] This would suggest that viral nucleic acid is not involved in the observations made in the hybrid strain and that the situation is more analogous to that observed in bacterial transformation where the DNA of the donor combines with that of the recipient, resulting in the appearance of donor characteristics in the recipient bacterium That is, the hybrid mice, after having received *AKR* nucleic acid, are more like their *AKR* parent, so far as tendency to develop leukæmia is concerned, than non-injected F_1 hybrids

The observation of the induction of parotid and adrenal tumours in $C3H_f/Bi$ mice injected into the thymus with nucleic acid from *AKR* leukæmic tissues would indicate that this nucleic acid preparation very likely contained DNA from polyoma virus This preparation was made from a different *AKR* leukæmic mouse than that utilized for the subcutaneous and intravenous injections An agent that induced parotid gland and other neoplasms in $C3H_f/Bi$ mice was found initially in cell-free extracts of tissues of the leukæmic *AKR* mouse[9] Latarjet[10] has carried out experiments using the detergent method of isolating nucleic acids from *AKR* leukæmic tissues He also found no increased incidence of leukæmia in *C3H* mice injected subcutaneously with these nucleic acid preparations at birth, but 5 per cent of the injected animals after an average latency of 202 days developed multiple tumours which were interpreted by the author as being due to the inclusion of DNA from the polyoma virus in his preparations The experiments of Di Mayorca *et al* [11] and Smith *et al* [12] have shown the polyoma virus to be a DNA virus

Experiments are now in progress to investigate the effects of nucleases on the induction of leukæmia and other tumours with these nucleic acid preparations A group of hybrid mice receiving nucleic acid preparations with litter-mate controls is under investigation

This work was supported by contract *AT*(04–1)–*GEN*–12 between the Atomic Energy Commission and the University of California at Los Angeles

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ENHANCED EFFECT OF TOTAL-BODY X-IRRADIATION IN MICE UNDER MILD HYPOTHERMIA

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It is well established that the sensitivity of a variety of biological species to damage by ionizing radiation is dependent on the presence or absence of oxygen in the environment at the time of irradiation An increase in tissue oxygen can be brought about through breathing oxygen under raised pressure or by lowering its consumption by means of hypothermia

The use of hypothermia in man to raise the cerebral oxygen concentration and thereby perhaps increase the radiosensitivity of intracranial tumours is being investigated at the Royal Marsden Hospital, London This has been undertaken to try to improve the extremely poor results obtained by conventional methods of treatment in patients suffering from malignant grades III and IV cerebral astrocytomata (glioblastoma multiforme) Under generalized mild hypothermia (30–32°) moderate doses of whole brain X-irradiation (300–450 r given in daily fractions of 50–150 r) appear to produce an exaggerated cerebral reaction[1] Many problems were encountered in this treatment and, while the clinical work was in progress, the present investigation was undertaken to investigate the influence of hypothermia on the response of mice to whole-body irradiation

Eight-week-old albino male mice of Theiler's original strain were anæsthetized with intraperitoneal sodium pentobarbitone (approximately 50 mgm /kgm body-weight) and then suspended by a plastic collar in a thermostatically controlled water-bath, the entire body with the exception of the head being immersed The bath was kept at various temperatures between 29° and 37° (Table 1) and the colonic temperature of each mouse was continuously recorded by a mercury thermometer *in situ* The temperatures of the anæsthetized mice fell to approximately 29° (room temperature, 20°) and remained in this region until they were placed in the water-bath, the colonic temperatures reached that of the bath within 10 min of immersion

The mice were irradiated five at a time in the water-bath and allowed to remain immersed for 20 min before and after the irradiation The X-ray factors were 140 kV , 5 m amp , focal skin distance 38 cm , no added filtration, half-value layer 2 mm aluminium, dose-rate 25 r /min After removal from the bath the mice were placed in a box at 37° to recover and dry out

The effects of total-body irradiation with a dose of 550 r was studied, but the head, not being shielded by the water, received 12 per cent more irradiation For each of the temperatures used, 10 control mice were treated under identical conditions, but shielded in each case from irradiation by a 3-mm thick lead screen

In these experiments the criterion of an irradiation effect was death of the animal within 30 days The majority of fatalities occurred between the tenth and twentieth day following irradiation There did not appear to be any difference in the mode of death at the different temperatures, nor with the number of animals involved could a significant relationship be established between time of death and temperature

The results (Table 1) show an increased mortality among the animals treated under mild hypothermic conditions (31–35°) At a temperature of 29° a protective effect was noted No deaths occurred in any of the control animal groups

Table 1 EFFECT ON MOUSE SURVIVAL OF COMBINED HYPOTHERMIA AND 550 R TOTAL-BODY X-IRRADIATION

Temperature	No. of mice	Survivals	Died (per cent)
29°	19	19	0
31°	19	7	63
33°	18	11	39
35°	18	13	28
37°	21	18	14

Ten control animals for each temperature, shielded from irradiation no deaths

Hypothermia, by lowering the metabolic rate and by increasing the amount of oxygen dissolved in the plasma, may lead to an increase in the oxygen environment of tissues and thereby perhaps an increase in radiosensitivity Working against these tendencies to an increased oxygen tension in hypothermia, however, may be (1) a reduction of blood-flow due to the decreased cardiac output, the increased peripheral resistance and the increased viscosity of the blood, (2) a shift of the oxygen dissociation curve to the left, which will interfere with the liberation of oxygen from hæmoglobin, (3) a fall in the rate and depth of respiration On the other hand, normal well-oxygenated tissues are considered to be practically at their maximum radiosensitivity In such circumstances no appreciable increase in response to ionizing radiation is expected to follow a further rise of oxygen tension, as opposed to relatively anoxic foci in tumours which have outgrown their blood supply[2] The oxygen tension in normal bone marrow, however, appears to be low compared with that of muscle and subcutaneous tissue[3] If this is true, hypothermia may increase the oxygen environment and therefore the radiosensitivity of normal marrow The chief cause of death in mice following 550 r whole-body irradiation is marrow failure Alternatively, the lowered temperature may increase radiosensitivity by interfering with the efficiency of detoxifying and repair mechanisms or the transport of tissue breakdown products A third possibility is that hypothermia, like mild whole-body irradiation, may increase the response to local irradiation[4] through the production of certain plasma factors[5] A recent report[6] suggests that a greater difference in litter size, in viability at birth, in developmental curves and in behaviour changes occur in chicks following whole-body pre-natal irradiation of the mother compared with localized direct irradiation of the gravid uterus

The influence of temperature on the degree and the timing of response to γ- and X-ray irradiation of various biological systems have been investigated by many workers with somewhat conflicting results In general, profound hypothermia (15° C or less) reduces the damage resulting from irradiation, and such observations have recently been reviewed by Weiss[7] Heat, on the other hand, appears to have an additive effect on irradiation[8,9]

Although most investigators report that hypothermia reduces radiosensitivity, an increased effect at reduced temperatures has been reported in the broad bean root[10], in *Tradescantia* microspores[11], in mouse skin[12] and in mouse carcinoma tissue[13] Changes of temperatures at the time of irradiation do not appear to influence the response of isolated mammalian cells *in vitro*[14–16], but may bring about pronounced effects in more complex tissue systems during *in vivo* studies[7,17–20]

The results of the present investigation appear to support a protective effect for hypothermia at 29° against ionizing radiation, but reveal an enhanced effect of irradiation with a lesser degree of hypothermia, the peak being at 31–32° The difference in survival-rate following whole-body irradiation at 31° and 29°, a change of only 2 degrees, is striking The results in mice reported here and the severe cerebral reactions observed in patients treated by whole-brain irradiation[1] suggest that the increased response to irradiation during mild hypothermia is more likely to be due to systemic humoral factors rather than a local oxygen effect (for example, stress endocrine changes, tissue breakdown products)

These experiments, including an investigation into the possible mechanisms involved, will be reported in greater detail elsewhere

We wish to thank Dr P Alexander for his helpful criticism and discussions

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SIMPLIFIED TECHNIQUE FOR ISOLATION OF SUB-CELLULAR PARTICLES

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THE heterogeneity of particles isolated in the classical sub-cellular fractions[1-3] makes it desirable that tissue homogenates be divided into a greater number of fractions each of which can be tested for enzymic activity and chemical composition Density gradient centrifugation has been widely used for this, but it has certain disadvantages These are difficulty in constructing reproducible gradients, especially of the continuous variety, difficulty in estimating the sizes of the particles collected[4], and difficulty in collecting the particles from the various levels in the gradient after centrifugation Moreover, in order to construct gradients of sufficient density to separate particles, many workers have used extremely hypertonic solutions which have probably had deleterious effects on the morphology and activity of the particles collected

My technique utilizes layering of aliquots of a homogenate in 0 88 *M* sucrose over relatively large amounts of clear 0 92 *M* sucrose solution, and centrifugation of a series of these preparations at successively greater forces Each increased force sediments all the particles sedimented by previous forces, together with some smaller particles not sedimented by the lower forces The value of using 'two phase' preparations has been demonstrated[5] Only particles which sediment completely are collected in the precipitate Smaller particles are decanted with the supernatant, and since all the particles start sedimenting at essentially the same level at the top of the tube, there is negligible contamination of desired particles in the precipitate with smaller particles The clear phase, moreover, washes the particles as they sediment and frees them of contaminants

DeDuve *et al* [6] have shown that the integral force applied to a preparation can be approximated by multiplying the force used by the time during which it was applied The product is expressed in gravity-minute (*g*-min) units I have found it desirable to use the swinging bucket rotor (*SW* 39) in the 'Spinco' model *L* centrifuge because sedimenting particles in angle rotors move first to the centrifugal side of the tube and then slide down the tube to the bottom In the horizontal rotor the distance that a particle must move from the thin layer of homogenate to the bottom of the tube can be measured accurately, and at the end of centrifugation there are fewer particles adhering to the sides of the tube

No special precautions were taken to prevent vibration during acceleration and deceleration of the rotor Such vibration would appear to be less critical in this procedure, with a packed sediment well separated from suspended particles, than in density gradient methods, where zones of suspended particles must be stabilized The acceleration and deceleration times were short relative to centrifugation time at top speed, and only the latter was used in the calculation To test the efficacy of our technique, a survey of uricase and cytochrome *c* oxidase in the particles of rat liver was done Particles were sedimented by layering 0 5 ml of 20 per cent rat liver homogenate in 0 88 *M* sucrose over 3 0 ml of 0 92 *M* sucrose and centrifuging a series of these preparations for varying periods of time at different speeds Aliquots of the supernatants were used for enzymic assays Cytochrome *c* oxidase was assayed by the technique described by Smith[7] Uricase was assayed by the technique of Hogeboom and Schneider[8] with modifications as suggested by Kalckar[9] The results are shown in Fig 1 The activity of the enzyme in each supernatant is plotted against the integral centrifugal force used to prepare it The centrifugal forces shown are those applied at the bottom of the tube The curves thus show the disappearance of these enzymes from the homogenate and indicate the forces required to precipitate the particles containing the enzymes

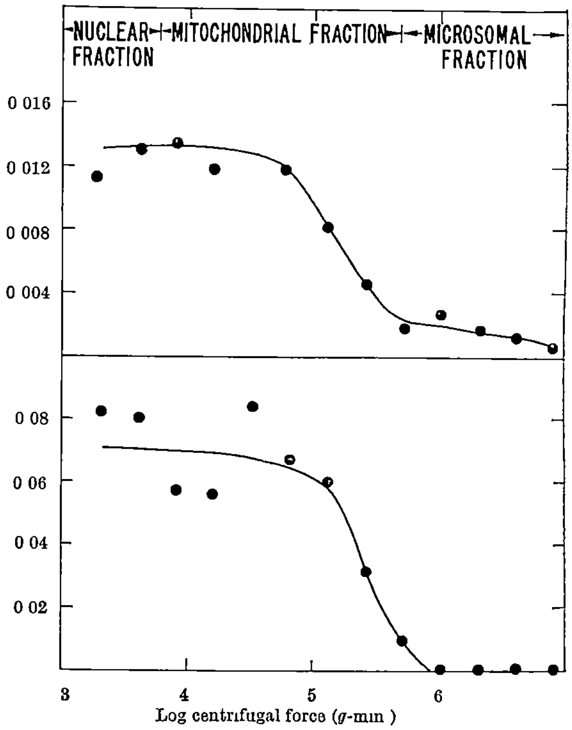

Fig 1 Distribution of uricase and cytochrome *c* oxidase in sub-cellular particles of rat livers

Ordinates, cytochrome *c* oxidase activity (top) is given as the constant for the first order reaction observed colorimetrically in sec $^{-1}$ units, uricase activity (bottom) as micromoles of uric acid destroyed by each fraction in 30 min

Comparison of the forces needed to precipitate these enzymes with those used to prepare classical sub-cellular fractions[10] (indicated at the top of Fig 1) shows that both of the enzymes are 'mitochondrial' The range of forces precipitating particles containing either of these enzymes is much narrower, however, than the range used to prepare the whole mitochondrial fraction From a knowledge of these forces, a fractionation method can be devised by which particles containing either of these enzymes can be isolated in the absence of other particles usually isolated with the 'mitochondrial fraction'

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Calculation of the diameters of the particles (assuming that they are spherical) can be made using the following expression, which may be derived from the equations given by DeDuve and Berthet[6]

$$d^2 = \frac{63\ \eta\ (\log_{10} r_2 - \log_{10} r_1)}{(\sigma - \rho)\ (\text{r p m})^2\ T}$$

where d = particle diameter (cm), η = viscosity of the suspending medium (poises), T = time for sedimentation (min), σ = particle density (gm /c c) ρ = density of the suspending medium (gm /c c), r_1 = the distance from the axis of rotation to the surface of the suspension in the tube (cm), r_2 = the distance from the axis of rotation to the bottom of the centrifuge tube (cm), and r p m = the speed of centrifugation (revolutions per minute)

The value for the density of these particles was that determined in sucrose and ordinary water by Beaufay *et al*[11] The approximate average density of 1 22 was used for both cytochrome *c* oxidase and uricase, although the uricase particles appear to be less homogeneous Since this value was determined by isopycnic centrifugation, the density of any particle undergoing osmotic shrinking will be increased relative to that in isotonic solutions or in 0 92 *M* sucrose used in the present experiments Its diameter, calculated from the above equation, will be underestimated Density values obtained in sucrose–deuterium oxide gradients[11] were not used because of the increased density of uricase apparently due to bound deuterium oxide The values for the density and viscosity of 0 92 *M* sucrose were obtained from DeDuve *et al*[12]

The diameters calculated from the data in Fig 1 showed a range of 0 52–1 58μ for cytochrome *c* oxidase-containing particles, and 0 26–0 75μ for those containing uricase These may be compared with the values reported by Beaufay *et al*[11], where calculation was made by reference to 'standard conditions' of 0 25 *M* sucrose and 0° C They found the diameter of cytochrome *c* oxidase-containing particles to be about 0 5–1 2μ and of uricase-containing particles, 0 2–0 7μ Other workers have also found the 'mitochondrial' oxidative enzymes to be associated with larger particles than the 'mitochondrial' (lysosomal) lytic enzymes[10,13]

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ALTERATIONS IN CONNECTIVE TISSUE AND INTESTINE PRODUCED BY HYPERVITAMINOSIS A IN *XENOPUS LAEVIS*

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RECENT experiments have indicated that vitamin A may liberate from intracellular lysosomes a cathepsin-like protease active on such extracellular materials as cartilage matrix[1]. Thus the depletion of cartilage matrix observed in rabbits made acutely hypervitaminotic A resembled that produced by an exogenously administered protease, papain[2] Considerable catheptic activity has been demonstrated in the tails of amphibian larvæ, this is greatest at the time of tail resorption during metamorphosis[3] If the hypothesis of vitamin A action on lysosomes is correct, resorption of the tails of amphibian larvæ might accompany hypervitaminosis A, since the vitamin would cause release of cathepsins from the lysosomes, which Novikoff has shown are abundant in amphibian tails[4].

Larvæ of *Xenopus laevis* were therefore exposed to high doses of vitamin A, and the effect of this treatment on gross and microscopical structure was observed. Although the predicted effects on connective tissue were seen, and will be briefly noted, this article is chiefly concerned with an entirely unexpected effect of this régime on the intestines of the larvæ

Larvæ of *Xenopus laevis*, obtained through the generosity of Dr. R T Sims, Department of Anatomy, University of Cambridge, were grown under conditions suggested by Nieuwkoop and Faber[5], at room temperature and fed with nettle powder thrice weekly Twenty-eight animals were given 2 mgm of synthetic vitamin A alcohol ground with their food twice a week the tank contained 800 ml of tap water for each group of 14 larvæ An equal number served as controls

Treatment was continued for three months, beginning with larval stages 49–50 By the end of the experiments, the treated animals had progressed to stage 59–61, while some of the controls had undergone metamorphosis After three weeks, six of the experimental animals were transferred to fresh water, and given the normal diet without added vitamin A At varying intervals, larvæ were killed with 'Nembutal', fixed *in toto* in acetic alcohol, embedded, and sections were stained with hæmatoxylin and eosin and with toluidine blue

Grossly, the first change noted was a drooping of the tentacles of the larvæ exposed to the vitamin This occurred on the fourteenth or fifteenth day of treatment, and resembled in many ways the drooping of the ears of rabbits made hypervitaminotic A, or of those given papain intravenously[2] After 18-20 days, however, another change became evident The stools of the animals, which had been well-formed, dark, and solid, assumed a mucinous, thready appearance and floated in strands at the surface of the tank This change persisted to the end of the experiment, and simple addition of vitamin A to a suspension of normal stools did not produce a similar

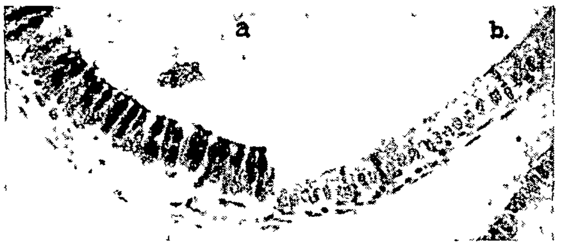

Fig 1 Photomicrographs of fore-gut of *Xenopus laevis* larvæ (stage 53–54) stained with toluidine blue (*a*) is from an animal exposed to high doses of vitamin A alcohol for 42 days There is a marked overgrowth of metachromatically staining goblet cells, and the epithelium is more columnar than in the control (*b*) (× 216)

change That this represented diarrhœa was readily observed by isolating individual normal and hypervitaminotic larvæ and noting the increased number of stools eliminated by the latter By the twenty-first day of the experiment, most of the hypervitaminotic larvæ had developed pronounced kyphoscoliosis and also showed definite resorption of the tail, with a loss of the fine flagellar structure of that appendage, several actually developed fractures of the tails, along with shrivelling and resorption Associated with the gross skeletal deformity was a change in the normal apposition of the upper and lower jaws, the experimental group having developed a prognathic appearance

Histologically, the most remarkable change was in the gut of the hypervitaminotic animals Sections of gut taken before the appearance of mucinous stools were unremarkable, but those taken two or three weeks later showed a great increase in the number of metachromatically staining goblet cells (Fig 1*a*) These, situated mainly in the proximal gut and in the lower colon, were increased not only in absolute number (per high-power field in sections cut at 7µ) but also in ratio to the normal cells of the epithelium These metachromatic cells were secreting discrete strands of metachromatic material into the lumen The fæcal material in the gut of treated animals was diffuse, mucinous, and mixed with metachromatic strands, thus contrasting with similar debris in the lumen of controls where it was compact and entirely orthochromatic

The whole character of the hypervitaminotic gut was altered At comparable larval stages, the intestines of the treated animals had better developed villi, abnormally deep columnar epithelium (Fig 1*a*, *b*) and at stages before metamorphosis (stages 49–60) the gut closely resembled the organ in control animals after metamorphosis, except for the remarkable overgrowth of goblet cells In other structures, especially in the resorbing tail and the collapsed tentacles, there was a decrease of the metachromatically staining material of the ground substance, in these chronically hypervitaminotic animals the metachromasy of cartilage was almost unaffected, unlike that of acutely hypervitaminotic rabbits[2] Those larvæ which were placed in the recovery group lost all the gross morphological stigmata of hypervitaminosis A within 21 days, and their gastro-intestinal tracts were indistinguishable histologically from those of the controls, despite the continuance of mucinous diarrhœa for 8–10 days after transfer to a medium without vitamin A

The above findings are in accord with two recently postulated effects of vitamin A Moretti and Wolf[6] have found that mucopolysaccharide-bound hexosamine is decreased in the colon of hypovitaminotic A rats, and have suggested that vitamin A is necessary for mucopolysaccharide synthesis It has been postulated that vitamin A causes the release of a proteolytic enzyme from cells or their organelles[2–7] which afterwards acts on the protein–polysaccharide complexes of connective tissue In amphibian tails and tentacles the means for their own resorption during metamorphosis must be 'built in' as an active lysosome–cathepsin system, and the observation that these structures are affected by vitamin A, would support the lysosomal theory of vitamin A action[1] If the degradation of mucoprotein complexes in the epithelium of gut or in the ground substance of connective tissue by cell-released proteases were to act as a stimulus for the resynthesis of protein-polysaccharide complexes by competent cells, then the lysosomal action would explain the intestinal as well as the cartilagenous changes

This work was done in the laboratory of Dr S Fitton Jackson during the tenure of a special research fellowship of the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service

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SEPARATION OF BACTERIA BY COUNTER-CURRENT DISTRIBUTION

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THE method of counter-current distribution has been successfully applied to the separation of important biochemical substances[1] Even very large particles, such as proteins of high molecular weight and viruses, may be studied by this method, if aqueous-phase systems containing two different water-soluble polymers are used[2–4] In this case, the use of organic solvents, which may cause denaturation, is avoided

In the present article we report some results of work on the distribution of whole cells, in this case bacteria, in these aqueous polymer phase systems It has been found that the nature of the distribution in a given phase system of this type is frequently different for different bacteria. On the basis of this observation, it has been found possible to separate even quite closely related bacterial types by means of counter-current distribution The

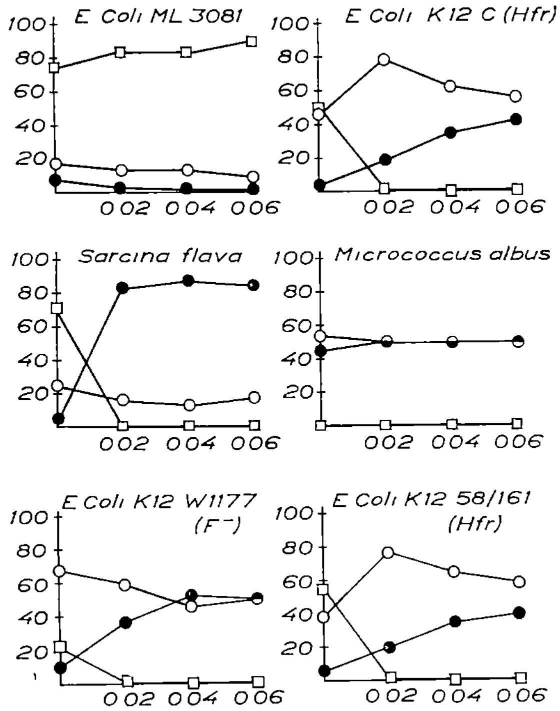

Fig 1 Distribution patterns of different types of bacterium in phase system (D_5^{48}–PEG_4^{6000}) in the absence and presence of sodium chloride Abscissa, concentration of sodium chloride (*M*), ordinate, quantity of cells present in each phase and at the interface (per cent of total added) Experiments carried out at 20° C Bacteria cultured overnight on Penassay broth at 37° C, collected in growing state and afterwards washed twice in 0 01 *M* potassium phosphate buffer, *p*H 6 9, at 5° C In general, the distribution of each bacterial type examined by mixing 1 ml of washed cell suspension of *E*(1 cm, 400 mμ) = 8 0 with 9 gm of aqueous polymer solution to produce about 10 gm of cell/phase system mixture, in which the polymers were at their correct concentration The bottom phase of this mixture had a volume of 4 6 ml and the top phase a volume of 5 2 ml
●, Bottom phase, ○, interface, □, top phase

results obtained may be of interest not only to microbiologists but also to other cell research workers, since in principle it should also be possible to apply the method to the separation of other types of whole cells

Phase systems consisting of aqueous solutions of dextran and polyethylene glycol have been used for examining the distribution of bacteria Systems containing these polymers have previously been employed mainly for studying the distribution of proteins[2,3] The particular phase system that was found most appropriate for the studies on bacteria was that containing 5 per cent w/w dextran ('Dextran 500', limiting viscosity number 48 ml /gm, obtained from A. B Pharmacia, Uppsala, Sweden) and 4 per cent w/w polyethylene glycol ('Carbowax 6000', average molecular weight 6,000–7,500, obtained from Carbide and Carbon Chemicals Co, New York) This phase system will be designated (D_5^{48} – PEG_4^{6000}) in this article The aqueous solution of these polymers was lightly buffered with 0 01 *M* potassium phosphate buffer, *p*H 6 9 The distribution of proteins in the dextran/polyethylene glycol systems was found to be markedly influenced by the presence of electrolytes[2,5] It seemed therefore of interest also to examine the effect of electrolytes on the distribution of bacteria Accordingly, the effect of increasing concentrations of sodium chloride on the distribution of each bacterial type in (D_5^{48} – PEG_4^{6000}) was also studied

The behaviour of a number of different types of bacteria under these conditions is illustrated in Fig 1 In the case of *Escherichia coli ML* 3081, it can be seen that in the absence of sodium chloride the majority of the cells are suspended in the top phase, and that increasing concentrations of salt scarcely affect this distribution pattern at all It appears that other *E coli ML* strains also behave in a similar fashion In the case of the *E coli K* 12 *C* (*Hfr*) strain, too, a large portion of the cells are present in the upper phase in the absence of salt Increase of salt concentration to 0 02 *M*, however, causes these cells to be transferred to the interface and the bottom phase It seems to be a general rule that the distribution of bacterial cells in this phase system is more sensitive to increasing concentrations of sodium chloride in the region 0 00–0 02 *M* than at higher salt concentrations Thus, the distribution of *Sarcina flava* is also altered markedly by increasing the concentration of sodium chloride to 0 02 *M* In the absence of salt, the majority of the cells of this bacterium are present in the top phase, but are transferred to the bottom phase when 0 02 *M* sodium chloride is added Increase of the salt concentration to 0 06 *M* does not appear to affect the distribution any further *Micrococcus albus* is another example of a bacterium the distribution of which is scarcely affected by increase of concentration of sodium chloride from 0 00 to 0 06 *M* In this case, about half the cells are present at the interface and about half in the bottom phase at all the salt concentrations used

It is thus clear that different types of bacteria are distributed in quite different fashions in this phase system However, on occasions different bacteria can also behave in the same manner For example, the *E coli K* 12 strains, 58/161 (*Hfr*) and C 600 (*F*⁻), and also *Bacillus megaterium* are distributed in this system in a very similar way to *E coli K* 12 *C* (*Hfr*) (*F*⁻ indicates standard female mating type, *Hfr* indicates male giving high frequency of recombination)

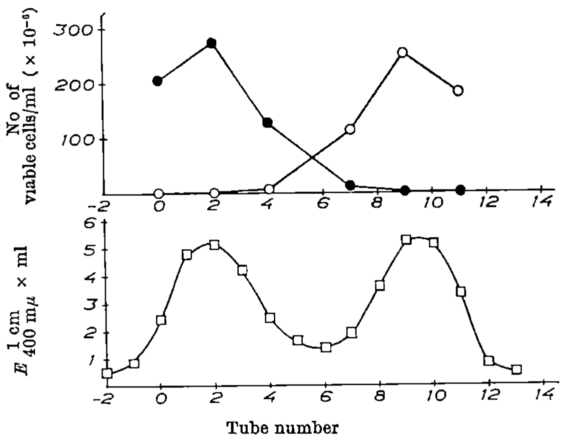

Fig 2 Counter-current distribution of a mixture of *E coli ML* 3081 and *E coli K* 12 *C* (*Hfr*) in phase system (D_5^{48}–PEG_4^{6000}), containing 0 06 *M* sodium chloride Cells cultured and washed as for Fig 1 1 ml samples of *ML* 3081 and *C*(*Hfr*), each of *E*(1 cm, 400 mμ) = 26 0, added to tube No 0 Twelve transfers carried out Lower graph, variation in *E*(1 cm, 400 mμ) × total volume of cell/phase system mixture per tube (7 5 ml) with tube number Upper graph, variation in viable count on *EMB* agar plates with tube number Volume of bottom phase 2 5 ml and of top phase 5 0 ml, of which 0 5 ml kept stationary Total volume per tube therefore 7 5 ml and stationary volume 3 ml. A hand-operated train of 20 glass tubes of the type described by Raymond (ref 6) (obtained from E-C Apparatus Co, Swarthmore, Penn, U S A) was used in this experiment, which was carried out at room temperature (20–23° C)
●, lactose +ve colonies, ○, lactose –ve colonies

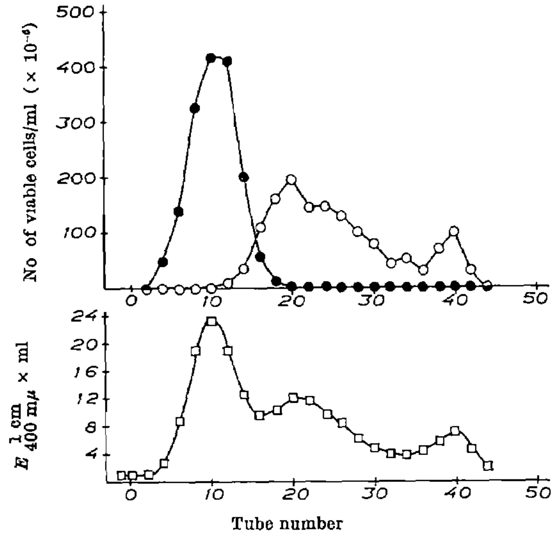

Fig 3 Counter-current distribution of a mixture of the *E coli K* 12 strains *W* 1177 (F^-) and 58/161 (*Hfr*) in phase system (D_5^{48}–PEG_4^{6000}) in the absence of sodium chloride Cells cultured and washed as for Fig 1 5 ml samples of *W* 1177 and 58/161, each of *E*(1 cm , 400 mμ) = 40 0, added to tube No 0 Forty-five transfers carried out Lower graph, variation in *E*(1 cm , 400 mμ) × total volume of cell/phase system mixture per tube (20 7 ml) with tube number Upper graph, variation in viable count on *EMB* agar plates with tube number Volume of bottom phase 9 5 ml and of top phase 11 2 ml , of which 0 5 ml kept stationary Total volume per tube therefore 20 7 ml and stationary volume 10 ml An automatically operated train of glass tubes of the type due to Craig (ref 1) was used in this experiment, which was carried out at room temperature (20–23° C)
●, lactose – ve colonies, ○, lactose + ve colonies

It can then be seen that it is possible to obtain a partial fractionation of cells from a mixture of bacteria which have different distribution patterns in the phase system merely by a one-step procedure To obtain a more complete purification of the cells of one particular type of bacterium from such a mixture, this mixture can be subjected to counter-current distribution The straightforward counter-current procedure, where the top phase is transferred and the bottom phase kept stationary, cannot, however, be used for bacterial cells, as they, in common with other whole cells, are usually to some extent adsorbed to the interface Instead, the whole bottom phase, the interface and a small layer of the upper phase above it can be kept stationary, while the rest of the upper phase is transferred[2] Alternatively, a small layer of the bottom phase, the interface and the whole of the top phase may be transferred, while the rest of the bottom phase is kept stationary In either of these cases, distribution of the particles occurs effectively between the interface and that phase which is moving relative to it

Several mixtures containing two different types of bacteria have been fractionated by this method of counter-current distribution, the results of two such experiments being illustrated in Figs 2 and 3 In both these experiments, the two bacterial types were cultured and washed separately and then mixed, together with phase system, in the first tube of the distribution chain The choice of pairs of bacteria for these two experiments was determined not only by differences in behaviour in phase system but also by the necessity of distinguishing between the members of each pair in a convenient fashion. This latter requirement was met by selecting pairs, one member of which could ferment lactose, and was therefore lactose-positive, while the other could not ferment this substance, and was therefore lactose-negative It was then possible to distinguish between the two by means of the well-known procedure in which bacteria are plated out on agar containing both lactose and indicator (*EMB* agar) As the result of acid production during lactose fermentation, lactose-positive colonies appear dark red when grown on this agar, whereas the lactose-negative remain light pink

From Fig 1, it is clear that in phase system (D_5^{48} – PEG_4^{6000}), the majority of *E coli ML* 3081 cells (lactose-negative) are found in the top phase in the presence of 0 06 *M* sodium chloride, while under these conditions *E coli K* 12 *C* (*Hfr*) (lactose-positive) is found either at the interface or in the bottom phase It should therefore be possible largely to separate a mixture of these two in the presence of this concentration of salt in one step, assuming that the distribution of each is unaffected by the presence of the other Alternatively, complete separation should be obtained by counter-current distribution after only a small number of transfers To demonstrate this, the experiment illustrated in Fig 2 was performed Here, the interface was kept stationary At the conclusion of the experiment it was possible, as described above, to determine the nature and proportion of the bacteria in each tube of the train by plating out on *EMB* agar From the plating results, it is clear that tubes 0–4 and 7–11 of the extraction train contained virtually uncontaminated samples of *K* 12 *C* (*Hfr*) and *ML* 3081 respectively

It is also possible to fractionate mixtures of bacterial cells which have distributions which are much more similar to each other than those of *ML* 3081 and *K* 12 *C* (*Hfr*) Thus, mixtures of the *E coli K* 12 strains *W* 1177 (F^-) (lactose-negative) and 58/161 (*Hfr*) (lactose-positive), the distributions of which are shown in Fig 1, can also be successfully separated by the counter-current procedure From Fig 1 it can be seen that a greater portion of the male cells than of the female cells partition in favour of the top phase of the (D_5^{48} – PEG_4^{6000}) system when no salt is present This implies that it is possible to separate the two strains using a stationary interface, if a sufficient number of transfers is carried out Fig 3 represents the results of an experiment carried out in this manner From the viable counts on *EMB* agar it is apparent that the two cell types were largely separated in this experiment One may assume that, with a larger number of transfers, complete separation could have been achieved The reason for the asymmetric distribution of the *Hfr* cells is under investigation

These results indicate, then, that different types of bacteria frequently have different patterns of distribution in the dextran/polyethylene glycol system It would seem that these distribution patterns, each of which can be expressed as a phase-interface distribution ratio, G_i, can be maintained through a number of transfers in the counter-current apparatus, implying in particular that adsorption of cells to the interface is reversible[2] Furthermore, the distribution of one type of bacterium seems not to be noticeably affected by the presence of another with a different sort of distribution It can be noted here that comparison of viable counts of untreated bacteria and of bacteria which had been subjected to counter-current distribution indicated that treatment with phase system did not apparently have any deleterious effect on the viability of the bacterial cells

It is of interest that even very similar strains, such as the *E. coli K* 12 strains *W* 1177 and 58/161, are distributed in manners which are sufficiently different to allow mixtures of the two strains to be separated by the counter-current procedure. *E. coli K* 12 strains of opposite sex do not seem always to have different distribution patterns, however. For example, as mentioned before, the *E. coli K* 12 female strain *C* 600 is distributed in a very similar fashion to the *E. coli K* 12 male strains 58/161 and *C*. A more detailed study of the behaviour of sex strains in aqueous polymer phase systems and of the influence of surface properties on the distribution of bacteria in these systems is in progress.

We wish to thank Prof. A. Tiselius for his interest in this work. Financial support has been received from the Department of Scientific and Industrial Research in the form of a research fellowship (to G. D. B.), and from the Swedish Technical Research Council.

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EFFECT OF TEMPERATURE DURING GERMINATION ON THE CRYSTALLINE TYPE OF STARCH IN SOYBEAN SEEDLINGS

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THE crystalline structure of native starch granules is not identical, and the three main types, the *A*-, *B*- and *C*-types[1], are known. The *U*-type, which has been found recently in *Ulva expana* starch[2], is an exceptional type. The *A*-type has been found mainly in cereal starches, the *B*-type in tuber starches, and the *C*-type in bean and root starches. Some exceptional cases have been found as, for example, in amylomaize (*B*-type)[3], wrinkled pea (*B*-type)[4] and *Colocasia antiquorum* (*A*-type)[5]. The *C*-type is a transition type between the *A*- and *B*-types and many varieties are known, some resembling the *A*-type (*Ca*-type), some being intermediate (*Cc*-type) and some resembling the *B*-type (*Cb*-type). A transition series from the *A*-type (rice) to the *B*-type (potato) through various *C*-types has been observed by Nikuni *et al.*[6,7]

Some hypotheses have been proposed as to why these various structural modifications occur, but this important problem has not been elucidated. We tried to attack this problem by studying the effect of environmental conditions on crystallization, using amylodextrin (short-chain amylose) as test material[8,9]. It was shown that the crystalline type of amylodextrin obtained depends entirely on crystallization conditions such as the temperature of crystallization[9], the concentration of amylodextrin[8] and the kind and concentration of inorganic ions in the mother liquor[8].

In this article, the effect of temperature on the crystalline type of starch in living plants is reported.

Mature soybeans (*Glycine soja*) contain practically no starch, though starch is produced during the germination period to the extent of several per cent of the weight of the seedlings[10,11]. Therefore, seedlings germinated at different temperatures were very suitable material for this work.

Batches of 50 gm. of soybeans were soaked in water at various constant temperatures (10–40° ± 0.5° C.—the temperature-range at which germination is possible). They were kept in a thermostat at these same constant temperatures for 24 hr., or for 48 hr. when the temperature was below 13.5° C. Then the soaked beans were transferred to wet purified cotton beds in deep Petri dishes (12 cm. in diameter and 5 cm. deep). Beans were germinated in the dark, keeping the thermostat at the same fixed temperatures. It took 3–10 days, depending on the temperature, for seedlings to germinate. During incubation, the seedlings were washed with water at the various temperatures and transferred carefully and quickly once or twice a day to new beds to prevent bacterial infection and to provide sufficient humidity. The water content of the cotyledons was 59–64 per cent and that of hypocotyls was 88–92 per cent.

These parts were homogenized with cold water (about 5° C.) for 6 min. in a homogenizer. The homogenate was filtered through filter paper (Toyo Roshi No. 2), with suction. Starch granules were small enough to pass through the filter paper (0.3–3.5μ in diameter) and were thus separated from the cell debris. The residual cell debris were homogenized twice more in the same way. The resulting filtrates were combined and centrifuged at 3,500 r.p.m. for 5 min. The starch fraction, which sedimented, was washed repeatedly with cold water, and finally with methanol, and was then dried to about 20 per cent moisture content. The resulting starch preparation was examined in an X-ray diffractometer. The X-rays used were nickel filtered copper radiations.

The results showed that the crystalline type of the starch obtained from the cotyledons and hypocotyls of soybean seedlings varied greatly with the temperature at which germination took place. A transition series from the *B*-type to one very similar to the *A*-type (*Ca*-type) was obtained with elevation of the germination temperature. The *B*-type appeared at germination temperature below 13.5° C., the *Cb*-type at 16° C., the *Cc*-type at 22° C., and the *Ca*-type at or above 28° C. There was no difference in the type of the starch in the cotyledons and hypocotyls of the same seedlings.

To investigate a mixed type of starch, an incubation was carried out at alternating temperatures. Thus, soaked beans were kept at 32° C. for the first 10 hr. and then the Petri dish was transferred to a 13.5° C. thermostat for the next 14 hr. This altera-

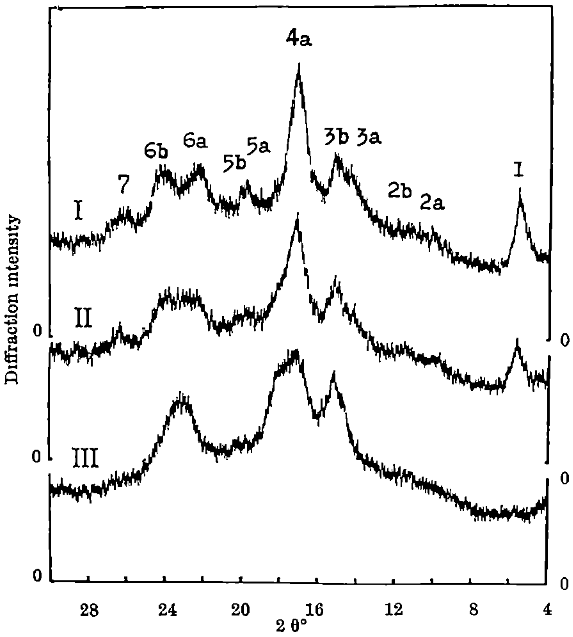

Fig. 1. X-ray diffractometer patterns of starch in soybean seedlings germinated at various constant temperatures. Germination temperature: I, 13.5° C (*B*-type); II, 22° C (*Cc*-type); III, 30° C (*Ca*-type, faint 1-line seen)

tion of temperature was repeated five times during the 5-day period of incubation. The starch obtained from these seedlings was the *Cc*-type, which corresponded to that formed at the intermediate temperature of 22° C. This is further evidence for the idea in ref. 7 that the *C*-type is a mixture of the *A*- and *B*-types.

Typical X-ray diffractometer patterns are shown in Fig. 1. The relation between the diffractometer patterns and the temperature are exactly reproducible, in the same batch of soybeans. Patterns of starch from younger and older seedlings at the same temperature were identical.

This relation between the crystalline type of native starch and the germination temperature coincided with the relation between the crystalline type of amylodextrin and the temperature of crystallization which was reported in ref. 9.

These facts indicate that the crystalline type of native starch is not always decided by the botanical classification of the plant and that it is very much affected by the environmental conditions during the formation of the starch. In fact, some variations in crystalline type have been found in peas[12], mung beans (*Phaseolus aureus*)[13] and sweet-potatoes[14].

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INDUCTION OF A LIGHT REQUIREMENT FOR THE GERMINATION OF LETTUCE SEED BY NARINGENIN, AND ITS REMOVAL BY GIBBERELLIC ACID

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NARINGENIN (5,7,4′-trihydroxyflavanone) has recently been isolated from dormant buds of peach[1], with the suggestion that it is a plant growth-inhibitor playing a functional hormonal role in the control of dormancy[2]. I have been examining some effects of naringenin on several growth and dormancy systems, during the course of which it has become apparent that naringenin exhibits certain properties very similar to that of coumarin, another well-known naturally occurring plant growth-inhibitor.

One of the most interesting effects of coumarin on plants is the observation that it can induce a light requirement for germination in varieties of lettuce that are normally not light-requiring[3], and that such an induced requirement can be removed by gibberellic acid[4]. Thus it was decided to investigate the possibility that naringenin, too, could induce a light requirement by non-dormant lettuce seed, despite its structure being very different from that of coumarin.

Commercially supplied lettuce seed (var. Great Lakes) was used in the following experiments. The seeds were allowed to germinate in either darkness or light in 9 cm. diameter Petri dishes on Whatman 1/64 in. seed-test papers, which had been moistened with either 4 ml. distilled water in the controls, or with 4 ml. test solution. Light was supplied by a bank of 'Daylight' fluorescent tubes 88 cm. above the Petri dishes, and the temperature was maintained at a constant 21° ± 1° C. Germination was allowed to proceed for 48 hr., at the end of which time the percentage germination per treatment was determined. There were approximately 300 seeds in each treatment.

Germination of seeds in a range of aqueous naringenin concentrations revealed that, up to 20 mgm./l. naringenin, there was no inhibition of germination

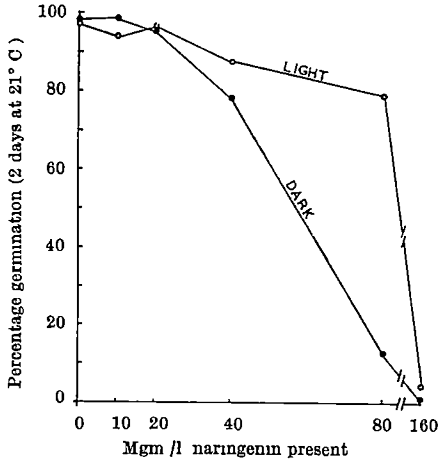

Fig 1 Result of germinating seeds of Great Lakes lettuce (approximately 300 seeds per treatment) at 21° ± 1° C, in aqueous solutions of naringenin, or in water Open points, seeds allowed to germinate in light, closed points, seeds germinated in darkness

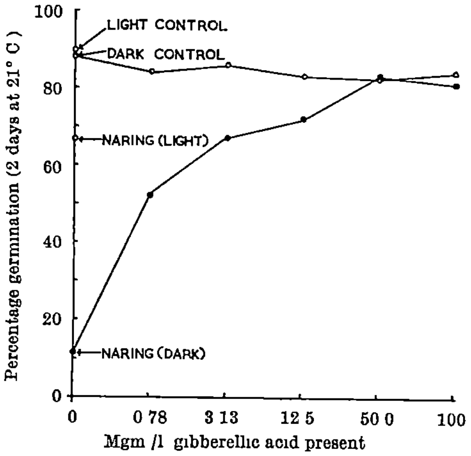

Fig 2 Effect of a range of concentrations of gibberellic acid on the dark-inhibiting effect of an 80 mgm /l solution of naringenin, on the germination of Great Lakes lettuce seed O, gibberellic acid alone, ●, gibberellic acid plus naringenin

in either light or dark, but that at 40 mgm /l, and particularly at 80 mgm /l there was a suppression of germination, with much more inhibition in darkness than in light (Fig 1) This result has been fully confirmed in other experiments Consequently naringenin behaves similarly to coumarin in this respect

The effect of a range of gibberellic acid concentrations on the dark germination of seeds in a solution of 80 mgm /l naringenin was measured, and the result shown in Fig 2 It can be seen that gibberellic acid reverses the light requirement induced by naringenin, and that the extent of the reversal is a function of the ratio of the number of gibberellic acid molecules present to molecules of naringenin It might be said, therefore, that there is a competitive relationship between gibberellic acid and naringenin in their effects on lettuce-seed germination in darkness This again is similar to what has been found for coumarin[4]

It is interesting that two such chemically dissimilar compounds as coumarin and naringenin have the same effects on lettuce-seed germination, and that these effects can be counteracted by gibberellic acid However, both compounds are naturally occurring plant-growth inhibitors, and there is an accumulation of evidence that such substances may be active in the control of dormancy It is conceivable that both are active as inhibitors of growth in dormant tissues by acting as competitive inhibitors of endogenous gibberellin of the tissues This hypothesis is supported by the evidence that gibberellins are present in plant tissues[5], and that inhibitors are found particularly in dormant organs However, in work on auxin-antagonists it has generally been found that where such a compound competitively inhibits auxin then the 'competition' for sites of action between antagonist and auxin can be related to chemical structure[6] Perhaps in the case of coumarin and naringenin there is a common basis in their molecular structures for their similar physiological effect, but elucidation of what that basis is must wait until the effects of other compounds on dormancy systems are investigated to enable a closer comparison to be made.

It is possible that such apparent competitive inhibition of gibberellic acid by either coumarin or naringenin as reported here may not be a true, simple competition of molecules for 'sites of action', as is the case for competitive enzyme inhibitors If this were so, then it might be that a wide variety of divergent types of compound will behave similarly to coumarin and naringenin, with no common structural basis to explain the similarity However, it has been reported[7] that any change in the molecular structure of coumarin, as brought about by substitution, will cause a decrease in the inhibiting effect of the molecule This would seem to indicate that there is some specific molecular requirement for inhibitory activity

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CHLOROGENIC ACID: AN ALLERGEN IN GREEN COFFEE BEAN

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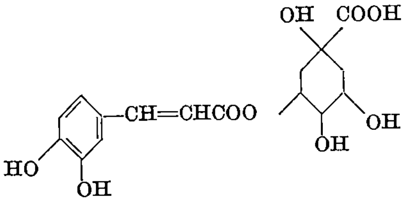
IT is well known that workers in the coffee industry frequently develop bronchial asthma, rhinitis or dermatitis on exposure to the fine dusty powder which fills the air of the factory as the chaff is mechanically stripped from the raw bean prior to roasting Previous investigations[1] showed that all workers so affected gave large positive immediate type intradermal reactions to aqueous extracts of green coffee chaff and green coffee bean Skin-sensitizing antibody (reagin) to green coffee was shown to be present in the sera of these allergic workers by passive transfer (Prausnitz–Kustner) experiments Extracts of roasted coffee, on the other hand, gave minimal or negative reactions on both direct and passive transfer testing

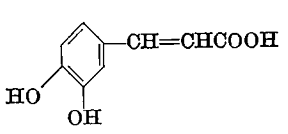
Chlorogenic acid, a compound of quinic and caffeic acids, is one of the major water-soluble constituents of green coffee, accounting for 5–8 per cent of the dry weight of the bean[2] It was first obtained in crystalline form from green coffee beans by Gorter[3] On the basis of a ferric chloride colour reaction, he concluded that this compound was widely distributed in the leaves and seeds of numerous plants, but it is now known that Gorter's test is not specific for chlorogenic acid since ferric chloride will give a colour reaction with most phenolic compounds Nevertheless, chlorogenic acid has been shown to be present in significant quantities in apples, pears, carrots, beans, celery, lettuce, cucumber, cabbage, plums, cherries, sweet potatoes and tobacco[4-8] Joslyn and Ponting have suggested that the darkening of certain cut fruits on exposure to air is due to the oxidation of chlorogenic acid to a quinone, rather than to the oxidation of true tannins[9]

The purpose of the present work was to identify the chemical nature of the allergen(s) present in green coffee bean The aqueous extract investigated was highly allergenic, producing a markedly positive wheal and erythema response on direct skin testing with 0 02–5 ml of a solution containing 5 mgm /ml and a positive passive transfer reaction at a serum dilution of 1 3,200

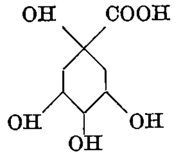
The aqueous extract of green coffee was fractionated by chromatography on dextran gel ('Sephadex *G*-25', purchased from Pharmacia, Uppsala). Forty-five fractions were collected and tested for allergenicity by the passive transfer method The middle fractions (18–29) gave strongly positive passive transfer reactions, while the remainder of the fractions gave minimal or negative reactions All the allergenically active fractions were coloured brown and gave a green colour reaction with ferric chloride, while the allergenically inactive fractions were colourless and gave a negative ferric chloride test These observations suggested that phenolic compounds were present in much higher concentrations in the allergenically active fractions



Chlorogenic acid (3-caffeoylquinic acid)



Caffeic acid



Quinic acid

A systematic evaluation of the known phenolic compounds in coffee was undertaken by injecting the suspected compounds into the skins of ten workers known to be allergic to green coffee Only chlorogenic acid produced positive reactions 1 µgm of chlorogenic acid was sufficient to induce a large wheal and flare response with pseudopods in all the subjects tested On passive transfer testing with the sera obtained from five of these workers, as little as 5×10^{-5} µgm of chlorogenic acid was required to elicit a reaction at a site sensitized with 0 05 ml of serum Identical results were found with chlorogenic acid obtained commercially and with chlorogenic acid isolated from the aqueous extract of green coffee by the method of Moores *et al* [2]

Cross-neutralization tests were performed with chlorogenic acid and an aqueous extract of green coffee in order to elucidate the allergenic relationship between these two substances In one series of tests, sites in the skin of normal human recipients were passively sensitized with sera obtained from individuals allergic to green coffee These sites were then challenged in 24 hr by the intradermal injection of 1 µgm of chlorogenic acid On the following day, the same sites were re-challenged with 100 µgm of green coffee extract There were no reactions on re-challenge, indicating that all the antibody directed against the green coffee had been previously neutralized by the chlorogenic acid (Table 1)

Table 1 RESULTS OF CROSS-NEUTRALIZATION EXPERIMENTS

Serum from patients sensitive to green coffee	Initial reactions on passive transfer to 1 µgm of chlorogenic acid	Reactions on rechallenge with 100 µgm of green coffee
P S	+ + + +	–
P S	+ + +	–
A S	+ + + +	–
M C	+ + +	–
W M	+ + +	–

Complete cross-neutralization was also obtained by another technique Chlorogenic acid and the serum of an individual allergic to green coffee were mixed *in vitro* in previously determined neutralizing proportions 24 hr later, this mixture was injected into the skin of a normal recipient Challenge of this same site on the following day with green coffee extract produced no reaction These results would suggest that chlorogenic acid (or chlorogenic acid complexed with other constituents of green coffee) may be the principal allergen in green coffee

In an attempt to identify the allergenically active portion of the chlorogenic acid molecule, passive transfer tests were performed with caffeic and quinic acids No positive reactions were obtained However, when a passive transfer site previously sensitized with allergic serum was injected with a mixture of caffeic and quinic acids in equimolar proportions at a relatively high concentration (300 µgm /ml. and 320 µgm /ml respectively), and the same site was re-challenged in 24 hr with 1 µgm of chlorogenic acid, the expected reaction was partially inhibited Similarly, when a solution of quinic and caffeic acids at the same high concentrations was mixed with allergic serum prior to injection, there was partial inhibition of the passive transfer reaction to chlorogenic acid

In order to explore the possibility of developing an *in vitro* test for precipitating or hæmagglutinating antibodies to green coffee, rabbits were immunized with a mixture of green coffee extract and Freund's adjuvant A positive precipitin test was obtained when the rabbit antisera were treated with green coffee extract or with a solution containing both chlorogenic acid and human serum albumin In the hæmagglutination procedure, green coffee extract was linked to rabbit erythrocytes using the *bis*-diazotized benzidine technique described by Gordon, Rose and Sehon[10] Hæmagglutinating antibodies against green coffee were detected in the rabbit antisera up to a dilution of 1 1,000,000 Chlorogenic acid, when used in the standard hæmagglutination procedure, produced non-specific hæmagglutination of rabbit erythrocytes and was therefore unsuitable for investigation by this method The hæmagglutination reaction with green coffee was completely inhibited by the whole extract and by the previously described allergenically active coloured fractions obtained on chromatography

No precipitation was obtained with human allergic sera, but one of the human sera so far tested was found to contain hæmagglutinating antibodies to green coffee up to a dilution of 1 1,280

In conclusion, it would appear that chlorogenic acid may be the principal allergen in green coffee The fact that an immediate skin reaction can be produced by a compound of low molecular weight would suggest that chlorogenic acid acts as a hapten in human skin or mucous membranes A simple phenolic compound of low molecular weight has thus been identified as a major allergenic component of a plant material responsible for human atopic disease Because polyphenolic compounds are widely distributed throughout the plant kingdom, it is possible that these observations may be useful in the search for the allergenic constituents of other foods, pollens, dusts or moulds

This work was supported by grants from the Imperial Tobacco Company of Canada, the National Institute of Allergy and Infectious Diseases, Bethesda, Md , and the National Research Council of Canada

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RESPONSE OF CHLOROPLAST CYTOCHROMES TO LIGHT AND SUBSTRATES

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THE introduction of a new spectrophotometric technique for investigation of oxidation-reduction processes in living material has promoted our knowledge of the respiratory enzymes in plants, for example, roots of cereals, and yeast[1–4] The presence and activity of a number of cytochromes, flavoproteins and enzymes linked to pyridine nucleotide (PN) has been demonstrated in live cells and in mitochondria and observations of the steady-state changes at variation of the oxygen supply are revealing certain general features of the structural organization of the respiratory unit[3,4]

Chloroplasts, too, contain cytochromes, which are spectroscopically very similar to those in mitochondria[5] Hill and Scarisbrick[6] found in chloroplasts a cytochrome *f*, characterized by an α-band at about 554 mμ and a prosthetic group similar to that of cytochrome *c* Its spectral bands are very close to those of cytochrome c_1 in mitochondria The cytochromes *c* and c_1 are predominantly oxidized in normally respiring mitochondria and are reduced rapidly in the absence of oxygen Cytochrome *f* remains partially reduced in the dark and the α-band at 554 mμ may be observed easily in de-chlorophyllized chloroplasts I showed in 1954 that it is oxidized if the chloroplasts are exposed to light[5] Similar observations of the oxidation by light of a cytochrome of *c*-type were made in a number of photosynthetic micro-organisms[8] During my work on the response of cytochrome *f* to light I also observed partly opposite reactions of a cytochrome *b* (ref 5) Hill reported[9] the presence of a cytochrome b_6 with an α-peak at about 563 mμ Finally, a cytochrome b_3 was distinguished with an α-peak of the reduced enzyme at about 559 and a γ-band at 429 mμ (ref 10) This cytochrome is very similar to a cytochrome b_3, which is probably a regular constituent of the respiratory unit in plant tissues Very little has hitherto been reported regarding an eventual response of the *b*-group in chloroplasts to light The whole problem thus called for a re-investigation, particularly in connexion with the recent discoveries regarding photophosphorylation

Arnon[11] first showed the actual existence of a light-induced phosphorylation in chloroplasts, linked, in his results, with the mentioned interaction of cytochromes in the light response According to Arnon's scheme of photophosphorylation, chlorophyll converts light-energy in a stream of electrons which are passing through a cycle of redox-enzymes, among these, cytochromes The cytochromes in chloroplasts are figured co-operating in phosphorylation in much the same way as in mitochondria

Chloroplasts also produce reduced pyridine nucleotide[11,12] (PN, preferably triphosphopyridine nucleotide (TPN)) and, simultaneously, oxygen It is commonly assumed that PN is reduced by the electrons expelled from the excited chlorophyll plus protons (H^+) from water The remaining OH^- ions may lose their electrons to the cytochrome system and simultaneously produce oxygen and water Water thus acts as a bridge connecting the reducing (Chl → TPNH) and oxidizing (cytochromes → Chl) moieties of the cycle The net effect of the water bridge is to move electrons in the direction of the potential gradient and protons in the opposite direction The formation of adenosine triphosphate (ATP) and reduced pyridine nucleotide (PNH) takes place in the pigmented grana[13] The reduction of $CO_2 + H_2O$ to carbohydrate (CH_2O) is a dark chemo-synthetical process which takes place in the non-coloured stroma of chloroplasts.

In continuing my work on the response of chloroplast cytochromes to light I have kept in mind the results of my recent investigations on steady-state relations of the respiratory enzymes in baker's yeast[14]. It was shown that the intensity of respiration and the apparent velocity of reduction of the involved cytochromes are synchronously lowered on the addition of ATP, and raised on the addition of ADP + PO_4. This is what would be expected if the steady-state balance ADP + $PO_4 \rightleftharpoons$ ATP were moved in one direction or the other on changing the concentration of the substrates, and if the process of phosphorylation serves as an efficient path of electron transfer between two cytochromes. A displacement of the steady-states of pyridine nucleotides may also have an influence. Because in chloroplasts both pyridine nucleotides and adenosine phosphates are directly or indirectly involved in the transfer of electrons between chlorophyll and water it may be expected that a rise of concentration of one of these substrates will exert a significant influence on the light response.

Cultivated and wild plants, including spinach, wheat, clover (*Trifolium repens*), *Agrostis stolonifera* and *Chenopodium album* served as experimental material. The leaves were ground with sand and 0·3 *M* sodium chloride with 0·02 mol. *tris* buffer and the brei squeezed through double cheese-cloth. For clover and spinach homogenization for 5–7 sec. also gave good results. Coarse particles were removed by centrifugation for 1 min. at 100–200*g*. The supernatant was spun for 7 min. at 1,400*g* and the pellet, containing the whole chloroplasts, was washed and re-centrifuged in 0·3 *M* sodium chloride. All manipulations, including the spectrophotometric observations, were carried out at approximately 0° C. The final suspension in 0·3 *M* sodium chloride contained about 30 mgm. chloroplasts (dry weight) in 10 ml. solution (0·3 per cent).

For the spectrophotometric analysis 10-mm. cuvettes were provided with an inset of gilded brass leaving a central 2-mm.-wide slit. The cuvettes were placed in a holder about 30 mm. from the entrance slit of a Bausch and Lomb monochromator. The enclosed portion of the suspension (2 × 10 × 10 mm.) was illuminated by focusing on it the straight band of a tungsten lamp (through a thick plate of heat-absorbing glass). By means of a suitable shutter the light was turned on immediately after the start of the spectrophotometric recorder. Two instruments of my own construction were used for this investigation. One instrument moves the wave-length screw 2 mμ, in intervals of 0·33 or 0·5 sec. and yields a record (high-speed pen recorder) of the region 540–570 mμ in 5·0–7·5 sec., which is repeated in intervals of 10 sec. The second instrument is of the scanning type and moves the wave-length screw 540–570 mμ in 0·25 sec. with immediate repetition of the movement. An oscillographic recorder yields 180 complete spectrograms in 1 min.

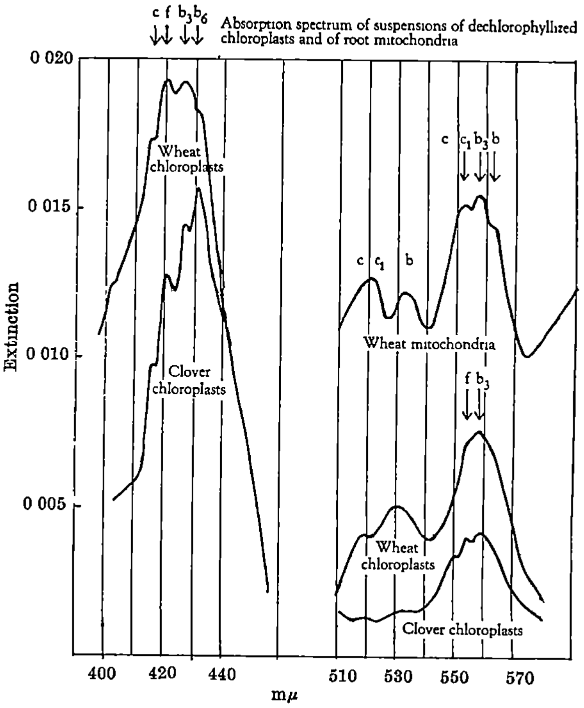

Fig. 1. Reduced cytochromes (dithionite) in chloroplasts of wheat and clover, from which the chlorophyll was removed by treatment with alcohol, compared with cytochromes in mitochondria

Occurrence and Percentage of Cytochromes in the Chloroplasts

Because the state of oxidation-reduction of the cytochromes is varying according to light intensity and availability of co-factors and substrates, measurements of the amplitudes of the reduced α-bands under varying conditions are revealing not only the types of involved cytochromes, but also the reacting quantities. Direct spectrograms of chloroplast suspensions or whole leaves show band-peaks or elevations at 550, 554, 556–558 and 562–564 mμ, corresponding to cytochromes *c*, *f*, b_3 and b_6. The close relation of cytochrome b_3 to a corresponding cytochrome in roots and of b_6 to the cytochrome *b* in mitochondria appears from records of the γ-region of de-chlorophyllized chloroplasts in dithionite (Fig. 1). Peaks are appearing here at 416 (*c*), 420 (*f*), 426–427 (b_3) and 430–432 mμ (b_6). The relation in height between the γ-bands and the α-bands amounts to 4–7, or about the same as in wheat roots and in preparations of cytochromes *c*, *f*, c_1 and *b*.

After removal of the chlorophyll with alcohol and re-suspending in 0·3 *M* sodium chloride, chloroplasts and whole leaves show a reduced band of cytochrome *f* which is further increased in dithionite. The weak bands at 416 and 550 mμ are not always clearly recorded, and the possibility cannot be *a priori* excluded that contaminations from protoplasm are at work. A comparison between the spectrograms from whole leaves and from suspensions of chloroplasts does not support this possibility, however, and it must be concluded that chloroplasts contain certain quantities of a cytochrome, the bands of which lie close to those of cytochrome *c*. The complete sets of cytochromes in chloroplasts and mitochondria are thus surprisingly similar to one another even if the proportions are different (see Fig. 1). Besides the above-mentioned differences, roots contain about 2–4 times as much cytochrome *b* as cytochrome c_1, whereas in wheat chloroplasts the relation b_3 to *f* is about 1, and b_6 is less abundant than *b* in plant mitochondria. A suspension of chloroplasts of clover contains 3–5 times as much cytochrome as a yeast suspension of equal concentration.

For calculation of the percentage of cytochrome in chloroplasts, measurements were made at the following wave-length intervals 550–542, 554–542, 558–542, or 558–570 and 564–570 mμ. The calculations are approximate, primarily because of the partial overlapping of the bands of *c* and *f* and of *f* and b_3. About 25 per cent of the α-band of *f* covers the α-band of b_3.

In 0.3 per cent chloroplast suspensions the amplitude of variations in the state of oxidation-reduction in maximum amounted to about $\varepsilon = \pm 0.03$, corresponding to about 2.5 per cent cytochrome $(c + f + b_3 + b_6)$ calculated per unit dry weight. Leaves of clover contain higher quantities than those of wheat seedlings. It was assumed that the group $c + f$ has a molecular weight of 15,000 and the group $b_3 + b_6$ 30,000 (ref. 15). These calculations are, of course, only tentative. De-chlorophyllized chloroplasts yield lower values, or 0.7 per cent, probably because some cytochrome is extracted or disorganized in alcohol. Alcohol also removes most of the lipids, which, together with chlorophyll, contribute about 50 per cent of the solid substance of the chloroplasts. Per unit of iron, group *c*–*f* and the group b_3–b_6 have about equal percentages of the total quantity of cytochromes.

For comparison, determinations were also made on mitochondria from wheat roots, yielding 0.5–1.0 per cent cytochrome per unit dry weight (earlier figures are somewhat higher, because it was then calculated with a molecular weight of cytochrome $b = 60,000$). We thus arrive at the conclusion that chloroplasts contain 2–3 times more cytochrome than mitochondria from the same plant. The observed rapid interaction of chlorophyll and cytochromes, and the fact that the grana are the sites of photophosphorylation, support the conclusion that all cytochromes are structurally linked to chlorophyll.

Response to Light

According to current opinions (see above) the excited chlorophyll maintains a flux of electrons through a cycle of redox enzymes and co-factors making up an internal circuit and, if $H^+ + e^-$ is consumed, water the external circuit. Warburg discovered [16] that chloride was acting as a co-factor. Chloride ions may here possibly act in a similar way to salt ions in respiration, in other words facilitating the shuffling of electrons over a water bridge. In photosynthesis the chlorophyll may be thought of as operating in the same way as the cathode of a photocell, expelling electrons in light, like many other dyes[17]. The conversion of electrical energy into chemical energy, namely, the synthesis of reduced pyridine nucleotide and high-energy phosphate, occurs at a few intermediate points.

The structural organization of the respiratory unit of mitochondria is mirrored in a rhythmic course of reduction at removal of the oxygen[2,3]. Reduction invariably starts with the enzymes of highest oxidation potential (cytochrome oxidase (a_3) and cytochrome *c*). After a gap of 1–2 sec (in yeast at room temperature) or a few minutes (in wheat roots) reduction of cytochrome *b* begins. Observations of the time course of reduction by means of rapidly recording spectrophotometers[4] thus serve as a useful tool for investigating the individual response of the single respiratory enzymes to environmental influences. At re-oxidation the time course is reversed, now starting at the enzymes of the highest reduction potential, but the rhythm is here less clearly observed, because re-oxidation, as a rule, proceeds very quickly.

Chloroplasts have no cytochrome oxidase and do not respire[11]. Their electronic state in darkness thus corresponds to that of mitochondria which lack oxygen. Light converts chlorophyll into an oxidase which attracts electrons from the adjacent cytochrome *f*. Electrons are now flowing through the whole cytochrome system and continuously replaced by those expelled from the excited chlorophyll. Each link in the cycle is steered by an on-reaction and an off-reaction $\left(\frac{e^-}{\text{on}} \rightarrow \text{cyt} \rightarrow \frac{e^-}{\text{off}}\right)$ and the single enzymes, at least some of them, are interspersed by speed-regulating 'factors'. A close observation of the time course might also reveal an individual response. I have in this article restricted myself to observing the sign and magnitude of the response of the cytochromes.

Recent investigations of Witt and co-workers[18] have shown that the immediate activation of chlorophyll may be observed a few milliseconds after turning on the light and also that activation of the water bridge probably starts very rapidly. My own oscillographic records show, in less than 0.1 sec., a rapid rise in extinction of the whole green trough between 540 and 570 mμ. The extinction is highest in the region 555–560 mμ, but this broad band does not belong to a cytochrome. A possible reactant is catalase. The role of catalase in chloroplasts has been widely discussed, but we cannot go into this problem here. The possibility of catalase guiding the de-ionization of OH^- and transference of electrons to cytochrome b_3 deserves serious consideration.

The response of the cytochromes to light is considerably slower than the initial reaction already mentioned. In my first publication the response was measured about 10 sec. from the start[5]. New records in time-intervals of 0.3–0.5 sec. afforded values down to 2 sec. but most of them centred around 6–8 sec.

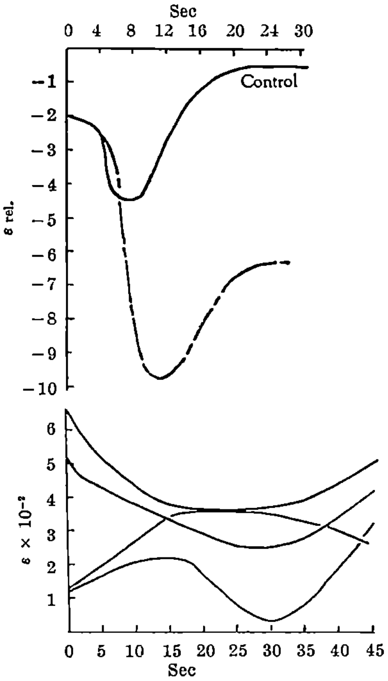

Fig. 2. Above, the response of cytochrome *f* to light. Washed chloroplasts of *Chenopodium album*. Upper curve in 0.3 *M* NaCl, lower curve 0.3 *M* NaCl + 0.015 *M* ADP as sodium salt. Below, response of chloroplast cytochromes of *Trifolium repens* to light. The light was in both cases turned on at 0 sec.

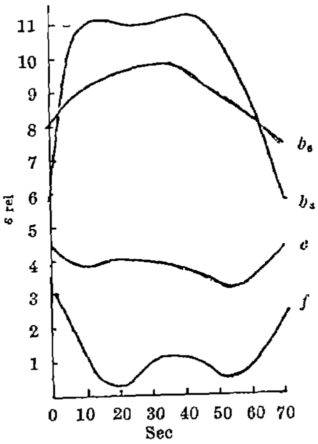

Fig 3 Response of chloroplast cytochromes of *Agrostis stolonifera* to light Whole leaves The light was turned on at 0 sec

(Fig 2) The existence of a starting lag was observed in all plants, both in whole leaves and in chloroplast suspensions In a number of cases different starting lags were observed regarding cytochromes *f* and b_3 The oxidation of cytochrome *f* in light started earlier than the oxidation of cytochrome b_3 (and b_6) by cytochrome *f* This result refers to washed chloroplasts and shows a more rapid supply of electrons from TPNH to b_3 and from *f* to chlorophyll than from b_3 to *f*, when phosphorylation is retarded for lack of substrates

Similar to the length of the starting period, the time course of the response to light may vary too All these observations were made on chloroplast suspensions in 0 3 *M* sodium chloride Ageing suspensions sometimes react differently to light and may show either an insignificant response or an increased reduction of the cytochromes instead of oxidation (Figs 4 and 5) Sometimes cytochromes *f* (and *c*) react in an opposite way to the group b_3–b_6 This counter reaction of the *b*-group, which was observed also in 1954 (ref 5), is shown in Fig 3 These observations also show a lowering of the conductivity between the *b*-group (primarily b_3) and the *f* (*c*)-group, a fact reminiscent of the relation between the cytochromes *b* and *c* in mitochondria, which is exposed to a number of influences, for example, inhibition by urethane and antimycin *A* (refs 1 and 19)

Variations in the response to light are always to be expected from a cyclic system in which the individual on- and off-reactions are regulated by interaction with different enzymes or 'factors' Some of the intermediate factors have been tested, for example, flavin adenine mononucleotide (FMN), vitamin K, etc [11] These co-factors are controlling the supply of electrons to the cytochrome system My experiments show that addition of FMN turns a negative response to light (oxidation of cytochromes), into a positive one (reduction in light) This may be explained as follows FMN lowers the resistance to internal transport of electrons from excited chlorophyll to the cytochromes, a result in agreement with observations on photophosphorylation[11] An opposite effect —an increase of the light-induced oxidation of the cytochromes—is observed on addition of ferricyanide This substance is obviously trapping the electrons before they reach the cytochromes, but does not impede the reduction of chlorophyll (Fig 4)

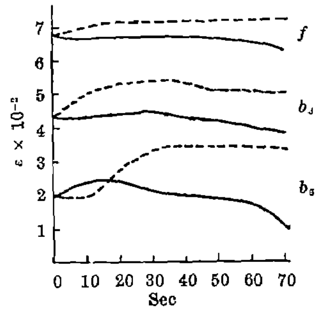

Fig 4 Response to light of unwashed wheat chloroplasts after addition of ferricyanide (10 mgm in 1 ml) The controls (broken curves) show in this experiment an increased reduction of the cytochromes after turning on the light (see text)

Influence of Substrate Factors on the Light Response

In these experiments sodium salts of DPN or DPNH (Sigma, acting similar to TPN and TPNH, but slightly weaker) and ATP or ADP were added in quantities corresponding to 0 01–0 02 *M* After a period of 10–60 min in the dark, the response to light was recorded as above For determination of the dark response to the substrates the recipients were placed in front of the photomultiplier tube. The weak light from the monochromator does not appreciably disturb the steady states

As shown in Table 1, addition of DPN induces increased oxidation of the cytochrome system The opposite effect of DPNH is notably observed as a reduction of cytochrome b_3 The weak response of cytochrome *f* to reducing agents is due apparently to its normally predominant reduction in the dark The results indicate the existence of a steady state between pyridine nucleotide and cytochromes, in which transferring co-factors (FMN, etc) may be involved, according to the scheme in Fig 6 and reaction 1 (see later) A rise in the concentration of PN moves the steady state in one direction, addition of DPNH promotes the reverse reaction The pronounced response of b_3 points to this cytochrome functioning as the switch-board between the reducing (Chl → PN → co-factors) and the oxidizing moieties (cytochromes → Chl) of the cycle

Table 1 DARK REACTIONS EFFECTS OF SUBSTRATES ON THE STATE OF OXIDATION-REDUCTION OF THE CYTOCHROMES CHLOROPLASTS FROM WHEAT SEEDLINGS

Cytochromes	*f* (+*c*)	b_3	b_6
+0 015 *M* DPN	Oxidation	Oxidation	Oxidation
+0 015 *M* DPNH	Weak reduction	Strong reduction	Weak reduction
+0 015 *M* ADP(+PO_4)	Weak oxidation	Weak oxidation	Weak reduction
+0 015 *M* ATP	Reduction	Reduction	Reduction

As to the adenosine phosphates, recent observations on yeast showed that a supply of ADP + (PO_4) accelerates the electron transference, notably from cytochrome *b* to *c* (and c_1), whereas addition of ATP retards it The effects were also observed as an increase or a decrease of the intensity of oxygen consumption It was concluded that variations in the mutual concentration of ADP and ATP cause the steady-state balance ADP + (PO_4) $\rightleftharpoons$ ATP to move forwards or backwards

In the dark chloroplasts the chlorophyll remains saturated with electrons and cannot absorb more from cytochrome *f* This cytochrome consequently remains reduced to a degree which is determined by the total oxidation–reduction balance between PN, PNH, co-factors, and cytochromes Substrate influences will thus, to a certain degree, be reflected in a displacement of the corresponding steady states

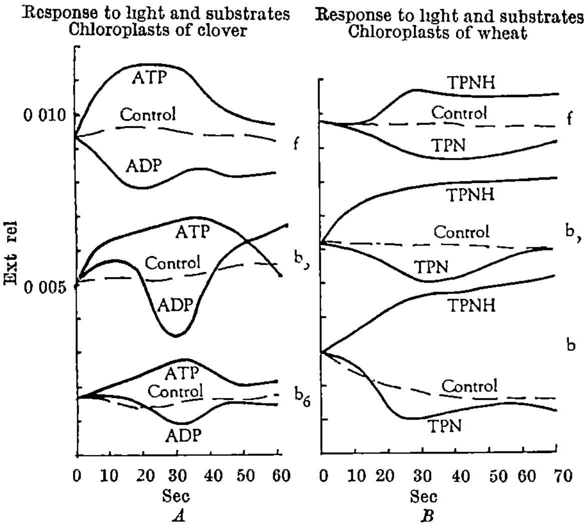

Fig 5*A* Response to light of washed chloroplasts of clover (*Trifolium repens*) in 0 3 *M* NaCl after addition of ATP or ADP (8 mgm in 1 ml) Exposure (at 0 sec) made 40 min after addition of substrates Controls (broken curves) in 0 3 *M* NaCl alone

B Response to light of washed chloroplasts of wheat in 0 3 *M* NaCl after addition of TPN and TPNH (as sodium salts, 6 mgm in 1 ml) Controls (broken curves) in 0 3 *M* NaCl alone

in the dark The response of cytochromes to ADP or ATP in the dark (Table 1) indicates the position of a point 2 of phosphorylation between b_3 (and/or b_6) and *f* (and/or *c*) Changes in the steady state of b_3 and b_6 indicate a point 3 of phosphorylation between these cytochromes (or one of them) and the dehydrogenizing co-factors (see also Fig 6)

The response to light (Table 2 and Fig 5) illustrates a further development of the dark effects, the reactions are still more intensified except in the case of b_6, which responds to ADP or ATP much in the same way as in respiring yeast The rising oxidation of yeast cytochrome *b* on the addition of ATP was interpreted as a retardation of the stream of electrons from dehydrogenases to cytochrome *b* This conclusion may be applied also to the chloroplasts The increased reduction of b_3 of chloroplasts on the addition of ATP indicates the existence of a point 2 of phosphorylation between b_3 and *f*, the inhibition of which retards the off-reaction of b_3 and consequently promotes its reduction The fact that cytochrome *f* is less oxidized in light after addition of ATP is interesting because it indicates the existence between *f* and Chl$^+$ of a point 1 of phosphorylation, which is absent in the transference $a_3 \rightarrow O_2$ in the respiratory chain

Table 2 RESPONSE TO LIGHT AVERAGE EFFECTS OF SUBSTRATES ON THE LIGHT-REACTION OF CHLOROPLASTS OF CLOVER AND WHEAT Period of illumination, 60 sec Spectral records at short intervals Controls without organic substrate showed weak oxidation

Cytochromes	*f* (+*c*)	b_3	b_6
+0 015 *M* DPN	Oxidation	Oxidation	Weak oxidation
+0 015 *M* DPNH	Weak reduction	Reduction	Reduction
+0 015 *M* ADP(+PO_4)	Oxidation	Oxidation	Weak oxidation
+0 015 *M* ATP	Weak oxidation	Reduction	Oxidation

DISCUSSION

The new experiments corroborate my work in 1954 regarding oxidation of cytochrome *f* in light[5] I claimed that "it is feasible to assume that radiant energy transformed into chemical energy may oxidize cytochrome *f* or alternatively a special cytochrome system connected to the chloroplasts and thus create a pool of chemically accessible hydrogen required for up-hill reactions in the path of carbon" It was assumed that the electrons flowing from cytochrome to chlorophyll are brought back again to cytochrome *b* by the transformed light energy Arnon's discovery of photophosphorylation strongly supports this idea of the chloroplast cytochromes acting as a mechanism for transforming electrical energy into chemical energy

The results of the present investigation are in agreement with Arnon's concept of a photophosphorylation and they also emphasize the similarities in enzymic composition of chloroplasts and mitochondria

With regard to Arnon's distinction of a cyclic and a non-cyclic phosphorylation, or, in other words, production of only ATP or of ATP + TPNH, my own results point to the combined activity of a chain of reduction, ending in TPNH, and a chain of oxidation (through cytochromes) producing ATP The following reactions may illustrate the main transferences (cf Fig 6)

$$\mathrm{TPN} + 2\mathrm{H}^{+} + 2\overset{\text{excited chlorophyll}}{\downarrow} e^{-} \xrightarrow{\text{TPN reductase}} \mathrm{TPNH}_2 \qquad (1)$$

$$\mathrm{TPHN}_2 + 2\mathrm{Fe}^{3+} \xrightarrow[\text{dehydrogenases}]{\text{co-factors}} \mathrm{TPN} + 2\mathrm{H}^{+} + 2\mathrm{Fe}^{2+} \qquad (2)$$

$$\mathrm{H_2O} + 2\mathrm{Fe}^{3+} \xrightarrow{\text{catalase ?}} \mathrm{O} + 2\mathrm{H}^{+} + 2\mathrm{Fe}^{2+} \qquad (3)$$

$$\mathrm{Fe}^{2+} + \text{excited chlorophyll} \rightarrow \text{chlorophyll} + \mathrm{Fe}^{3+} \qquad (4)$$

Chloroplasts which are not performing chemosynthesis of carbohydrates only form ATP (Arnon's cyclic phosphorylation) The electrons which are relinquished from the excited chlorophyll are, together with protons from the surroundings, reducing TPN (reaction 1) and are from here (via dehydrogenase co-factors) transferred to cytochrome *b* (reaction 2) Because in reaction 2 the same quantity of protons is released as is consumed in reaction 1, hydroxyl ions are not produced There is consequently no need for eliminating OH^- and producing oxygen The electrons are flowing directly to the cytochromes from reduced pyridine nucleotide without accumulation of anything except ATP

The need of a continuous supply of protons arises if $TPNH_2$ is used for an external purpose, such as the chemosynthesis of carbohydrates Protons are, in this case, continuously pulled from water leaving OH^- ions which would be accumulating in unphysiological quantities unless they are eliminated by reac-

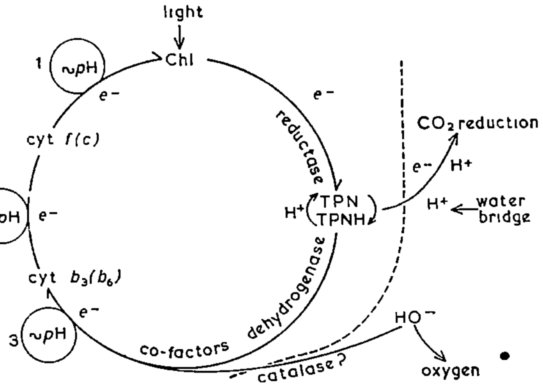

Fig. 6 Scheme of the photosynthesis in chloroplasts The broken line marks the effect of switching carbohydrate synthesis on or off

tion 3 This process implies a de-ionization of OH^- by the hæm enzymes, possibly in co-operation with catalase, leaving reduced cytochrome *b* and oxygen Reaction 3 is similar to the well-known Hill reaction[22], with the experimental difference that in the reaction observed by Hill, electrons from the excited chlorophyll are reducing external ferri-ions (cf trapping of electrons in potassium ferrocyanide (Fig 4)) What is producing oxygen is, however, in this case not the reducing moiety of the cycle but the oxidizing one, namely, the cytochrome system in co-operation with catalase or some other enzyme catalysing the electrolysis of water If electrons are trapped in the reducing moiety the excited chlorophyll will pull electrons from the cytochromes and thus keep the electrolysis of water going The relinquished protons (see reaction 3) are, in the Hill reaction, accumulating to unphysiological levels[23], but in normal photosynthesis they are feeding the transference of $H^+ + e^-$ from TPNH to the chemosynthetic processes producing carbohydrates from $CO_2 + H_2O$

Introduction of water in the electron cycle means an extension of the electron transference to an outer circuit (the water bridge) The reducing moiety is here acting as the cathode and the oxidizing moiety as the anode The electrons expelled from the excited chlorophyll are thus passing over the water bridge back again to the chlorophyll molecule (reaction 4)

The observed oxidation of the cytochromes on addition of TPN indicates a retardation or partial reversal of reaction 2 It may be expected that this initial retardation will decline slowly because of a simultaneous stimulation of reaction 1 The experiments show that the initial oxidation of the cytochromes soon disappears and even may turn over to reduction (Fig 4)

On addition of TPNH, reaction 2 is going from left to right. This initial response, which results in an increased reduction of the cytochromes, soon declines after the speed of photophosphorylation has adjusted itself to the increased supply of electrons (Fig 4)

The mere fact that the sudden start of the electronic cycle at turning on the light does not affect uniformly all links in the chain proves the existence of a spatial organization and of factors or zones of specific conductivity interspersed between the enzymes Similar to the respiratory enzymes, the corresponding enzymes in chloroplasts are very probably organized in sub-micro-multimolecular groups[3] (layers, rod-like or helical formations, etc) which are reacting at specific points ('reaction centres'[7]) or surfaces A number of observations furthermore indicate the existence of intermediate factors[19], lipids or quinones, which are acting as electrical machines transforming the motive power of electrons into chemical energy Most important are those machines which produce ATP The intermediate factors are largely responsible for the internal conductivity of the system and the observed specific response of the various cytochromes may be interpreted as a reflexion of local changes in their conductivity

The three points of phosphorylation are probably individually sensitive to variations of substrate concentrations, a circumstance that might explain negative results regarding the 'exchange reaction' between ADP and ATP [20] It is important to distinguish between the initial response, which, according to the present article, mirrors the points of substrate sensitivity, and the adaptation of the total cycle to the changed conditions, which, as shown here, may be completed in less than 1 min Conventional biochemical methods are insufficient for studying the initial effects In yeast the mechanisms of oxidative phosphorylation were shown to be regulating the internal conductivity and hence the speed of electron transference from reduced substrates to oxygen From the present results it may be inferred that in chloroplasts, too, the internal conductivity varies with the intensity of the ATP formation

It has been shown that the external circuit (the water bridge in chloroplasts which are consuming both water and ATP during the chemosynthesis of carbohydrates) is sensitive to the presence of salts, in particular chloride, whereas salts are reported as not affecting the internal circuit[11] This points to the existence of alternate pathways between TPN and the cytochromes as shown in Fig 6 It appears from this scheme that the internal stream of electrons is automatically switched over to the water bridge the moment $H^+ + e^-$ are consumed for reduction of carbon dioxide

In 1954 I advanced the idea that anions may, in chloroplasts, play a similar part as carriers of electrons as they are doing in the respiratory chain[5] What is going on in the water bridge may be characterized as a hidden electrolysis of water (only oxygen is evolved, $H^+ + e^-$ is hidden in reduced products) Salts are promoting *in vitro* the electric conductivity and stimulating the electrolysis, and the same may be true regarding chloroplasts The fact that chloride serves preferably as an electron carrier in photosynthesis may depend on the ability of chloride ions to be adsorbed firmly to the structure[21] Also, the stimulating influence of salts on respiration may be specific, owing to hydration, specific mobilities, etc , of anions and cations, but the specificity is generally less pronounced than in the case of photosynthesis

These investigations have been supported by the Swedish Research Councils for Natural Science and Agriculture and by a grant (*RG*7737) from the U S Public Health Service

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LETTERS TO THE EDITORS

RADIOPHYSICS

Riometer Observations during a Solar Eclipse

During the solar eclipse of October 2, 1959, recordings of cosmic noise (extra-terrestrial radio waves) intensities were made with riometers at the Sagamore Hill Radio Observatory of the Air Force Cambridge Research Laboratories in Hamilton, Massachusetts. The observing site is 243 ft. above sea-level at 70° 48′ 56″ west longitude and 42° 37′ 51″ north latitude and was directly in the path of totality.

The riometer consists of a servo-controlled, self-balancing receiving system which compares the incoming cosmic noise signals with the noise output of a diode vacuum tube. Any change in the value of the input signal to the receiver is detected by an internal balancing system, and a corresponding change in the filament temperature of the noise diode is effected. The actual recorded trace is driven by the filament current of the servo noise diode and represents the power-level of the incoming cosmic noise at the antenna. Variations in the recorded signal strength from the normal day curve are due to fluctuations in the charged particle population of the atmosphere, in particular changes in the electron density of the ionosphere. The attenuation curve of a 27·5 Mc./sec. radio wave passing through the atmosphere is shown in Fig. 1 and was derived from electron density profiles and electron-neutral particle collision cross-sections (personal communications from T. J. Keneschea and G. Gassman).

On the day of the eclipse, the Sun rose approximately 10 min. before second contact. Totality began at 5 hr. 50 min. 1 sec. and continued until 5 hr. 51 min. 32 sec. E.S.T. The solar eclipse occurred at an elevation of about 1° above the horizon and was of magnitude 1·02. The Fort Belvoir and planetary *K* indices were 5 during the observing period.

Two riometers were used in the measurements. One was tuned to a frequency of 30·25 Mc./sec., with its antenna oriented toward the zenith. The other was tuned to 27·5 Mc./sec., and its antenna was pointed toward the eclipse. The antennæ were three-element Yagis with lobe dimensions of approximately 110° horizontally by 60° vertically. The 27·5 Mc./sec. riometer antenna was mounted a half-wave-length above the ground with its axis of maximum sensitivity about 30° above the horizontal. The azimuth position of this beam was 95° E., the azimuth angle at which the eclipse would take place. The beam-width of the 27·5 Mc./sec. antenna was sufficiently large to enable signals to be well received before and just after totality. Since the antenna was stationary and did not track the Sun, incoming signals were received in different parts of the antenna reception lobe as time progressed. The recorded fluctuations in signal strength, therefore, indicate only relative changes in absorption. Because of the large antenna beam-width, however, the 27·5 Mc./sec. record provided an excellent indication of the variation of atmospheric absorption as the eclipse progressed.

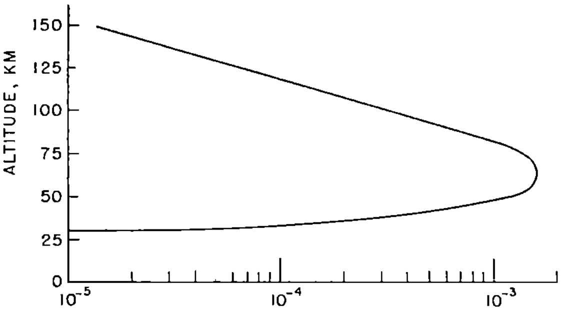

Fig. 1. Attenuation curve of a 27·5 Mc./sec. radio wave passing through the atmosphere

Fig. 2 shows the records of both the zenith and the eclipse riometers. The zenith riometer (30·25 Mc./sec.) shows no deviations from the normal quiet-day curve which was obtained from the records of the control days. The eclipse riometer record (27·5 Mc./sec.) shows slight absorption occurring before sunrise and an increase in cosmic noise of about 2 db. nearly coincident with optical totality. The pre-sunrise absorption may be due to the presence of auroral ionization observed in the backscatter records[1] of the Plum Island Observatory. It is of particular interest that peak cosmic noise intensity, corresponding to minimum electron density, is observed to follow optical totality by less than 30 sec.

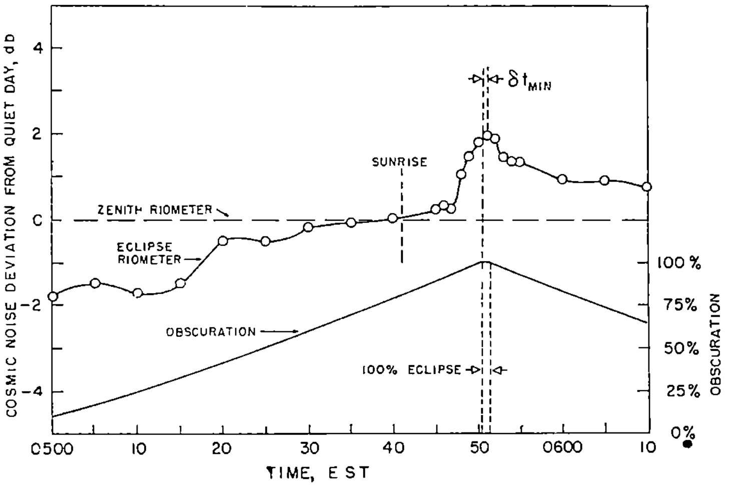

Fig. 2. Variation of cosmic noise from normal quiet day curve on October 2, 1959, during a solar eclipse. Percentage of obscuration also shown

As can be seen from Fig. 1, maximum attenuation for signals received by the eclipse riometer occurs at approximately 70 km., the *D* region. Because of the low angle at which the eclipse occurred, the path-length of the 27·5 Mc./sec. cosmic noise through the *D* region is about 200 km. Therefore, an average change of attenuation of 0·01 db./km. will explain the over-all change in attenuation of cosmic noise of

2 db At the zenith, the path-length is considerably less than the path-length in the direction of the eclipse Therefore, the small change in attenuation observed in the eclipse riometer would not result in a noticeable change of cosmic noise intensity measured in the zenith

Theoretical investigations of the E and F_1 regions during a solar eclipse have been made[2,3] Expressions involving the delay in time between optical totality and the minimum electron density have been derived Measured times of delay for the F_1 region during total eclipses are approximately 5 min , for the E region, 3 min[4] From my measurement, the delay time, $\delta_{\min}$, is determined to be about 30 sec for the D region Such a value is consistent with theory

This work has been sponsored by a National Science Foundation grant to the Arctic Institute of North America It was carried out at the Ionospheric Physics Laboratory, United States Air Force Cambridge Research Laboratories, Massachusetts I thank H Strick, of the Radio Astronomy Branch, for operation of the Zenith riometer and analysis of the zenith observations

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GEOPHYSICS

World-wide Distribution of Diurnal Variation of the Neutron Intensity of Cosmic Rays

IT is important to glean information on ways in which cosmic rays are influenced in interplanetary space If it were possible to find world-wide distributions of cosmic rays and their variations with time, one might be able to obtain information on modulation of cosmic rays by magnetic clouds in interplanetary space As it is impossible, however, to normalize the cosmic-ray intensities at every station in the world at present, I investigated the world-wide distributions of local times of maximum intensity in the diurnal variations and their variations with time during the period of the International Geophysical Year, and the following results were obtained

Data of the thirty-nine stations in the world were used during the period July–December 1957, forty-five stations during January–June 1958, and forty-five stations during July–December 1958 These stations were classified into two groups, one is the mountain altitude group ($\geqslant$ 2,000 m above sea-level), the other the sea-level group ($<$ 2,000 m above sea-level)

T_m, the local time of the maximum intensity in the diurnal variation, was derived as follows

(1) The bi-hourly readings, represented by the procedure of Wada[1], were averaged over a period of one month, excluding all data of the imperfect days, and only the months having more than ten perfect days were used The harmonic analyses were carried out on these monthly mean diurnal variations, and the average value of T_m of each month was derived from the vector sum of the first and second harmonics

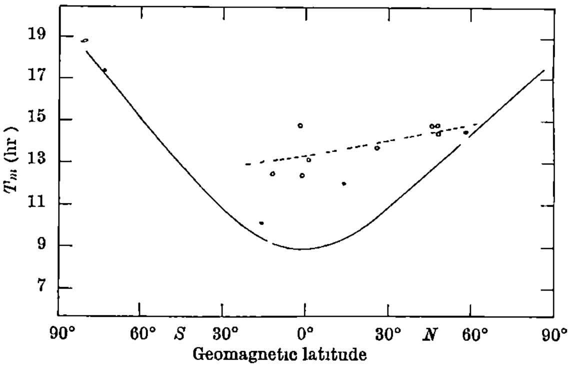

Fig 1. Relation between T_m and the geomagnetic latitude on the average, during the period July–December 1957 Full line and ●, sea-level group, dotted line and ○, mountain altitude group

(2) The average values of T_m during the period of each half-year were derived from the averaged values of the vector sum during six months of each period

Variations of T_m with the geomagnetic latitudes during the period of July–December, 1957, are shown in Fig. 1 It seems that there is a latitude effect in T_m, which has the minimum value around the geomagnetic equator Also the minimum values of T_m and the differences of T_m ($\Delta T_{m,\ 80°-0°}$) between 80° and 0° (geomagnetic latitude) of the latitude-effect curves belonging to the sea-level group have both clear correlations with the sunspot numbers determined by the Zurich Astronomical Observatory during each month It appears that minimum T_m advances and $\Delta T_{m,80°-0°}$ increases as the solar activity becomes intense Since many points deviate from the curve in Fig 1, lines of equal T_m were drawn in the world map in order to find reasons for this From the lines of equal T_m in the world map (Mercator's projection), it is seen that world-wide distributions have special tendencies there is a minimum value of T_m in the region from the Pacific Ocean to South Africa passing through South America, there are longitudinal effects, and the distributions tend to move every month, as shown in Fig 2

If it is possible to define broadly the minimum point between the northern hemisphere and the southern hemisphere as producing the equator, it is then possible to consider the minimum point of T_m as a kind of cosmic-ray equator, instead of the minimum point of the intensity, and this may be important, since one cannot normalize the intensity of stations Thus it may be plausible to suggest that the cosmic-ray equator is moving with time

From the results mentioned before, it may be suggested that the cosmic-ray intensity is controlled by three factors the first is due to the inner terrestrial magnetic dipole, the second due to the modulation by magnetic clouds in interplanetary space, and the third due to deformation of the lines of force of the geomagnetic field by contact with magnetic clouds

It may be suggested that, from the first factor, there exists the latitude effect on the average, from the second, minimum T_m advances and $\Delta T_{m,\ 80°-0°}$ becomes large as the solar activity becomes intense, and from the third are derived variations with time of the world-wide distribution of T_m and the cosmic-ray equator

It was pointed out by Simpson *et al* [2] and others[3] that a difference between the geomagnetic equator and

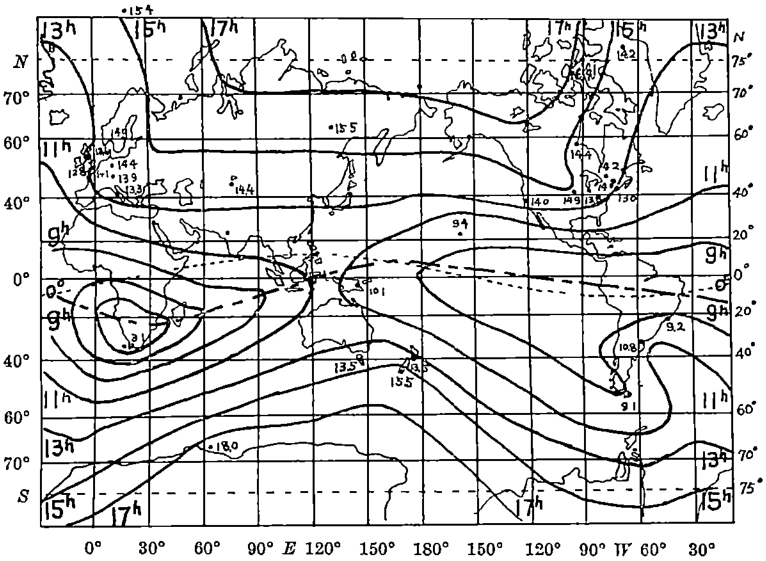

Fig 2 The average world-wide distribution of T_m during the period July–December 1957 The heavy chained line shows the cosmic-ray equator determined by T_m The fine dotted line shows the geomagnetic equator Numbers at stations show T_m (units/hr) belonging to them

the cosmic-ray equator exists, but the reason for this has not been made clear until now The cosmic-ray equator, that was established by them, was produced from data obtained at different times If it is true that the location of the cosmic-ray equator may also vary with time because the cosmic ray is modulated in interplanetary space, it may be permissible to determine the location of the cosmic-ray equator without consideration of the variation with time

I thank the directors and staffs of all the stations from which data were obtained for this work These data were obtained through *WDC*–C2 by Dr Y Miyazaki *et al* , whom I wish to thank too Also my thanks are due to Prof Y Kato for his suggestions and continuous encouragement, to Prof Y Sekido, the chief director of the Primary Cosmic-Ray Co-operative Research Group in Japan, and members of this group for their help

This investigation was financially supported by the Scientific Research Fund of the Ministry of Education

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Effect of a Lake on Distribution of Permafrost in the Mackenzie River Delta

THE thawing effect of water in contact with permafrost is of considerable importance to engineers engaged on construction work that may involve flooding areas of perennially frozen ground There is little information at present available, however, to indicate the magnitude of the effect which might be expected An investigation of the present level of permafrost under natural bodies of water in the north, such as lakes and streams, is one means of improving our knowledge of this effect and of providing some guidance to engineers During April 1961 a drilling programme was carried out by the Division of Building Research of the Canadian National Research Council to determine the distribution of permafrost under a lake in the Mackenzie River Delta (68° 18′ N , 133° 50′ W) at the new town-site of Inuvik, Northwest Territories, near the arctic coast

A small lake was selected for investigation following a preliminary study of aerial photographs and a field reconnaissance, in 1960, of a portion of the delta within a 10-mile radius of Inuvik This lake, located about 5 miles south-west of Inuvik, is approximately circular, having a diameter of about 900 ft Like all delta lakes it is shallow At the time of investigation the ice cover was about 2 25 ft thick and the average depth of water was 1 5–2 ft The snow cover was on the average 12–15 in The colour of the lake water was light brown and a very strong odour of hydrogen sulphide was noticed when the bottom sediments were disturbed The dense forest vegetation around the lake is typical of the climax developed in the lower valley and delta of the Mackenzie River The dominant species is white spruce, the largest trees growing to a height of 50 ft Also present are black spruce, willow and alder with scattered stands of tamarack The apparently continuous moss cover is about 1 in thick

A diamond drill-bit was used to drill and sample the various materials encountered The operation was greatly complicated by the presence of permafrost and the subnormal air temperatures which occurred during April Water, obtained from the lake at a temperature of 33°–35° F , was used as a circulating medium except for an unsuccessful attempt to use arctic grade fuel oil in one hole When using water, drilling techniques had to be slightly modified and precautions taken to prevent freezing of equipment in the hole Hot water was circulated periodically to keep the hole free of ice

During the course of the investigation, four holes were drilled at the locations and to the depths noted No 1, centre of lake—to 240 ft , No 2, 33 ft west of lake—to 115 ft , No 3, 132 ft west of lake—to 196 ft , No 4, 550 ft west of lake—to 260 ft

To measure ground temperatures, two thermocouple cables were installed to depths of 100 and 200 ft in holes Nos 3 and 4 respectively

Good undisturbed cores were obtained which indicated the following soil profile 0–100 ft , thinly stratified sandy silt with layers of decomposed organic material throughout, 100–180 ft , fine to medium sand with thin layers of organic material in the upper 40 ft , 180–240 ft , very dense, clay-sized soil (free of stones except for last few feet)

Representative samples were taken for identification and classification testing of the soils, moisture (ice) content, pollen analysis, carbon-14 age determinations and petroleum pigment analysis Bedrock (a dolomitic limestone) was encountered in hole No 3 at a depth of 230 ft and in hole No 4 at a depth of 260 ft

Although testing of samples has not been completed nor the information fully analysed, a number of observations can be made. No frozen ground was encountered throughout the depth of the hole under the lake (No. 1), but did occur for the full depth of each of the three other holes. Initial ground temperature measurements suggest that the permafrost is about 250 ft. thick at the location 130 ft. from the lake and about 300 ft. thick at a distance of 550 ft. from the lake.

Probings showed that the permafrost table extended out from shore under the lake and sloped down steeply. 10 ft. from the shore the permafrost table occurred 15·5 ft. below the ice surface; 12 ft. from the shore at a depth of 19·7 ft.; at 15 ft. it was more than 22 ft. below the ice. Although the soils encountered in the three holes adjacent to the lake (Nos. 2, 3 and 4) were solidly frozen, little ice was observed in the soils below the 20-ft. depth. Although flooding of the lake and surrounding area probably does not occur each year, it appears that it may occur at intervals of a few years. Marks on tree trunks indicate an extreme high-water mark about 5 ft. above the present ground surface at the lake, that is, about 20 ft. above the winter ice-level of a nearby delta channel.

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METEOROLOGY

Size Distribution of Raindrops

A. C. BEST[1] has found an empirical formula for the size distribution of raindrops. If $F(x)$ is the fraction of liquid water in the air comprised by drops of diameter less than x

$$F = 1 - \exp[-(x/a)^n]$$

where a and n are constants for any particular rainfall. Contrary to the general supposition that the above formula applies to the average size distribution of a number of samples of rain, I have found that the formula holds for individual samples in 90 out of 104 cases of general monsoon rains and in 134 out of 169 cases of thunderstorm rains recorded at Poona, provided the samples are sufficiently representative. The formula fails where the liquid water distribution is multimodal (generally bimodal) or where the distribution curve has a long tail with a sharp maximum either at the beginning or at the end.

The parameters a and n are found to be independent for general rains but are connected by the average relation $an = 7\cdot1$ mm. for thunderstorm rains. There is also a correlation between the intensity of precipitation I (mm./hr.) and the parameter n. For general rains the average relation found is

$$I = 6\cdot9 \exp[-0\cdot14(n - 5\cdot5)^2]$$

and that for thunderstorm rains is $I = 975 \exp(-n)$.

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PHYSICS

Stark Broadening of the Spectrum Line 4603 Å. of Lithium

THE Stark broadening of spectrum lines, in particular the lines of hydrogen, has been of interest to astrophysicists for many years. Recent advances in plasma physics on a laboratory scale have emphasized the need for a better understanding of the relationship between the number density of charged particles in a plasma and the profiles of the spectrum lines from the plasma. The broadened hydrogen lines from a water-stabilized carbon arc have been studied carefully by workers at Kiel[1-3], and the latest refinements of the theory of line broadening describe the profile of Hβ quite accurately[4].

The shock tube is a very useful device for studying spectrum lines from plasmas, since the degree of ionization and the temperature behind the shock wave can be varied over a wide range and calculated from measurements of the shock speed. The shock tube used for the present work has a low-pressure section 18 ft. 7 in. long, and a high-pressure section 3 ft. long. Argon is used as the test gas in the low-pressure channel, and hydrogen as the driver gas in the high-pressure section. Diaphragms of aluminium or 'Mylar' are used, depending on the bursting pressure desired. Spectra are obtained with a Raman glass spectrograph. Spectra can be resolved in time by sweeping the spectrograph slit with a rotating disk having a number of radial slots.

Lithium was chosen for initial Stark broadening studies, since this shows a large Stark effect for some of its lines, particularly the line at 4603 Å. Hydrogen and helium both have large Stark shifts, but were not used since their presence in quantities large enough to give easily photographed spectrum lines would reduce the shock strength quite markedly. The lithium was introduced into the shock tube in the form of lithium chloride which was supported on a short rod mounted on the closed end of the tube. A time-resolved spectrum showed that the lithium was excited behind the shock wave reflected from the closed end of the tube. A time-integrated spectrum is shown in Fig. 1. The argon lines are broader than those emitted from an argon discharge lamp and they are also shifted a little to the red. Hβ appears very faintly; this is excited near the contact surface between the hydrogen driver and the argon. The lithium line at 4603 Å. is very broad. The blackening profile of this line is shown in Fig. 2.

In the analysis of this line it has been assumed that the line is broadened solely by the statistical micro-fields due to ions. The broadening of the lithium line at 4603 Å. has been treated approximately by Holstmark using some experimental results obtained by Stark on sparks through lithium vapour[5]. Since then, more accurate Stark results have become available for lithium mainly through the experimental work of Poirot and these results have been used for the present analysis[6].

The lithium line at 4603 Å. arises from a $2p$–$4d$ transition. Under the action of a uniform electric field this line is displaced to longer wave-lengths and two other lines appear, one due to a $2p$–$4p$ transition and the other to a $2p$–$4f$ transition. The $2p$–$4p$ transition gives a weak line at 4636 Å. for vanishingly weak electric fields, shifting to longer wave-lengths at increasing field strengths.

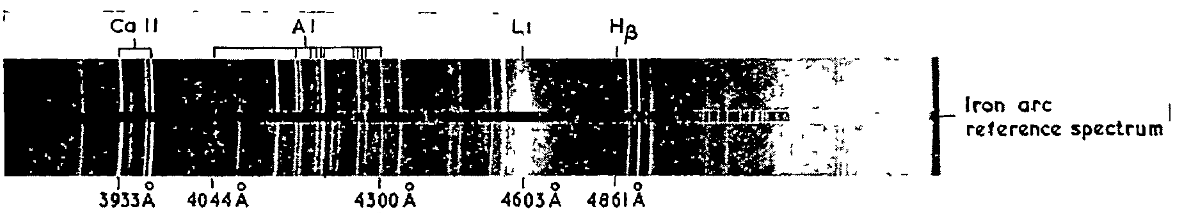

Fig. 1. Lithium excited in the shock tube, showing Stark-broadened line at 4603 Å. The Stark-broadened hydrogen line Hβ is just visible also

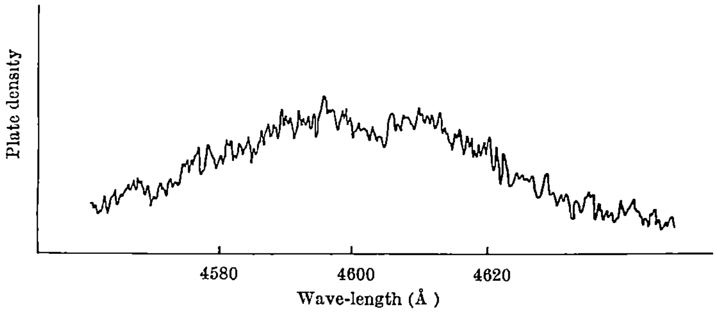

Fig. 2. Density profile of Stark-broadened line of lithium at 4603 Å

The $2p$–$4f$ transition gives a line at 4602 Å for vanishingly weak fields, shifting to shorter wave-lengths at increasing field strengths. Data on the intensities of these Stark components are not available and therefore the full-line contour cannot be worked out as it has been done for the hydrogen lines by Schmaljohann, who applied Holtsmark's statistical theory to the known Stark data for hydrogen[7]. In order to overcome this difficulty it is assumed that the long wave-length part of the contour, having a maximum at 4611 Å, is due solely to the $2p$–$4d$ transition, and the weaker $2p$–$4p$ transition, which is much farther into the red, is neglected. This part of the line contour cannot be encroached on by the line due to the $2p$–$4f$ transition, since that cannot affect any part of the line at wave-lengths greater than 4602 Å. Poirot gives only rough estimates of the intensities of the components, but from these estimates it appears that the line due to $2p$–$4d$ transition does not vary in intensity over the field strengths he used, which did not exceed 95 kV. per cm.

Poirot's results show that the Stark displacement varies very nearly as the square of the electric field strength. For the quadratic Stark effect, the statistical theory of line broadening for a single line leads to a line profile given by (ref. 8, equation 3.14)

$$I = \frac{W(\beta)}{2\nu_0\beta} \qquad (1)$$

where

$$\beta = \left(\frac{\Delta\nu}{\Delta\nu_0}\right)^{1/2} \qquad (2)$$

where $\Delta\nu$ is Stark displacement in cm.$^{-1}$ due to a field F, $\Delta\nu_0$ is Stark displacement in cm.$^{-1}$ due to a field F_0 and W is a probability function that has been tabulated by Schmaljohann[7].

F_0 is related to the number density of ions, N, by

$$F_0 = 2.61\, eN^{2/3} \qquad (3)$$

where e is the electronic charge (4.80×10^{-10} E.S.U.).

From Schmaljohann's tabulated values of $W(\beta)$, it is readily found that the maximum of I occurs when $\beta = 1.1$. Substitution in (2) for β gives the position of the maximum as $1.21\ \Delta\nu_0$. From direct measurement of the blackening contour (Fig. 2), this is 38 cm.$^{-1}$, which gives $\Delta\nu = 31.4$ cm.$^{-1}$. From an extrapolation of Poirot's results this corresponds to $F_0 = 122$ kV./cm.

Equation (3) then gives

$$N = 1.85 \times 10^{17} \text{ per c.c.}$$

In the actual experiment the shock wave Mach number was 9.2, and the initial pressure of the argon 5 mm. mercury. By calculation this gives a temperature of 11,500° K. and ion density of 1.6×10^{17} per c.c. behind the shock wave reflected from the end of the tube. There are two effects which would lead to a higher observed ion density than that computed behind the reflected shock wave: (1) the reflected shock wave undergoes a second reflexion at the contact surface before the radiation is finally quenched by the spreading contact surface. This second reflexion is shown by a time-resolved spectrum, and has the effect of further heating and compressing the gas, thus raising the number density of electrons and ions. Computations have not been carried out for this second reflexion. (2) The lithium itself almost certainly provides a large number of electrons and ions since this has a much lower ionization potential (5.39 eV.) than argon (15.75 eV.).

This work is proceeding further in order to investigate the effect of varying the number density of ions and electrons. The most recent work on hydrogen line broadening also shows the need to take into account the effect of electrons on the broadening, which has been neglected in this treatment[8].

This work is published by permission of the Director of the Laboratory. I acknowledge with thanks the assistance of Mr. J. Townsend in the experimental work.

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Polarized Thermal Emission from Narrow Tungsten Filaments

FROM experimental work carried out by Fresnel many years ago and a theoretical investigation made by K Schwarzschild[1], it is well known that light transmitted through a narrow slit is more or less polarized Though conditions are rather different in the case of thermal emission from a narrow filament the idea has occurred to me that polarized emission may be obtained in this way in addition to inclined-surface-layer-effects described by Worthing[2] and others (Arago effect[3]) Through the courtesy of Dr G Siljeholm of the Lumalampan Aktiebolag in Stockholm I have been able to test recently some filaments no thicker than 4 8μ The experiments were carried out at the Swedish Solar Observatory in Anacapri, and a polarization as high as 28 per cent has been found in the red The plane of vibration (electric vector) is perpendicular to the direction of the filament Considering the fact that the 4 8μ filament had not been polished as in the case of Worthing's measurements, the high value obtained in the red (28 per cent instead of 19 per cent found by Worthing) gives some indication that a 'slot-aerial' effect might be present To some extent this view is supported by measurements of a 6 5μ filament showing already a smaller degree of polarization than a polished broad filament. Furthermore, when examining with a Savart polariscope the interference fringes appearing in highly magnified images, it has been found that the polarization appears to be nearly the same in the central part of the wire as at the limb Though the resolving power may have been insufficient for separating the regions clearly, it seems hard to explain the observation by means of the Arago effect alone

By using different colour screens I have been able to establish an increase of polarization with wave-length. The determinations are presented in Table 1 The effective wave-length λ transmitted through the filters has been estimated with a spectroscope The degree of polarization was measured with a Cornu polarimeter, except in the case of infra-red radiation for which photographic observations were made Special precautions were taken to avoid polarization effects due to reflexion in the glass tube, etc

In Table 1 the quantity $\frac{d}{\lambda}$ is given as well as $P\frac{d}{\lambda}$, d being the thickness of the wire As is evident from the last column $P\frac{d}{\lambda}$ is remarkably constant and approximates to 2

In Fig 1 the degree of polarization is plotted against $\frac{d}{\lambda}$ using dots for $d = 6\ 5\mu$ and crosses for $d = 4\ 8\mu$ The dots follow closely a straight line All values follow closely the hyperbola $P\frac{d}{\lambda} = 2$ presented as a dashed curve When using instead $1/\lambda$ as abscissa the crosses and the dots give different relationships This indicates again that an aerial effect may be present The probable behaviour close to $\frac{d}{\lambda} = 1$ has been indicated by the dotted curve by taking into account the phenomena produced by a narrow slit If the relation $P\frac{d}{\lambda} = 2$ is valid also for smaller values of $\frac{d}{\lambda}$ than those examined here, the highest polarization would be expected when the thickness of the filament is about twice the wave-length of light

Table 1

$d(\mu)$	$\lambda(\mu)$	$\frac{d}{\lambda}$	P	$P\frac{d}{\lambda}$
● 6 5	0 48	13 5	0 14 ± 0 02	1 9
	0 55	11 8	0 16 ± 0 01	1 9
	0 64	10 2	0 16 ± 0 02	1 6
	0 66	9 8	0 19 ± 0 02	1 9
	0 82	7 9	0 22 ± 0 02	1 7
4 8	0 48	10 0	0 22 ± 0 02	2 2
	0 66	7 2	0 28 ± 0 02	2 0

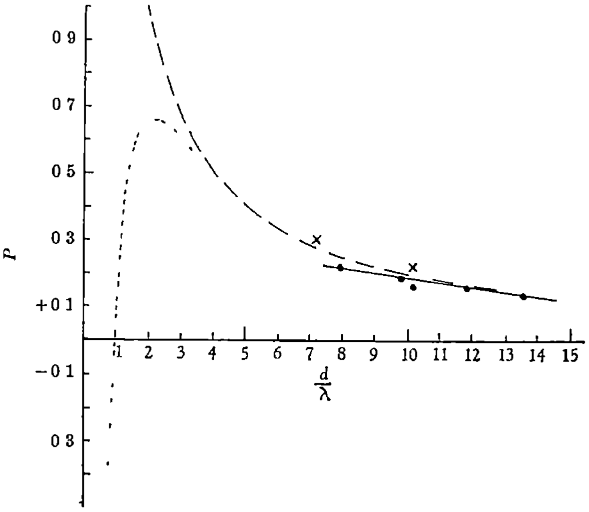

Fig 1

Preparations are being made for extending the measurements to narrower filaments It is planned to ascertain also whether narrow bolometer filaments may be used for the purpose of analysing polarization in the infra-red

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CRYSTALLOGRAPHY

Crystallographic Data for Urea Calcium Nitrate and Urea Cadmium Nitrate

THE crystal structures of urea calcium nitrate and urea cadmium nitrate are being investigated as part of a survey of hydrogen bonding in urea inorganic salt complexes Preliminary work has shown that urea calcium nitrate and urea cadmium nitrate are isostructural and belong to the monoclinic system

By using small single crystals mounted on a Seifert diffractometer and copper K_α radiation, accurate values for the translations d_{001}, d_{100}, d_{101} and b were obtained The accurate estimates were made by plotting the values of these translations, determined from the $00l$, $h00$, $h0l$ (for $h = 1$) and $0k0$ reflexions, against the function[1] $\frac{1}{2}\left(\frac{\cos^2\theta}{\sin\theta} + \frac{\cos^2\theta}{\theta}\right)$ and extrapolating to zero

The monoclinic angle β was calculated by means of the equation

$$\beta = \text{arc cos}\ \frac{d^2_{101}(d^2_{100} + d^2_{001}) - (d_{100}d_{001})^2}{2d_{100}d_{001}d^2_{101}}$$

By using the calculated value of β and the observed values of d_{001} and d_{100}, the unit cell translations c and a were derived

The systematic absences were deduced from indexed Weissenberg photographs taken about the unit cell axes

The densities were determined by flotation of the crystals in toluene/bromoform mixtures

A summary of the crystallographic data for urea calcium nitrate and urea cadmium nitrate is given in Table 1

Table 1

	Urea calcium nitrate, $Ca(NO_3)_2 4(NH_2)_2 CO$	Urea cadmium nitrate, $Cd(NO_3)_2 4(NH_2)_2 CO$
a	7 683 ± 0 002 Å	7 607 ± 0 002 Å
b	12 665 ± 0 002 Å	12 772 ± 0 002 Å
c	9 245 ± 0 002 Å	9 040 ± 0 002 Å
β	113° 32′ ± 2′	114° 4′ ± 2′
No of molecules per unit cell	2	2
Measured density	1 634 gm cm $^{-3}$	1 968 gm cm $^{-3}$
Calculated density	1 628 gm cm $^{-3}$	1 973 gm cm $^{-3}$
Linear absorption coefficient for copper K_α radiation	41 9 cm $^{-1}$	123 0 cm $^{-1}$
Systematic absences		
$h0l$	$h = 2n + 1$	$h = 2n + 1$
$0k0$	$k = 2n + 1$	$k = 2n + 1$
Space group (uniquely determined)	$P2_1/a$	$P2_1/a$
$a/2 - c \cos \beta$	0 150	0 118

All the crystals of urea cadmium nitrate that were examined were found to be twinned, with (010) forming the composition plane. Examination of the (010) Weissenberg photographs of urea cadmium nitrate showed that twinning had occurred as a result of a two-fold rotation about the a axis Since the relation $c \cos \beta = \frac{a}{2}$ holds fairly closely for urea cadmium nitrate, this is an example of twinning according to the Carlsbad Law[2]

No evidence of twinning has been observed in the isostructural urea calcium nitrate complex, for which the value of $\frac{a}{2} - c \cos \beta$ is equal to 0 150 as compared with the value of 0 118 for urea cadmium nitrate.

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CHEMISTRY

A Transition in the Dielectric Properties of Solid Secondary Alcohols

Since the discovery of the large dielectric absorption in aliphatic secondary alcohols[1], there have been several papers[2–4] published on this subject The only definite conclusion about the mechanism is that the presence of some lattice defect or arrangement of defects is required for this large absorption[3,4]

In the belief that further progress can be made only from measurements on single crystals grown from pure material, attempts have been made to grow single crystals from the melt The solid grown to date has been below the standard it is hoped to obtain eventually Nevertheless, measurements made on the oriented polycrystalline material obtained has revealed a sharp transition in the dielectric properties of certain alcohols at a temperature about − 4° C The transition was not apparent in samples pressed from powders, although a suggestion of its presence was noted in samples annealed for long periods

The solid was that used by Meakins and Sack[1], zone-refined and grown from the melt by a Bridgman method The secondary alcohol was contained in a glass tube which was evacuated to a pressure of 10^{-5} mm mercury. The tube was lowered through a sharp and highly stable temperature gradient at 0 5 in a day After removal from the tube, plane parallel sections for measurement were cut in three perpendicular directions, using a wet string saw One of these directions was perpendicular to the growth axis In some experiments 'Aquadag' electrodes were painted on the samples

In the case of 9-heptadecanol ($C_8H_{17}CHOH\,C_8H_{17}$), the absorption at room temperature is comparable in all three directions However, the change in dielectric absorption at the transition is three times greater in the two directions for which the measuring field is perpendicular to the growth axis than when the field is in the direction of the growth axis In Fig 1 the dielectric absorption for one of these samples is

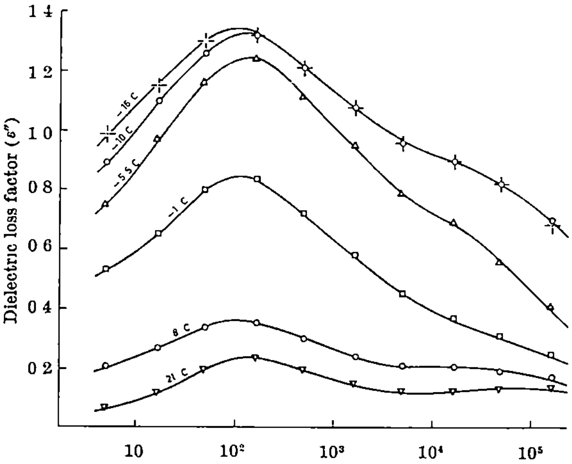

Fig 1 Dielectric-loss factor as a function of frequency for the symmetrical secondary alcohol 9-heptadecanol

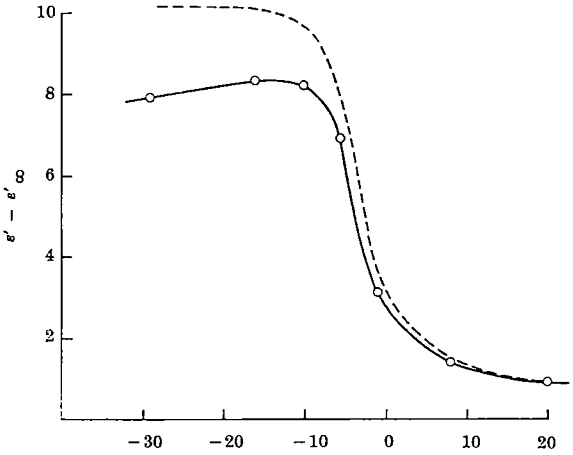

Fig 2 $\varepsilon' - \varepsilon'_\infty$ for 9-heptadecanol as a function of temperature The full line refers to ε' measured at 4 8 c /s , the dotted line to an estimated ε'_0, that is, the permittivity below the absorption region plotted as a function of frequency at several temperatures In Fig 2 the relative permittivity at a frequency of 4 8 c /s is plotted as a function of temperature, the dotted curve is an estimated ε'_0 obtained by extrapolating the absorption curves to frequencies below 4 8 c /s and measuring the area under the extrapolated portion It can be seen that the dispersion ($\varepsilon'_0 - \varepsilon'_\infty$) increases by a factor of 10(0 95–10) in passing through the transition

A similar transition at approximately the same temperature has been observed in 8-pentadecanol ($C_7H_{15}CHOH\ C_7H_{15}$), and evidence has been obtained of a less-sharp transition in 10-nonadecanol ($C_9H_{19}CHOH\ C_9H_{19}$) The best sample obtained of the latter material was considerably more polycrystalline than those of the other compounds

A direct-current voltage of 30 kV /cm was applied to the samples at temperatures both above and below the transition, the permittivity was linear up to this voltage

Further purification and growth of better crystals of these compounds are being attempted

We wish to acknowledge the contributions of Mr J S Cook and Mr K G Weir to the crystal-growing apparatus

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1,2-Benzanthracene Derivatives in a Kuwait Mineral Oil

THE isolation of a number of polymethylanthracenes from fractions of a Kuwait crude oil has been reported by one of us[1] The hydrocarbons were obtained by treatment of acetone extracts of the oil with maleic anhydride and chromatography of the material recovered from the acidic adducts by distillation from sodium hydroxide We have now applied the same procedure to higher-boiling fractions of acetone and furfural extracts of the Kuwait oil (corrected boiling points in the range 390–440° C) and have obtained crystalline fractions consisting essentially of alkyl derivatives of 1,2-benzanthracene This is evident from their ultra-violet absorption spectra, which closely resemble those of typical alkylbenzanthracenes[2] Vapour-phase chromatography of some of the fractions indicates, however, that they are mixtures containing varying proportions of benzanthracene and other components with retention times similar to those of mono- and dimethylbenzanthracenes 1,2-Benzanthracene itself was isolated from several fractions by further chromatography on alumina as colourless plates, m p 154–156° undepressed, when mixed with an authentic specimen, m p 158–159° The ultra-violet spectrum of the isolated material (λ_{max} 228, 256, 267, 277, 287, 299, 314, 327, 342, 358, 374, 384 mμ, log ε, 4 54, 4 51, 4 64, 4 89, 4 98, 3 95, 3 70, 3 80, 3 85, 3 69, 2 69, 2 86), and the infra-red spectrum[3], were identical with those of 1,2-benzanthracene, and vapour-phase chromatography gave one peak with retention time similar to that of benzanthracene under the same conditions The 2,4,7-trinitrofluorenone addition complex formed red needles, m p 217–221°, not depressed when mixed with an authentic specimen, m p 220–223° [4], and the identification was confirmed by preparation of the *s*-trinitrobenzene complex, orange needles, m p 161–163°, and comparison of this with an authentic specimen, m p 162–163° (found C, 65 3, H, 3 6, $C_{24}H_{15}N_3O_6$ requires C, 65 3, H, 3 4 per cent)

A second product, m p 192–194 5°, obtained in very small amount after purification by chromatography and sublimation, did not depress the melting point of 4′-methyl-1,2-benzanthracene (m p 199–200°)[5], and the ultra-violet spectrum (λ_{max} 226, 228–9, 256, 271 5, 281 5, 292 5, 302, 316, 328 5, 344, 361, 387 mμ, log ε, 4 55, 4 55, 4 57, 4 65, 4 78 4 79, 4 02, 3 77, 3 80, 3 81, 3 66, 2 94) was identical with that of this hydrocarbon The infra-red spectra were also identical in the region 650–900 cm $^{-1}$ (there was insufficient material to obtain a well-defined spectrum over the remainder of the normal range)[3] The 2,4 7-trinitrofluorenone complex formed brown needles, m p 223–227°, and when mixed with the derivative of 4′-methyl-1,2-benzanthracene (m p 233–235°)[6] the melting point was 224–230° The *s*-trinitrobenzene complex gave red needles, m p 158–160°, mixed with the derivative of the 4′-methyl compound m p 165 5–168°, the melting point was 160–168° Because of the very small amounts of material available it was not possible to obtain the derivatives completely pure, but the results reported, taken as a whole, may be considered to justify the identification of the substance from the oil as 4′-methyl-1,2-benzanthracene

The presence of derivatives of 1,2-benzanthracene in fractions of the Kuwait oil is of interest with regard to the carcinogenic activity of the oil[7] We have not as yet been able to obtain an accurate estimate of the amount of benzanthracenes present, but spectroscopic evidence indicates that, in one fraction of corrected boiling point 390–405°, it is certainly less than 0 25 per cent of the acetone extract, disregarding possible losses during decomposition of the maleic anhydride adducts 1,2-Benzanthracene itself is generally considered to be non-carcinogenic or only very weakly so when tested by skin painting on animals[8,9], but it has been reported to be moderately active by subcutaneous injection in mice[10] 4′-Methyl-1,2-benzanthracene has been shown in one series of tests to be a very weakly active carcinogen towards mouse skin[11]

Several other crystalline substances have been obtained by chromatography of those fractions of the oil which did not react with maleic anhydride One of these, obtained as colourless needles, m p 195–199°, was shown to be triphenylene by mixed melting point with an authentic specimen, m p 202–204° [12], by ultra-violet absorption measurements (λ_{max} 249, 257 5, 273, 284, 320, 328, 335 mμ, log ε, 4 93, 5 15, 4 37, 4 32, 2 87, 2 70, 2 72)[13] and by elementary analysis (found C, 94 6, H, 5 3, calc, for $C_{16}H_{12}$ C, 94 7, H, 5 3 per cent) Another substance, m p 252–256°, was identified as chrysene (m p 256°)[14] by mixed melting point and ultra-violet absorption (λ_{max} 241, 258, 267, 294, 306 5, 320, 344, 352, 361 mμ, log ε, 4 62, 5 18, 5 36, 4 39, 4 44 4 43, 3 12, 2 84, 3 10) The infra-red spectrum was identical with that of chrysene, and on vapour-phase chromatography one peak was obtained with retention time similar to that of chrysene The *s*-trinitrobenzene complex formed yellow needles, m p 189°, not depressed when mixed with an authentic specimen, m p 191–193° (lit m p 192–194°)[4]

None of the hydrocarbons reported here has previously been isolated from an uncracked petroleum fraction Claims have been presented, based on spectroscopic evidence, for the occurrence of chrysene and of 1,2-benzanthracene in the aromatic fraction of a clarified oil[15], and of chrysene, triphenylene and 1,2-benzanthracene in a paraffin wax of unspecified origin[16]

We are indebted to Dr J. W Cook for his interest and encouragement, and to Prof F Morton, of the Manchester College of Science and Technology, for supplying us with the oil fractions used in the investigation

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Partition Isotherms and Gas-Liquid Chromatography

The various well-known theories of gas-liquid chromatography all assume a linear 'partition' isotherm, that is, the concentration of a solute in the stationary liquid phase is a linear function of the concentration in the gas phase, also the theories are unanimous in predicting symmetrical elution peaks Asymmetrical peaks, when they occur, are variously ascribed to 'overloading' of the column, lack of attainment of equilibrium, adsorption on the solid support and at the gas-liquid interface, and finally, non-ideal solution behaviour[1] Non-ideality is taken to mean that the solutions show deviations from Raoult's law We wish to make two points, first, that partition isotherms are frequently markedly non-linear and, second, that non-ideal solution behaviour is not important in determining elution peak symmetry, but that isotherm shape is We base our arguments on the results of experiments which were aimed at the determination of partition isotherms by a static method

Using a modified McBain–Bakr balance we have measured the partition isotherms of a number of vapours in a variety of liquids commonly used as stationary phases in gas-liquid chromatography The method was as follows a small sample of 'Celite' coated with the liquid stationary phase was weighed into a small glass bucket and suspended from a silica spring (extension approximately 1 m per gm) and the system degassed to a vacuum of 10^{-4}–10^{-5} mm of mercury Small charges of the vapour were then admitted to the system and the uptake by the liquid determined by measuring the extension of the spring In this way we have investigated the solubility of the following vapours at a number of different temperatures cyclohexane, benzene, dichloromethane, chloroform and carbon tetrachloride in dinonyl phthalate and squalane, and additionally, benzene in benzyl diphenyl, diglycerol and silicone oil Similar measurements have been made by Everett and Ashworth[2] Some of the isotherms determined at 30° C are shown in Fig 1 With the exception of the system benzene–diglycerol, in which there was negligible uptake of benzene by the diglycerol, even at relatively high pressures, all the isotherms obtained were of the same general shape, that is, all were curved in a direction convex to the concentration axis This establishes our first point It is only fair to point out, however, that these isotherms approximate to linearity at low concentrations, hence, provided that only small samples are used in gas-liquid chromatography, symmetrical elution peaks should be obtained In passing, it is interesting to note that the isotherms curve in the opposite sense to that usually observed in adsorption at a solid surface

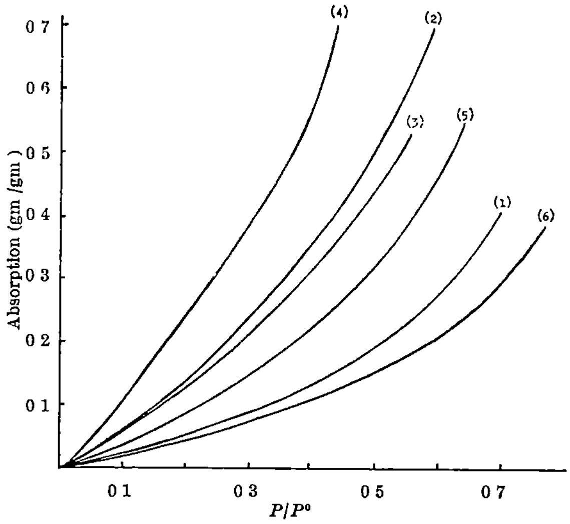

Fig 1 Isotherms at 30° C (1) Cyclohexane in dinonyl phthalate, (2) carbon tetrachloride in dinonyl phthalate, (3) dichloromethane in dinonyl phthalate, (4) chloroform in dinonyl phthalate, (5) cyclohexane in squalane, (6) dichloromethane in squalane

It has been shown theoretically by de Vault[3] and experimentally by Gregg and Stock[4] that systems giving rise to isotherms of the shape illustrated in Fig 1 will, if used in chromatography, tend to give elution peaks with diffuse front boundaries ('fronting') and sharp tails, and there is no doubt that such peaks are frequently observed in gas-liquid chromatography when rather large samples are used (as may be necessary when a comparatively insensitive detector is employed) Now Raoult's law defines an ideal solution as one which gives a straight line when the partial vapour pressure of a component is plotted against its mole fraction, x (In the systems under consideration only one of the components exerts an appreciable vapour pressure, that of the stationary

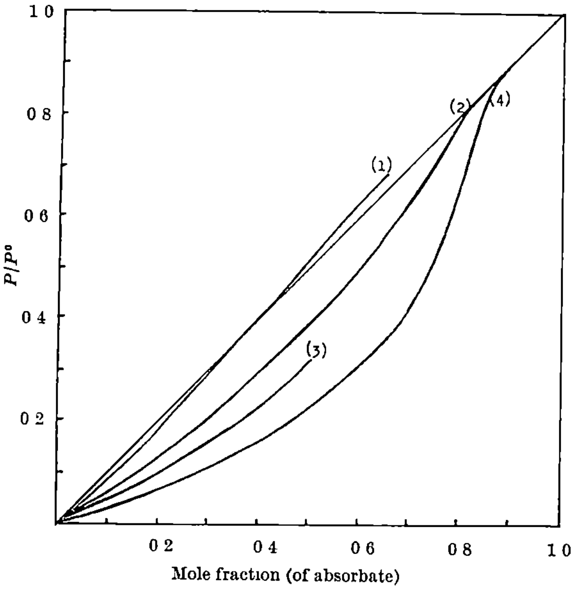

Fig 2 Deviation from Raoult's law at 30° C, all in dinonyl phthalate (1) Cyclohexane, (2) carbon tetrachloride, (3) dichloromethane, (4) chloroform

liquid can be neglected) If we now replot the isotherms involving dinonyl phthalate in Fig 2 using mole fractions instead of concentrations, we observe that three of the systems show negative deviations from Raoult's law and one shows both a positive and a negative deviation, in spite of the fact that the corresponding isotherms are all the same general shape. It can easily be ascertained that systems which obey Raoult's law or give positive deviations will still give curved isotherms of the type illustrated To obtain isotherms which are curved in the opposite sense, that is, are concave to the concentration axis, and which would therefore give rise to tailing, the most pronounced negative deviations from Raoult's law are necessary Such a system has never been observed by us. We are therefore of the opinion that 'fronting' is an inherent tendency in gas-liquid chromatography, but will not be marked, or even noticeable, when low concentrations of the solute are used Tailing is seldom, if ever, caused by deviations from ideality of the gas-liquid system, and is most probably due to the adsorption on the solid support, or perhaps at the gas-liquid interface

Finally, we would like to record that our results for the system showing both positive and negative deviations from Raoult's law, namely, cyclohexane–dinonyl phthalate, confirm the measurements and predictions of Everett and Ashworth[2] Very careful measurements were made to establish the deviation at low concentrations of cyclohexane, and the relevant data are given in Table 1 Further investigations have shown that this type of system is by no means uncommon in gas-liquid chromatography

Table 1 Results for the System Cyclohexane and Dinonyl Phthalate at 30° C

P/P^0	X	$\log_{10} P/P^0X$	$\frac{B(P^0 - P)}{2\ 303\ R\ T}$	$\log_{10}\gamma^{\circ}f$
0 02628	0 02841	$\bar{1}$ 9661	0 0041	$\bar{1}$ 9701
0 06483	0 06904	$\bar{1}$ 9726	0 0039	$\bar{1}$ 9765
0 11903	0 12504	$\bar{1}$ 9786	0 0037	$\bar{1}$ 9823
0 20486	0 21123	$\bar{1}$ 9867	0 0033	$\bar{1}$ 9900
0 25321	0 25772	$\bar{1}$ 9923	0 0031	$\bar{1}$ 9954
0 30065	0 30318	$\bar{1}$ 9964	0 0029	$\bar{1}$ 9993
0 35461	0 35424	0 0004	0 0027	0 0031
0 41186	0 40826	0 0033	0 0025	0 0057
0 48327	0 47396	0 0086	0 0021	0 0107
0 56548	0 54729	0 0140	0 0018	0 0158
0 66598	0 63829	0 0182	0 0014	0 0196

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A Cyclic Tetramer of Chloral

Treatment of chloral or its hydrate with concentrated sulphuric acid yields a number of polymeric materials which were identified by Chattaway and Kellett[1] and studied by Novak and Whalley[2] From the mixture obtained in this Laboratory by treating some 300–400 gm of chloral hydrate, about 2 gm (0 5 per cent) of a new polymeric compound was isolated as a white crystalline solid (m p 219–220° corr) It crystallized from absolute ethanol as short needles slightly less soluble in this solvent than chloralide or α-parachloral The compound is very soluble in acetone, pyridine and ether, moderately soluble in boiling ethanol, slightly soluble in carbon disulphide, carbon tetrachloride and cold ethanol and insoluble in water Boiling with an aqueous ethanol–20 per cent sodium hydroxide solution liberates chloroform and formic acid Further work gave the following results Analysis, calculated for $(CCl_3\text{–}CHO)_n$ C, 16 28, H, 0 68, Cl, 72 17 Found C, 16 43, H, 0 73, Cl, 71 78 Molecular weight (Rast), calculated for $(CCl_3\text{–}CHO)_4$, 589 5 Found 614, 616, 571 (average 600 3) Infra-red spectrum the compound was examined in 'Nujol' mull (0 025 mm) and in saturated (18° C) carbon tetrachloride and carbon disulphide solutions (1 mm), on a model 137 'Infracord' spectrophotometer (with sodium chloride prism) The bands observed are recorded in Table 1

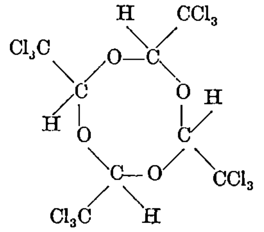
Table 1

Wave-length (μ)

Solid	Solution
3 49, weak	3 49, weak
6 60, medium	6 60, medium
7 23, medium	7 23, medium
7 50, medium	
7 50, medium	7 52, strong
7 57, strong	
	8 55, sharp
8 60, very strong	
	8 75, very strong
8 78, very strong	
9 22, strong	
9 37 strong	
	9 40, medium
	9 50, medium
9 80, very strong	9 80, strong
10 30, medium	
11 78, very strong	11 78, very strong
12 30, very strong	12 30, very strong
12 55, sharp	
13 90, sharp	13 90, sharp
14 10, strong	14 10, strong
	14 50, strong
15 00, strong	

The results in Table 1 indicate (*a*) the absence of hydroxyl and carbonyl groups, (*b*) the presence of a cyclic ether linkage due to the series of bands in the 950–1,200 cm.$^{-1}$ (8.3–10.5μ) and 800–860 cm.$^{-1}$ (11.6–12.5μ) regions, and (c) a shift towards lower frequencies as compared with bands of other known cyclic ethers[3]. It is a well-established fact that there is a shift to lower frequencies whenever there is an increase in ring-size among cyclic ethers.

These results are consistent with the existence of a cyclic tetramer having the following formula:



A report on the structure of this compound, 2,4,6,8-tetrakis-(trichloromethyl)-1,3,5,7-tetroxocane, will be published elsewhere.

I am indebted to Dr. Oscar H. Saggese of this Department for his interpretation of the infra-red spectra.

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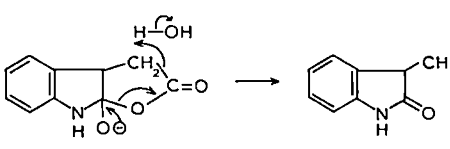
BIOCHEMISTRY

Oxidative Decarboxylation of Indole-3-acetic Acid by Mangani-Versene and by Wheat Leaf Enzyme

THERE is little agreement concerning either the pathway or the final products of the enzymic oxidation of heteroauxin[1]. The stoichiometry of the oxidative decarboxylation seems to be reasonably well established[1], one mole of oxygen being taken up and one of carbon dioxide evolved for every mole of indole-3-acetic acid (IAA) reacting. Galston[2] reported that no indole-3-aldehyde was formed when IAA was destroyed by a pea brei (see, however, Racusen[3]), two principal products were formed with R_F very close to that of *o*-aminoacetophenone (I) and *o*-formamidoacetophenone (II) and giving similar colour reactions. On the basis of a ferric chloride test, Galston concluded that these compounds were hydroxylated derivatives of (I) and (II). Indole-3-aldehyde has an R_F intermediate between those of (I) and (II) and does give a brownish-pink colour with ferric chloride, the colour reported by Galston is undoubtedly due to the spot for the indolealdehyde overlapping on to the other two spots. It also seems most unlikely that the R_F of phenolic derivatives of both (I) and (II) should be the same as those of (I) and (II). Finally, should such phenols be formed, the uptake of oxygen should be at least 1.5 times that actually reported. Using an oxidizing enzyme from *Omphalia flavida*, Stowe *et al.*[4] rejected the possibility that (I), (II) or indolealdehyde was formed in any amount and suggested that the product (not isolated) might be 3-methyldioxindole. From their chemical evidence it seems rather unlikely that their product was 3-methyldioxindole. Most of their observations can be explained on the basis of our present results. With *Lupinus* seedlings Stutz[5] obtained a number of products (one of them being indolealdehyde) chromatographically similar to those obtained by Galston[2]. Using substituted 3-indoleacetic acids Stutz concluded that the ring system (probably C_2) and not the side chain was the primary site of dehydrogenation.

MacLachlan and Waygood[6] reported that the oxidative decarboxylation of IAA by mangani-versene in a phosphate buffer at *p*H 6 gave a product which appeared to be chromatographically identical with that obtained by the oxidation of IAA in the presence of wheat leaf enzyme, or by using a peroxidase in the presence of manganese ions and 2,4-dichlorophenol. Waygood *et al.*[7] also suggested a radical chain mechanism for this reaction involving the formation of a skatyl radical leading to a product $C_9H_9O_2N$ (not established) (cf. Hewitt[8]). No pure compound was actually isolated, all the conclusions being based on the rates of absorption of oxygen and evolution of carbon dioxide.

We have now oxidized IAA with oxygen in the presence of mangani-versene at *p*H 6 and isolated the basic, neutral and acidic fractions. The neutral fraction (57.5 per cent based on IAA) was analysed using paper, column, and vapour phase chromatography. By preparative vapour phase chromatography two compounds could be identified (in all cases here by analysis, melting points, mixed melting points with authentic samples, R_F values, and infra-red spectra) as *o*-aminoacetophenone (I) (very small amounts) and *o*-formamidoacetophenone (II) (9–13 per cent). Column chromatography of the neutral fraction gave *o*-aminoacetophenone (10–13 per cent) (*o*-formamidoacetophenone is hydrolysed to (I) under these conditions), followed by a small amount of unidentified crystals, melting point 164–165° (no C = O bands in the infra-red). Indole-3-aldehyde was eluted next (12.3 per cent crude yield), and, finally, a low-melting solid (25 per cent) which was chiefly 3-methyloxindole (III). It would thus seem as though all the postulated modes of primary attack are occurring in this system. Phenolic products are probably also formed (cf. ref. 9) and are present in the acidic fraction together with some unreacted IAA, but this was not investigated. Formation of 3-methyloxindole probably proceeds by primary attack at C_2 of the indole ring followed by the assisted decarboxylation of a cyclic intermediate such as (IV).



(IV) (III)

The formation of (I) and (II) is analogous to the conversion of tryptophan to kynurenine and probably involves attack at C_3 (ref. 10). The formation of indole-3-aldehyde necessitates primary attack at C_α of the side-chain.

In the case of the enzymic oxidation products[6,7] (reaction mixtures kindly supplied by Dr. E. R. Waygood) neither (I) nor (II) could be detected. Column chromatography on alumina led to the isolation in low yield of 3-methyloxindole mainly together with indole-3-aldehyde.

A preliminary investigation (with Mr. K. A. H. Adams) of the mangani-versene catalysed oxidation of

IAA at *p*H 4 gave a coloured product (melting point 160° with decomposition) which was very difficult to purify and the molecular weight of which was approximately 576 This compound gave only one spot on paper chromatography, but decomposed on alumina into three fractions A small amount of the same product also precipitates out in the *p*H 6 reaction

We wish to thank Prof Waygood for generous supplies of enzymic oxidation mixtures and the National Research Council of Canada (Prairie Regional Committee) for financial support

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Complement-fixing Antibodies to Human Growth Hormone and Sheep Interstitial Cell Stimulating Hormone

PREVIOUS reports from this Laboratory[1-3] have shown that human growth hormone (HGH) and sheep interstitial cell stimulating hormone (ICSH) produce, in rabbits, precipitin antibodies that are specific to the respective hormones Immunochemical techniques have been used to characterize these hormones as well as to perform quantitative determinations of small quantities of the hormone Hæmagglutination inhibition has also been used to study and detect HGH in human serum[4] The purpose of the present investigation was to study HGH and sheep ICSH by another immunochemical reaction, namely, complement fixation It may be noted that the complement fixation method is capable of determining quantitatively as little as 0 01 μgm of the hormone and is an objective procedure which is more reproducible than the hæmagglutination technique

Rabbit antisera to HGH and sheep ICSH were produced and the γ-globulin was obtained as previously described[2,3] The procedure for the complement fixation tests was modified from that described by Kabat and Mayer[5] For a typical experiment, 0 02 μgm HGH was dissolved in 0 5 ml saline solution and 20 μgm anti-HGH γ-globulin plus 500 μgm lyophilized guinea pig serum in 0 5 ml veronal buffer of *p*H 7 3 were added. With HGH, complement fixation was carried out by incubation for an initial interval of 20 min at 37° C, followed by further incubation overnight in the refrigerator at 4° C In the case of ICSH, incubation for 1 hr at 37° C was sufficient

For the determination of uncombined complement, a suspension of hæmolysin-sensitized sheep red blood cells was added and the contents of the tubes were allowed to incubate for 1 hr at 37° C The non-hæmolysed intact red blood cells were removed by centrifugation, the clear supernatant fluid was diluted to the proper concentration for estimation of hæmoglobin concentration at 540 mμ in a Beckman spectrophotometer, model DU Increased complement fixation resulted in less hæmolysis and, consequently, a lower optical density Since the quantity of complement added is a critical step for reproducible results, a single lot of complement (Difco) with periodic titration was used in this work The amount of complement used was sufficient to lyse about 60 per cent of the sheep red blood cells in the tubes containing the lowest concentration of antigen.

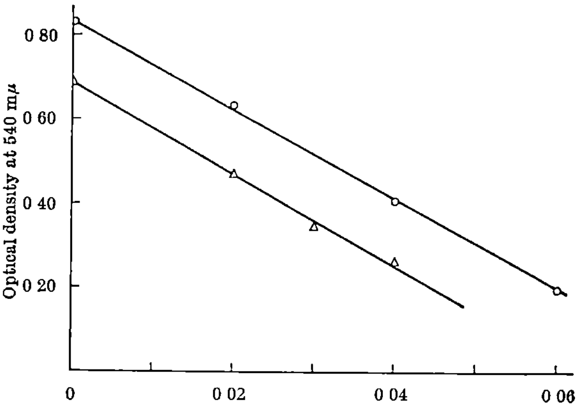

Fig 1 Amount of complement fixed as a function of human growth hormone (HGH) and sheep interstitial cell stimulating hormone (ICSH) concentrations △, HGH, ○, sheep ICSH

The standard curves, which were obtained by varying the concentration of the hormone while keeping constant the amounts of all other components of the system, are shown in Fig 1 A linear response was observed up to 0 06 μgm of ovine ICSH and 0 04 μgm of HGH The complement fixation tests were found to be reproducible if care was used in all phases of the test As a routine practice, it is always necessary to use a sufficient number of controls to detect anti-complement or synergistic activity and non-specific hæmolysis

The amount of complement fixed as a linear function of the hormone concentration suggested that the complement fixation reaction could be used as a quantitative method for determining HGH and ovine ICSH This technique has thus been employed for estimation of hormonal content in the pituitary gland Fresh pituitaries were homogenized in saline solution and the clear supernatant fluid was used The HGH content of two glands was found to be 21 3 and 31 8 μgm per mgm of fresh tissue by complement fixation and 19 3 and 36 1 μgm per mgm by quantitative precipitin tests[2] By complement fixation the HGH in three acetone-dried glands was estimated as 4 4 μgm per mgm of dry tissue and 4 1 μgm per mgm by the precipitin test[2] These results were in good agreement with values estimated on the basis of the results of the tibia test in hypophysectomized rats[6]

Similar studies were carried out for the estimation of the ICSH content in sheep pituitaries It was found that two sheep pituitaries contained 0 9 μgm and 0 8 μgm, respectively, of ICSH per mgm of fresh tissue when the content was determined by complement fixation and 1 0 μgm and 0 9 μgm, respectively, of ICSH per mgm by the precipitin test[3]

The results of this investigation demonstrate that antibodies, prepared from rabbit antisera, to highly purified preparations of human growth hormone and sheep ICSH fix complement when allowed to react with the respective antigens On a quantitative basis, the complement fixation reaction will detect about one-hundredth of the quantity of hormone measured by precipitin reactions The close agreement of the values obtained by the technique of complement fixation with those obtained by quantitative precipitin tests indicates that complement fixation can be used as a quantitative immunochemical technique for measuring minute quantities of HGH and sheep ICSH

This work was supported in part by a grant from the American Cancer Society and the United States Public Health Service (*RG*-4092) Two of us (A T and K K S) thank the United States Public Health Service and the Population Council of the Rockefeller Foundation, respectively, for research fellowships

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Effect of Riboflavin on Liver Histomucoid

Isenberg and Szent-Gyorgyi[1] studied the reaction between tryptophan and tryptophan metabolites and riboflavin It was observed that free tryptophan or this amino-acid in proteins formed a complex with riboflavin The vitamin molecule takes up one electron from tryptophan and a semiquinoid riboflavin free radical is formed Those authors speculated that this complex occurs *in vivo* Since it was established by Winzler *et al* [2] that seromucoid contains tryptophan, and in view of the hepatic origin of that fraction[3], experiments were conducted by us to investigate a possible effect of riboflavin on liver histomucoid *in vivo*

Male Wistar rats with mean body weight of 135 gm were injected intraperitoneally with a freshly prepared riboflavin solution (20 mgm /100 ml of physiological saline) The dose used was 0 2 mgm of riboflavin per 100 gm of body weight One group of animals received only one injection and the other was treated with a daily dose for 7 days Control rats were injected with the same volume of the saline solution All the animals were fed *ad libitum* a standard balanced diet, and liver histomucoid determinations were carried out, by the method previously reported[4], 24 hr after the last injection A summary of the results is shown in Table 1

Table 1 clearly shows that in both riboflavin groups lowered levels of liver histomucoids were observed The differences between riboflavin-injected rats and the controls were statistically significant (1 treatment t = 3 733, P < 0 01, 7 treatments t = 4 711, P < 0 001) From the results presented it is obvious that one dose of riboflavin is sufficient for the production of the full effect

Table 1 LIVER HISTOMUCOID IN RATS TREATED WITH RIBOFLAVIN

Treatment	No of injections	No of rats	Histomucoid (mgm hexose/100 gm acetone powder) Range	Mean ± S D
Saline	1	6	105–191	152 ± 36
Riboflavin	1	6	89–108	96 ± 7
Saline	7	8	110–187	146 ± 24
Riboflavin	7	11	67–127	97 ± 20

Seromucoid determinations[3] in the same animals were carried out and no differences were disclosed between control and riboflavin-treated rats This result warrants the conclusion that the lowered liver histomucoid observed was not produced by a mobilization of mucoproteins from that organ to the plasma

On the other hand, incubation of extracts from liver acetone powders[4] or liver homogenates in physiological saline with riboflavin at 37° C up to 3 hr gave no change in the histomucoid-level

Further investigations are being undertaken to elucidate the mode of the action of riboflavin on liver histomucoids

This work was carried out with research fellowships from the National Research Council of Brazil

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D-Penicillamine as an Antidote to 8-Hydroxyquinoline and Alloxan

Owing to the chelate-forming effect of its —SH and NH_2 radicals, DL-penicillamine has hitherto been used for removing copper deposited in brain tissues in cases of Wilson's disease and for preventing intoxication caused by heavy metals[1] It is, however, undesirable to use L-penicillamine as a remedy or a preventive, because of its strong toxicity which causes acute and chronic deficiency of vitamin B_6 (refs 2–4)

D-penicillamine, on the other hand, has no toxicity We have examined its preventive and remedial effects on intoxication by 8-hydroxyquinoline and alloxane, which are diabetogenic agents

0 03 mgm /gm of body-weight was determined as the lethal dose of 8-hydroxyquinoline for mice 10 hr after intramuscular injection 0 2 mgm /gm of D-penicillamine, injected 5 min before the injection of lethal doses of 8-hydroxyquinoline, prevented death of 60 per cent of the experimental animals or showed effects in retarding death Injection of 0 5 mgm /gm of D-penicillamine prevented death completely but could not prevent toxic symptoms

Table 1

No of Mice	10	10	10	10	10
D-Penicillamine mgm / body-weight (gm)	1 0	0	0 2	0 5	1 0
8-Hydroxyquinoline mgm / body-weight (gm)	0	0 03	0 03	0 03	0 03
Toxic symptoms	—	+ + +	+ +	+	—
No of mice dead	0	10	4	0	0

Table 2

No. of mice	10	10	10	10	10	3
Tested compounds mgm./body-weight (gm.)	—	D-penicillamine 1.0	DL-cystine 1.0	Vitamin B_6 1.0	Glucose 1.0	Vitamin B_{12} 0.15
Alloxan mgm./body-weight (gm.)	0.45	0.45	0.45	0.45	0.45	0.45
Toxic symptoms	+ + +	+	+ + +	+ + +	+ + +	+ +
No. of mice dead within 15 hr.	10	3	10	10	10	2

such as dyspnœa, tremor and paralysis. Injection of D-penicillamine (1.0 mgm./gm.), however, was successful in preventing death as well as all toxic symptoms (Table 1). Further, D-penicillamine injection in the same amount, 1 hr. before the lethal dose of 8-hydroxyquinoline, could also save the animals from death.

On the other hand, injection of 1 mgm./gm. of vitamin B_6, DL-cystein (hydrochloride) or glucose or of 0.15 mgm./gm. of vitamin B_{12} 5 min. before the injection of lethal doses of 8-hydroxyquinoline could not prevent death in these animals.

1.0 mgm./gm. D-penicillamine injection 5 min. before the injection of alloxane in the lethal doses (0.45 mgm./gm.) retarded death, but DL-cystein, vitamin B_6, glucose or vitamin B_{12}, injected in the amount mentioned above, could not prevent or retard death (Table 2).

8-Hydroxyquinoline injection in an amount less than the lethal dose caused the blood sugar in mice to increase by approximately 100 per cent in 1–3 hr. and, thereafter, in 6–16 hr., to decrease by approximately 40 per cent, during which time some of the mice died of intoxication. The blood sugar returned to the normal level in 24 hr. and, thereafter, in most cases showed slightly higher values than the normal. In order to test the influence of D-penicillamine upon the changes of blood sugar values brought about by 8-hydroxyquinoline, 1.0 mgm./gm. of D-penicillamine was injected 5 min. before 8-hydroxyquinoline injection, but no influence whatever was observed. Accordingly, it is considered that death caused by 8-hydroxyquinoline and the antidotal effect of D-penicillamine against 8-hydroxyquinoline has nothing to do with the changes of blood sugar values.

With regard to the mechanism of the preventive action of D-penicillamine, we are still conducting tests.

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Preparation and Determination of Sulphosalicylo-soluble Proteins using Tannin

In previous papers[1–3], regeneration of proteins from insoluble protein-tannin compounds (PT) after treatment with caffeine, without loss of physico-chemical and biological properties, was reported. The present communication is concerned with determination and preparation of sulphosalicylo-soluble proteins (SSP). The proteins belonging to that group are, for example, gonadotropins, thyreotropin, orosomucoid, haptoglobin, submaxillary mucoprotein, ovomucin, lysozyme, uromucoid and acid mucopolysaccharide-containing proteins.

In this communication the turbidimetric micromethod[4] is applied to estimation of both groups of proteins, soluble and insoluble ones, in sulphosalicylic acid (SSA). It has been shown that 10–100 μgm. of dried gelatin, lysozyme, orosomucoid or mucin, dissolved in 1 ml. of 0.9 per cent sodium chloride or 0.15 *M* SSA, produce with tannin reagent similar turbidity as hæmoglobin, fibrinogen or egg albumin dissolved in 1 ml. of 0.9 per cent sodium chloride. Under conditions of the method (*p*H about 0, excess of tannin with reference to weight of protein), irreversible PT compounds are formed. Turbidities, measured as optical density, are directly proportional to the weight of proteins and do not depend on molecular weight, nitrogen content or on presence of a non-protein moiety. In this way it is possible to apply the same criterion for the determination of proteins which are soluble or insoluble in SSA, and an appropriate conversion factor should be used.

It has been shown that as well as previously investigated proteins, orosomucoid and lysozyme form reversible PT compounds at their isoelectric points and below, at a definite ratio of weight of the reactants[5]. For the precipitation of proteins at *p*H about 1(0.15 *M* SSA), 400 mgm. of tannin should be added to each 100 ml. of the solution and then an amount of tannin equal to protein content. The precipitate is centrifuged and washed with water until it is free from SSA. Lysozyme and orosomucoid, after regeneration with caffeine, retain their electrophoretic mobility and solubility in SSA unchanged. SSP can be recovered with 90 per cent yield.

Concentrations of total protein as well as that remaining in SSA filtrate have been determined in different biological materials. For example, in 100 ml. of gastric juice, normal serum or urine (without uromucoid), 0.2, 6.0 and 0.005 gm. of protein and 0.2, 0.07 and 0.002 gm. of SSP, respectively, were found[5]. Very small amounts of SSP in normal serum and urine require to be concentrated before paper electrophoresis. For that purpose, the tannin and caffeine procedure was applied[1]. SSP of serum had been concentrated 50-fold and those of urine 1,000-fold, and they were separated afterwards by paper electrophoresis. The results of one experiment in which both

Table 1. CONTENT OF THE SULPHOSALICYLO-SOLUBLE PROTEINS (SSP) IN THE ELECTROPHORETIC FRACTIONS OF NORMAL HUMAN SERUM AND URINE

	Serum			Urine*		
		SSP			SSP	
Fraction	Protein gm./100 ml.	gm./100 ml.	Per cent of fraction	Protein gm./100 ml.	gm./100 ml.	Per cent of fraction
Albumin	3.40	0.0045	0.13	0.00073	0.00013	18.0
α_1-Globulin	0.34	0.0470	13.90	0.00048	0.00034	72.6
α_2-Globulin	0.72	0.0097	1.34	0.00054	0.00016	28.8
β-Globulin	0.92	0.0066	0.72	0.00158	0.00043	26.9
γ-Globulin	1.14	0.0142	1.25	0.00048	0.00029	60.5
Protein without electrophoresis	6.6	0.0820	1.24	0.00381	0.00135	35.5

* Urine without uromucoid of Tamm and Horsfall.

Paper electrophoresis in veronal buffer of *p*H 8.6 and ionic strength 0.09 (ref. 8). Protein was concentrated by the tannin–caffeine procedure (ref. 2). Amount of SSP in 100 ml. of serum, amount of protein in 100 ml. of urine and SSP in 100 ml. of urine were, after the concentration procedure, 3.1, 1.8 and 2 gm., respectively. The results are obtained by dividing the obtained values by degree of concentration.

the protein and regenerated SSP of normal human serum and regenerated protein and SSP of normal urine were determined after paper electrophoresis are shown in Table 1 The values obtained for orosomucoid of normal human serum were strikingly similar to those of Winzler[6]

Electrophoretic paper strips of regenerated proteins and SSP of normal urine were stained for acid mucopolysaccharides with alcian blue according to Heremans and Vaerman[7] Three fractions were found with anodic mobility greater than albumin even though chondroitic acid and heparin are not precipitable with tannin The presence of acid mucopolysaccharides proves that they are precipitated together with the protein component and are then dissociating during electrophoresis

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Comparative *in vitro* Activities of Phosphorylase *b* and Cytochrome Oxidase in Preparations from Two Ox Muscles

IT is generally observed that glycolysis in mammalian muscles ceases when the *p*H falls to 5 4–5 5 and that this *p*H is usually reached unless there is insufficient glycogen present in the muscle at the moment of death In some muscles, however, as much as 1 per cent of glycogen may remain unconverted to lactic acid at an ultimate *p*H of 5 9–6 0 [1,2] One such muscle is ox *sternocephalicus* It has been shown that during post-mortem glycolysis in this muscle the length of the external glycogen chains diminishes significantly Thus, while the mean number of glucose residues in external chains of glycogen molecules for ox *sternocephalicus* glycogen is 12 before the onset of *rigor mortis* (pre-rigor), the mean number of glucose residues for the residual glycogen[3] (post-rigor) is 9 the latter is apparently insusceptible to further glycolysis *in situ* In these circumstances, it was of interest to compare the behaviour of the glycogen isolated *post rigor* from ox *sternocephalicus* when exposed *in vitro* to the action of phosphorylase *b* preparations from ox *sternocephalicus* and *psoas major* muscles respectively The rate of *in vitro* breakdown of some other muscle glycogens by these phosphorylases has also been considered

The methods for preparing pre- and post-rigor samples of muscle glycogen have been described earlier[3] Phosphorylase *b* was prepared by the method of Fischer and Krebs[4], and its enzymic activity in forming glucose-1-phosphate from glycogen was measured as described by Illingworth and Cori[5] The activity of phosphorylase *b* prepared from ox *psoas major* muscles against pre-rigor glycogen from this same source has been given an arbitrary value of 100 Findings are summarized in Table 1

Table 1 RELATIVE *in vitro* ACTIVITY * OF OX PHOSPHORYLASE *b* FROM TWO OX MUSCLES WITH VARIOUS SAMPLES OF MUSCLE GLYCOGEN †

Source of phosphorylase *b*‡ preparation	Source of glycogen *Psoas major* (ox) pre-rigor	*Psoas major* (ox) post-rigor	*Sternocephalicus* (ox) post-rigor	Heart (horse) pre-rigor	Heart (horse) post-rigor
Psoas major§	100	100	80	130	95
Sternocephalicus§	100	110	75	145	115

* Rate of glucose-1-phosphate formation in a reaction mixture of 0 2 m*M* adenylic acid, 1,000 mgm per cent glycogen, 25 mgm per cent inorganic phosphate and 5 mgm per cent phosphorylase *b* protein in 0 07 *M* glycerophosphate and 0 06 *M* cysteine buffer, *p*H 6 8 Activity of *psoas major* phosphorylase *b* with *psoas major* (pre-rigor) glycogen taken as 100 = 850 units/mgm protein

† As prepared by Lawrie *et al* (ref 3)

‡ As prepared by Fischer and Krebs (ref 4)

§ Mean values of three determinations except for *psoas major* (post-rigor)

It is clear that phosphorylase *b* preparations from *psoas major* and *sternocephalicus* muscles of the ox break down a given glycogen at comparable rates Different glycogens, however, are attacked at markedly different rates by both phosphorylase *b* preparations Thus pre-rigor glycogen from horse heart is broken down at about twice the rate of post-rigor glycogen from ox *sternocephalicus* (Table 1)

The results indicate that post-rigor glycogen from ox *sternocephalicus* is broken down *in vitro* by phosphorylase *b* prepared from this same muscle This suggests that its apparent insusceptibility to attack during post-mortem glycolysis cannot be entirely explained by those structural changes in the molecule represented by the shortening of the mean number of glucose residues in external chains of glycogen molecules In this context it may be mentioned that pre- and post-rigor glycogens from horse diaphragm and *psoas* muscles have similar mean number of glucose residues, yet in both muscles there is 0 6–1 per cent of residual glycogen at ultimate *p*H values above 5 85 [3] On one hand it could mean that phosphorylase is more readily inactivated during post-mortem glycolysis in ox *sternocephalicus* than in ox *psoas* On the other hand, it could signify that, apart from the magnitude of the store of muscle glycogen, intracellular organization is a factor in determining the extent of post-mortem glycolysis It is not inconceivable that glycogen is functionally differentiated *in vivo* and that there may be concomitant specificity of localization Thus, in muscles such as ox *sternocephalicus*, some of the glycogen present *in vivo* may be inaccessible to attack under the conditions prevailing in the immediate post-mortem period

The relatively high activity of phosphorylase *b* with pre-rigor glycogen from horse heart is compatible with the rapidity of glycolysis in the latter post mortem[6] and under anaerobic conditions *in vivo*[7] Pre- and post-rigor glycogens from ox *psoas major* are apparently converted into glucose-1-phosphate at much the same rate by phosphorylase *b* preparations *in vitro* This accords with their similar branching structure[3] and with the fact that no residual glycogen is found in this muscle above an ultimate *p*H of 5 4–5 5 (ref 8).

The potential respiratory capacity of the ox *sternocephalicus* was compared with that of the *psoas major* by determining cytochrome oxidase in enzyme preparations[10] made from each (Table 2) Both the concentration of enzyme solids, and their

Table 2 COMPARATIVE DATA ON CYTOCHROME OXIDASE ACTIVITY IN ENZYME PREPARATIONS FROM OX *psoas major* AND OX *sternocephalicus* MUSCLES

Source of enzyme preparation	Cytochrome oxidase* Qo_2 mean and *S E*	Mgm dry fat-free solids/ml mean and *S E*	Calculated oxygen uptake on whole-tissue basis (µl /gm / hr)	Ultimate *p*H of muscle
Psoas major	1,146 ± 51 (5)	37 0 ± 6 0 (5)	4,770	5 51 ± 0 04 (5)
Sterno-cephalicus	1,554 ± 222 (5)	48 6 ± 6 8 (5)	8,400	5 91 ± 0 08 (5)

* Cytochrome oxidase Qo_2 max = µl oxygen/mgm dry fat-free enzyme solids/hr at infinite cytochrome *c* concentration Experimental conditions of Slater (ref 9)

intrinsic activity, were greater in the *sternocephalicus* preparations than in those from the *psoas major* As a result the potential rate of respiration due to cytochrome oxidase in the former was found to be about twice as high as that in *psoas major*, when calculated on a whole-tissue basis There tends to be an inverse relationship in muscle between respiratory capacity and the magnitude of the initial glycogen store[1,3,11] (Initial glycogen is an approximation to the *in vivo* resting value It refers to the quantity at a hypothetical initial *p*H of 7 2, calculated from the *p*H, glycogen content and buffering power of a muscle sample) Since the *sternocephalicus* has a markedly greater initial store of glycogen than *psoas major*[3], its greater cytochrome oxidase activity is unexpected This finding does suggest, however, that the glycogen store in a muscle such as ox *sternocephalicus* may serve other purposes than the production of energy by glycolysis or respiration solely from a quantitative aspect The resistance of part of this glycogen to post-mortem glycolysis, its relative resistance to phosphorylase *b* *in vitro* and its unusual branching characteristics lead to a similar conclusion

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Prostatic Cytochromes

THE endogenous respiration of adenomatous human prostatic tissue is known to be low[1] In the present work the respiratory activity and lactic acid production of normal rat prostate and adenomatous human prostate have been compared

The rate of oxygen uptake of the human gland was about one-third that of the dorsolateral lobe or the ventral lobe of the rat It was considerably increased by 10^{-3} *M* succinate and to a lesser extent by 10^{-3} *M* fructose Except for pyruvate, which had a slight stimulatory effect on the ventral lobe, the oxygen uptake of either lobe of the rat was uninfluenced by 10^{-3} *M* succinate, pyruvate, glucose or fructose (Table 1)

Table 1 EFFECT OF EXOGENOUS SUBSTRATES ON THE RATE OF OXYGEN UPTAKE OF PROSTATIC TISSUE

Substrate	Oxygen uptake µmoles/gm dry weight/hr Tissue Rat dorsolateral lobe	Rat ventral lobe	Human gland
Nil	80 ± 10 (4)	96 ± 5 (12)	33 ± 7 (8)
Succinate	82 ± 8 (3)	119 (2)	59 ± 5 (5)
Fructose	87 ± 13 (3)	101 ± 21 (3)	53 ± 5 (6)
Pyruvate	84 ± 8 (4)	126 ± 9 (8)	42 ± 6 (6)
Glucose	79 ± 7 (3)	99 ± 12 (8)	32 ± 2 (18)

Slices of tissue were incubated in a calcium-free Krebs–Ringer-phosphate buffer at *p*H 7 4 with 10^{-3} *M* substrate Equilibrated with air at 37°

Results are given as means ± *S E* with the number of experiments shown in parentheses

The rate of lactic acid production, which was greater in the human gland than in either lobe of the rat prostate, was increased by added fuctose All the substrates tested on the rat glands except fructose (which was without effect) slightly raised the rate of lactic acid production These findings might be due to qualitative or quantitative differences in the cytochrome systems of the tissues (Table 2)

Table 2 EFFECT OF EXOGENOUS SUBSTRATES ON THE RATE OF LACTIC ACID PRODUCTION OF PROSTATIC TISSUE

Substrate	Lactic acid production µmoles/gm dry weight/hr Tissue Rat dorsolateral lobe	Rat ventral lobe	Human gland
Nil	23 ± 4 (3)	17 ± 1 (5)	90 ± 19 (9)
Succinate	—	24 (2)	65 ± 14 (5)
Fructose	19 (2)	15 (2)	120 ± 10 (6)
Pyruvate	39 ± 6 (4)	27 ± 3 (6)	86 ± 25 (7)
Glucose	31 ± 3 (3)	24 ± 4 (3)	80 ± 10 (18)

Conditions were as shown in Table 1

Homogenates in phosphate-saline buffer at *p*H 7 4 were prepared from the glandular portions of human adenomatous prostate, and from the separated dorsolateral and ventral lobes of the rat prostate, reduced with sodium dithionite and examined by the Beck–Hartree microspectroscope Both lobes of the rat gland showed absorption bands with maxima at 550 mµ, 562 mµ and 600 mµ, corresponding to the maxima of cytochromes *c*, *b* and *a* The bands were also produced by the addition of succinate to cyanide-treated preparations, suggesting enzymic reduction of cytochromes Homogenates from human glands chemically or enzymically reduced showed strong bands of cytochrome *b* and weaker bands of cytochrome *a* Cytochrome *c* was not detected with certainty, and, if present, its concentration was considerably less than those of cytochromes *b* and *a* The cytochrome *b* bands in all preparations disappeared on heating in a boiling water-bath for 5 min

Fresh normal human prostates have not been available for examination, but the absence or relatively low concentration of cytochrome *c* in the human adenomatous tissue might either be related to the mechanism of adenomatous change, or an adaptive response to hypoxia in the poorly vascularized hypertrophied gland (cf the decrease in the concentration of cytochrome *c* in diaphragm during hypoxia[2])

We wish to thank the British Empire Cancer Campaign for a grant to support the work

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An Unusual Major Fatty Acid Component in Human Depot Fat

THE technique of gas chromatography has shown that naturally occurring fats and waxes contain saturated and unsaturated fatty acids with both even and odd numbers of carbon atoms. So far, however, the acids with odd numbers of carbon atoms have been reported to occur in only relatively small amounts in natural materials.

During analysis of human depot fat being carried out at this Institute, one specimen was shown to contain 8.7 per cent of a branched chain fatty acid containing thirteen carbon atoms. Other specimens of depot fat we have examined have always contained less than 0.1 per cent of this acid. The analysis showed no other abnormal features. The fat was obtained from the abdominal wall of a male African of the Sukuma tribe, whose approximate age was 60 years, admitted to hospital suffering from a strangulated hernia. The material was treated by the method described by Kingsbury *et al.*[1] and 0.1 µl. of the resulting methyl esters was applied to a 4-ft. column of 20 per cent 'Apiezon *L*' grease on 100–120 mesh 'Celite' held at a temperature of 186° C. Argon was used as the carrier gas and the effluent gas was passed through a Pye ionization detector. The methyl ester of the component emerged from the column with a retention volume of 0.256 relative to methyl palmitate. With these conditions the relative retention volume of *n*-tridecanoic acid was 0.307. The amount of material available was too small to allow the nature of the acid to be determined accurately, but as the peak corresponding to the acid still appeared on the trace after bromination by the method of James and Martin[2] it must be considered to be a saturated acid.

We are unable to explain the presence of the large amount of this acid in this specimen, but since it is most unlikely to occur to any extent in the diet, we suggest that its presence is due to an unusual method of fatty acid synthesis in the body.

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Blood Glucose-6-Phosphate in Heterozygous Carriers and Patients with Liver Glycogen Disease (Von Gierke's Disease)

Hsia and Kot[1] have reported that children with liver glycogen disease (von Gierke's disease) and their parents, presumably heterozygous for the disease trait, both have high levels of glucose-6-phosphate and fructose-6-phosphate in red blood cells. If correct, this observation would have very important practical as well as theoretical implications. It would greatly simplify the diagnosis and study of this disease and would open the possibility of its control through a eugenic approach. However, Hsia and Kot presented no evidence that their analytical methods were appropriate or that the materials they measured were in fact glucose-6-phosphate and fructose-6-phosphate. Furthermore, the values these authors cited for their normal control population (glucose-6-phosphate, 50–370 mgm. per 100 gm. red blood cells, fructose-6-phosphate, 120–570 mgm. per 100 gm. red blood cells) are much higher than those reported by other investigators[2,3] or expected from theoretical considerations. Accordingly, their findings seemed questionable. We have re-examined this problem with respect to glucose-6-phosphate, using a specific enzymic analytical method. Our results are in sharp disagreement with those of Hsia and Kot.

Samples of whole blood or sedimented erythrocytes were deproteinized with equal volumes of 7 per cent perchloric acid. The filtrates were neutralized with potassium hydroxide and aliquots were incubated with glucose-6-phosphate dehydrogenase and triphosphopyridine nucleotide (TPN). The procedure of Aisenberg[4] was followed, except that 300 µgm. of TPN was used and the total volume of the incubation mixture was reduced to 0.5 ml. The reaction was conducted in microcuvettes. The procedure was applied to standard solutions of glucose-6-phosphate, production of reduced TPN was within 6 per cent of the expected amount. The method was then tested by determining glucose-6-phosphate in whole blood to which different amounts of glucose-6-phosphate had been added. The results are shown in Table 1. There was some loss of added glucose-6-phosphate, presumably due to catabolism during the interval between addition of the material and inactivation of enzymes in the blood. However, recoveries were in a satisfactory range and reproducibility was good, down to concentrations of about 1 mgm./100 ml. Below this level, the procedure proved inaccurate because the increases in optical density during incubation were very small.

Table 1. RECOVERY OF GLUCOSE-6-PHOSPHATE ADDED TO WHOLE BLOOD

Manipulations were carried out at room temperature, time interval before addition of perchloric acid was about 2 min. In each experiment the glucose-6-phosphate concentration was determined in an aliquot of blood to which none had been added and the recovery results were appropriately corrected

Glucose-6-phosphate (mgm./100 ml.) Added	Recovered	Recovery (per cent)
6.4, 6.4, 6.4	5.5, 5.7, 5.6	84, 89, 88
3.2, 3.2, 3.2	3.1, 2.6, 2.9	97, 81, 91
1.6, 1.6	1.4, 1.4	88, 88
0.64, 0.64, 0.64	0.72, 0.53, 0.53	112, 82, 82
0.32, 0.32, 0.32	0.21, 0.34, 0.37	66, 107, 115
0.16, 0.16	0, 0.21	0, 131

Table 2 presents the results obtained on whole blood and/or sedimented erythrocytes in 3 children with von Gierke's disease, 4 parents and 12 other blood relatives of such children, and 11 healthy adults without a family history of the disease. In all cases, the concentrations of glucose-6-phosphate were near the lower limit measurable by the method and much below those reported by Hsia and Kot. There were no major differences among affected children, their parents, other relatives, and normal controls. Because our analytical method was inaccurate at these low concentrations, however, it was not deemed worth

Table 2. GLUCOSE-6-PHOSPHATE IN BLOOD OF CHILDREN WITH VON GIERKE'S DISEASE, RELATIVES OF SUCH CHILDREN, AND NORMAL ADULTS

Relatives examined include 4 parents, 3 grandparents, 4 aunts or uncles and 5 siblings

	Whole blood (mgm./100 ml.)	Red blood cells (mgm./100 ml.)
Affected children		
Case 1	0.4	1.0
Case 2	0.7	—
Case 3	—	0.8
Relatives of affected children, range	< 0.3–0.7 (8 individuals)	< 0.3–0.5 (8 individuals)
Normal controls, range	< 0.3–2.0 (11 individuals)	< 0.3–0.8 (8 individuals)

while to calculate means and standard errors, and our results do not exclude the possibility that small differences between some of these groups may exist

The recovery data shown in Table 1 indicate that we would not have failed to find the concentrations reported by Hsia and Kot for their normal controls, to say nothing of the very high values these authors reported for affected children and their parents Our values are roughly of the same order of magnitude as those reported by Bartlett[2], who undertook a painstaking analysis of glycolytic intermediates in human red blood cells, using carefully controlled column chromatographic techniques for the separation of individual compounds and identifying each fraction obtained by appropriate chemical methods Bartlett obtained the following values in 14 healthy adults glucose-6-phosphate, 0 08–0 10 µmole per ml red blood cells (2 1–2 6 mgm per 100 ml), fructose-6-phosphate, 0 01–0 02 µmole per ml red blood cells (0 26–0 52 mgm per 100 ml)

Hsia and Kot reported normal erythrocyte concentrations of glucose-6-phosphate and fructose-6-phosphate which are up to six times as high as the normal level of glucose The concentrations proposed for heterozygous carriers (who have no obvious clinical abnormalities) and for children with von Gierke's disease are ten to fifteen times the normal glucose concentration These values are far outside the range expected from theoretical considerations or found by other investigators Since Hsia and Kot offered neither recovery data as a check on their analytical technique nor evidence that the materials measured were indeed glucose-6-phosphate and fructose-6-phosphate, we are forced to conclude that they were measuring something other than these phosphorylated hexoses and that a method for the detection of heterozygous carriers of von Gierke's disease is not yet at hand

This investigation was supported in part by grant *A*–1646 (*C*) from the National Institutes of Health, U S Public Health Service

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DURING the past year, we have attempted to improve the methodology using two different approaches I would like to summarize the results obtained up to the present time

In all experiments, equal volumes of 10 per cent trichloroacetic acid were added to whole blood and the precipitate removed by centrifugation The supernatant was then extracted four times with ether or until neutral, and the ether removed by evaporation

In the first series of experiments, glucose-6-phosphate was measured directly by incubation with yeast glucose-6-phosphate dehydrogenase (Sigma) and TPN, and observing the conversion of TPN to TPNH in the Beckman spectrophotometer at 340 mµ Recoveries of glucose-6-phosphate added to normal red cells was somewhat variable, with ranges from 76 to 111 per cent being obtained in successive experiments Using this technique, the range of values for 6 normal controls was 0 23–0 74 mgm /100 ml red blood cells (all values have been expressed on the basis that the hexose phosphates are intracellular) In two patients with von Gierke's disease, the values were respectively 0 44 and 0 72 mgm /100 ml red blood cells These findings are essentially in agreement with those of Sokal *et al*

In the next series of experiments, the total hexose monophosphates were determined in the following manner The evaporated ether extract was passed through 'Dowex 50 × 8 (50–100)' and washed with 25 ml water This was neutralized with ammonium hydroxide and put through a 'Dowex 1 × 8 (50–100)' column and washed with 50 ml water The resin was eluted with 80 ml. 0 02 *N* hydrochloric acid and the total hexose determined by anthrone Recovery for glucose-6-phosphate was 101 per cent Using this technique, the range for normal controls was 1 04–1 77 mgm /100 ml red blood cells The values for two mothers were 1 74 and 1 85 mgm /100 ml red blood cells, and for five children with von Gierke's disease were 1 04, 1 59, 1 87, 2 07, and 2 20 mgm /100 ml Thus, three of the affected children and one of the mothers had levels in excess of the upper limits of the normal range These findings are compatible with those previously reported by Wagner *et al* [1] and from our laboratory

In reviewing the communication by Sokal *et al* , one should evaluate critically two points First, there is considerable doubt as to whether the conversion of TPN to TPNH at 340 mµ can be used as a satisfactory means of measuring glucose-6-phosphate at such low ranges In particular, I feel that the values by this method tend to run low Secondly, the authors have failed to take into account the action of phosphatases and other enzymes on glucose-6-phosphate in the erythrocytes of normal individuals[2] and in those with von Gierke's disease In experiments with normal red cells, we have observed changes of glucose-6-phosphate upon standing at room temperature during a period of 1 hr The results of Sokal *et al* in Table 2 would seem to support this In their experiments, the whole blood was deproteinized within 2 min , while the packed red cells had to be separated by sedimentation or centrifugation involving at least 20–30 min It can be seen that in case 1, this procedure had little or no effect, since the packed red cell values are approximately twice that in whole blood On the other hand, in the normal controls the value in the packed red cells, instead of being about twice the range in whole blood, is in fact about half, indicating roughly a four-fold decrease

We[3] have recently prepared an enzyme system in red cells which hydrolyses glucose-6-phosphate Assays were carried out using a mixture of 1 ml of 1 1 hæmolysate and 1 ml 0 04 *M* histidine buffer at 5 0 containing 75 μM of glucose-6-phosphate and 100 μM of ethylene diamine tetraacetate and incubated for 2 hr at 37° C The glucose that was liberated was measured by glucose oxidase Duplicate assays showed variations of less than 5 per cent by this method The activity in 15 normal controls averaged 17 ± 2 7 mgm glucose liberated/hr /100 ml red blood cells, while in 8 patients with glycogen storage disease and their parents, it averaged 14 ± 2 7 The demonstration of an erythrocyte enzyme system which hydrolyses glucose-6-phosphate and a significant decrease of activity among patients and their parents explains our earlier findings.

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PHYSIOLOGY

Prolonged Biological Half-Life of Sodium-22 in Patients with Essential Hypertension

IN the treatment of hypertension, the effectiveness of sodium depletion by either dietary restriction or natriuretic agents is now fully documented It is sufficient to say that the response to such treatment led to the hypothesis that hypertensives might suffer from an abnormality in sodium metabolism The first measurements of the sodium content of individuals with hypertension were done in 1951 by the technique of isotopic dilution using sodium-24[1] With the exception of one report these observations have been confirmed repeatedly by ourselves and by others · an increase in sodium was not evident In the single discordant report[2], some cases of severe hypertension were noted to have an increase in exchangeable sodium, but this was interpreted as being secondary rather than primary to the disease, because it was observed almost exclusively in individuals with advanced hypertension

In 1953 it was reported that when the average salt-consumption of rats was increased the mean systolic pressure increased linearly[3] Observations on human beings have shown a similar type of correlation as the average salt-consumption of a community increases, the prevalence of hypertension increases linearly[4] Such observations have been interpreted to indicate that chronic excess salt consumption plays a primary part in the ætiology of human hypertension[5] In view of these considerations, the apparent absence of measurable increase in body sodium in patients with hypertension has been subjected to new scrutiny

During the past two years we have explored further the sodium metabolism of patients with and without hypertension using sodium-22 (physical half-life, 2 6 years) instead of sodium-24 The results suggest that hypertensives probably do have an abnormality in sodium metabolism

Fifteen adult patients, none of whom had had symptoms or signs of cardiac or renal failure, were studied Seven patients had 'essential' hypertension whereas the other 8 had unrelated diseases with normal blood pressure These investigations were carried out while the patients were in a metabolic ward and were consuming a constant diet which contained about 250 mgm of sodium chloride by analysis After the dietary regimen had been established, sodium-22 (usually 2–4 μc) was given orally and the total body burden of this isotope was recorded every 1–3 days by means of a whole-body counter[6] Thereafter the patient's daily intake of salt was increased by 2, 5 or 10 gm, at which level it remained for 2–4 weeks without other change in the regimen Some patients served as subjects for more than one level of salt increment The addition of this salt resulted in a prompt increase in the rate at which sodium-22 was lost from the body, manifested by a decrease in total body counts On any given salt intake, the rate of decline in counts proved to be steady and relatively predictable for each patient At each of the three levels of salt ingestion, the hypertensive individuals, without exception, lost sodium-22 significantly more slowly than did those without hypertension ($P < 0\ 01$) This is shown in Table 1, where the rate of loss of sodium-22 is described in terms of its 'biological half-life'

There are at least three possible explanations for the slower excretion of the sodium-22 in hypertensive subjects (1) The metabolism of sodium-22 differs from that of sodium-23 in hypertensives This seems most unlikely (2) Hypertensives absorb less of the supplementary sodium chloride This was disproved by measurements of the urinary excretion of sodium. (3) Hypertensives have more sodium in the body, that is, they have a larger 'metabolic pool' of sodium Obviously, the last-named hypothesis merited re-investigation in spite of the repeated evidence to the contrary cited above

The original calculations of total exchangeable sodium were based on actual weight[1] However, recent unpublished observations which were made on obese hypertensive females during sharp decline in

Table 1

Hypertensive subjects

Patient	Age	Sex	Diagnosis	Weight (kgm)	Biological half-life of sodium-22 (days) Sodium chloride per day added (gm) 2	5	10
RB	51	M	EHT	83	61 (56, 66)*	28 (26, 30)	13 5 (13, 14)
BC	42	F	EHT	51	65		
EEr	45	M	EHT	62	62		13
AK	56	F	EHT	73	63		
ML	48	M	EHT + P	73			13
PD	58	M	EHT	84	63	26	
NS	55	M	EHT	63	71 (78, 64)	25 5 (26, 25)	13 5 (13, 14)
				Mean	64 2	26 5	13 3
				S D	± 3 6	± 1 2	± 0 3
			Normotensive subjects				
AE	51	F	Ob	59	48 7 (45, 46, 55)		12 (12, 12)
RG	25	F	Ob	79	41 (40, 42)	20	11
AR	17	F	Ob	55	30	23	11
MW	50	F	ND	64	37 (35, 39)		
MP	52	F	A	47	41	17	9
LL	56	M	Sch	51	39	21	11
CB (coloured)	39	M	Sch	65	45	22	11
EE	60	M	CVA	52	44	20	12
				Mean	40 7	20 5	11 0
				S D	± 5 3	± 2 1	± 1 0
				t†	9 27	4 58	± 4 37
				P†	< 0 01	< 0 01	< 0 01

EHT, Essential hypertension, EHT + P patient also had Parkinson's disease, Ob, obesity, ND, no disease, A, periodic alcoholism, no liver disease, Sch, schizophrenia, CVA, cerebrovascular accident—no known antecedent hypertension

* More than one value indicates additional periods of study which were not performed sequentially For such individuals the average of these values was used in calculating mean and standard deviation of the group

† For hypertensive versus normotensive subjects on equal sodium chloride intakes

weight had frequently shown negligible loss of exchangeable sodium despite weight losses of 20–50 kgm This indicated that the fat deposits in such people had a low sodium content Therefore the calculations based on actual weight might have been misleading if the average hypertensive person had been fatter than the average non-hypertensive subject Hence, body sodium content was recalculated on the basis of two approximations of 'lean body mass' This brought into evidence that the hypertensives appeared to have an increase in tissue sodium One approximation was based on 'desirable' body weight as defined from a recent large actuarial study[7], while in the second, exchangeable sodium was expressed with reference to body potassium The latter was derived from the amount of naturally occurring potassium-40 present in each patient as determined by a total body radiation counting technique[8]

The evidence seems unequivocal that individuals with essential hypertension have a prolonged biological half-life for sodium 22 Our preliminary calculations suggest that this is the result of hypertensive patients having a large metabolic pool of sodium These findings lend support to the thesis that sodium is intimately involved in the hypertensive process[4,5]

This work was supported by the United States Atomic Energy Commission

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Modification of the Inotropic Activity of Calcium by Potassium

In previous communications from this laboratory[1,2], results have been presented which suggest that the accumulation of calcium at specific sites on or about the membranes defining individual cardiac cells may provide the fundamental basis for the conditions of enhanced contractility recorded during post- and extra-systolic potentiation and throughout the classical staircase[3] These results confirmed the earlier observations of Niedergerke[4] and support the hypotheses of Sandow[5], of Csapo[6], and of Bianchi and Shanes[7], who maintain that entry of calcium associated with the depolarization stage of activation is involved both in the link between excitation and contraction and in the regulation of the contractile force Hajdu and Szent-Gyorgyi[8], however, having observed that the staircase in isolated perfused frog hearts was abolished by reducing the potassium concentration, $[K^+]$, of the perfusate suggested that progressive loss of potassium from the myocardium during successive beats of the staircase provided the basis for the increased contractility

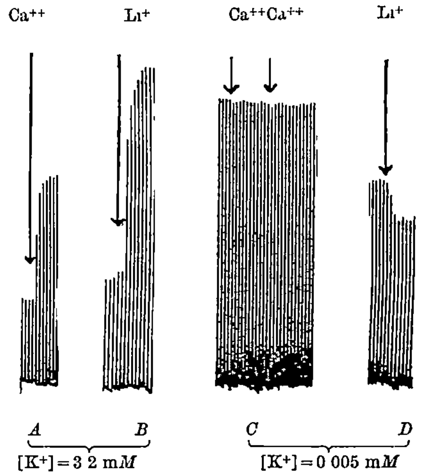


Fig 1 *A* and *B* Positive inotropic response recorded following *A*, increase of perfusate $[Ca^{++}]$ from 0 65 to 2 6 m*M* when $[K^+]$ was 3 2 m*M* (Ca^{++} added at arrow), *B*, replacement of half the perfusate Na^+ by Li^+ (at arrow) when the perfusate $[K^+]$ was 3 2 m*M*

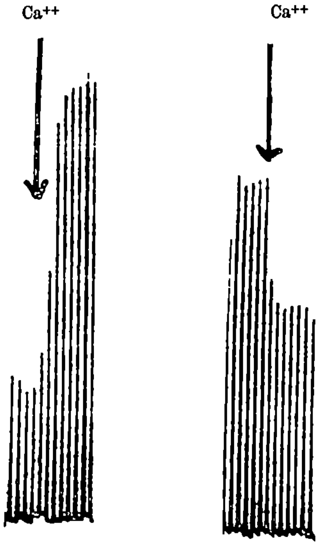
C and *D* Change in contractile force recorded in same preparation as used in *A* and *B* following *C*, increase of perfusate $[Ca^{++}]$ from 0 65 to 2 6 m*M* when the perfusate $[K^+]$ was reduced to 0 005 m*M*, *D*, replacement of half the perfusate Na^+ by Li^+ when the perfusate $[K^+]$ was 0 005 m*M*

A possible link between the hypothesis involving changed $[K^+]$ formulated by Hajdu and Szent-Gyorgyi, and that involving the accumulation of calcium as postulated by Niedergerke and others, has recently been noted in a series of experiments in which the action of calcium on the contractile behaviour of isolated toad ventricles perfused with Ringer's solution containing varying $[K^+]$ was studied

In these experiments ventricles removed from summer, non-hibernating toads of the species *Bufo marinus* were stimulated at a constant rate of 6 beats/min using supra-maximal rectangular pulses delivered from a 'Tektronix' square-wave generator Isotonic contractions detected by the photo-electric method previously described[9] were recorded on a direct-writing recorder and the ventricles initially perfused for a standard 60 min equilibration period with Ringer's solution containing 3 2 m*M* K^+ and 0 65 m*M* Ca^{++} Following this preliminary perfusion, addition of Ca^{++} (as $CaCl_2$) to give a final $[Ca^{++}]$ of 2 6 m*M* resulted in an immediate well-defined positive inotropic response (see Fig 1*A*) Such an increase in contractile force would be anticipated from the classical experiments of Ringer[10] Similarly, replacement of half the perfusate Na^+ by an isotonically equivalent amount of Li^+ (as LiCl) evoked an immediate positive inotropic response (Fig 1*B*)

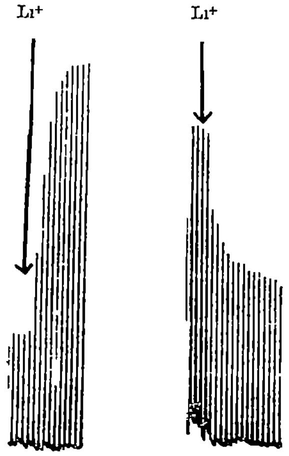
Reduction of the perfusate $[K^+]$ from the original level of 3 2 m*M* to 0 005 m*M* profoundly modified these positive inotropic responses recorded in the presence of 3 2 m*M* K^+ when the perfusate $[Ca^{++}]$ was increased or the $[Na^+]$ decreased Thus when the perfusate $[K^+]$ was reduced to 0 005 m*M*, increasing the $[Ca^{++}]$ from the original 0 65 m*M* to 2 6 m*M* either failed to alter the contractile force or in some preparations slightly decreased it (see Fig. 1*C*) In the presence of this low $[K^+]$, replacement of half the perfusate Na^+ by Li^+ without any attendant change in tonicity reduced the amplitude of contractions (see Fig 1 *D*)

The negative inotropic response which was recorded when the $[Ca^{++}]$ of the perfusate was raised from a



A $[K^+] = 3{\cdot}2$ m*M*　　*B* $[K^+] = 0$ m*M*

Fig. 2. Changes in contractile force recorded in a single preparation following the addition (at arrow) of Ca^{++} to give a final concentration of 2·6 m*M* when the perfusate contained *A*, 3·2 m*M* K^+, *B*, K^+ deleted



A $[K^+] = 3{\cdot}2$ m*M*　　*B* $[K^+] = 0$ m*M*

Fig. 3. Changes in contractile force recorded from a single preparation following replacement (at arrow) of half the perfusate Na^+ by an isotonically equivalent amount of Li^+ when *A*, perfusate contained 3·2 m*M* K^+, *B*, K^+ deleted from perfusate

preliminary level of 0·65 m*M* to 2·6 m*M* or when the $[Na^+]$ was halved was further enhanced by the use of a K^+-free perfusate. A typical result is shown in Fig. 2*A* and 2*B*, where the positive inotropic response recorded, following the above increase in perfusate $[Ca^{++}]$ in the presence of 3·2 m*M* K^+, is compared with that recorded from the same preparation following a similar increase in $[Ca^{++}]$ in the absence of any perfusate K^+. Similarly, replacement of half the perfusate Na^+ by Li^+ depressed the contractile activity when K^+-free perfusate was used (see Fig. 3*A* and 3*B*). In these particular experiments using the K^+-free solution it was necessary to replace repeatedly the K^+-free perfusate throughout the preliminary perfusion so that K^+-loss from the myocardium associated with successive contractions would not ultimately result in an appreciable perfusate $[K^+]$.

From these results it seems possible that the failure of Hajdu and Szent-Gyorgi to record the classical staircase in the absence of K^+ may reflect the need for the presence of a critical perfusate $[K^+]$ for Ca^{++} to exert a positive inotropic effect on cardiac muscle. The findings of Hajdu and Szent-Gyorgyi, therefore, do not preclude the involvement of calcium in the staircase and other conditions of enhanced cardiac contractility.

This work was supported by a grant from the Life Insurance Medical Research Fund of Australia and New Zealand.

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Free Amino-Acids in Rachitic Epiphyseal Cartilage of Albino Rat and the Influence of Vitamin D

It was suggested by Dikshit and Patwardhan[1] that administration of vitamin D imparted an enhanced calcifying capacity to rachitic bone and cartilage. Histochemical work by Ramalingaswami *et al.*[2] on cartilage in rickets and during healing under the influence of vitamin D, revealed an alteration in the staining characteristics of the cartilage matrix on the basis of which they suggested that vitamin D influenced the glycoprotein of the rachitic cartilage matrix. Dikshit[3] observed a decrease in the hexosamine content of the rachitic cartilage during healing induced by the administration of vitamin D. It was felt desirable, therefore, to investigate whether vitamin D influenced the protein metabolism of the cartilage. The effect of vitamin D on the free amino-acids of rachitic cartilage was studied as a preliminary to such investigations.

Young albino rats, 4 weeks old, were fed a low-phosphorus rachitogenic diet[4] for 14 days and then divided into 3 groups taking litter and sex-distribution into account. Each rat in group 3 received a single dose of 4,000 i.u. vitamin D orally on the fourteenth day and those in group 2 on the sixteenth day. Rats in group 1 received no vitamin D and served as rachitic controls. On the eighteenth day, 4–12 rats from each group were killed and their tibial epiphyseal cartilages removed, pooled according to groups, and weighed. The weighed cartilage was extracted exhaustively with 75 per cent ethanol, the collected extracts were evaporated and dried, and the residue was extracted again with 95 per cent ethanol. The residue from the second extract after evaporation was dissolved in 10 per cent isopropanol.

Using phenol/isopropanol/water (70 : 5 : 25) in presence of 0·3 per cent ammonia as the developing solvent, serine, glycine, threonine, glutamic acid and glutamine were separated from the mixture of free amino-acids contained in the isopropanol solution of cartilage extracts. Circular paper chromatographic procedure was used as described by Giri *et al.*[5] for

Table 1 EFFECT OF VITAMIN D ON THE FREE AMINO-ACIDS OF RACHITIC RAT CARTILAGE

Amino-acid		Rachitic controls (no vitamin D) I	Treated rats (4,000 I U vitamin D/rat) After 48 hr II	After 96 hr III
Glutamic acid	Mean	222	194*	222†
	S E	± 4 1	± 2 5	± 7 2
Serine	Mean	14 3	18 8*	24 2*†
	S E	± 1 45	± 0 39	± 0 51
Glycine	Mean	68	80	99*
	S E	± 2 1	± 4 7	± 6 5
Threonine	Mean	17 0	10 8*	15 5†
	S E	± 1 49	± 0 78	± 1 12
Glutamine	Mean	124	104*	122†
	S E	± 1 8	± 2 3	± 4 2

Values denote μgm of amino-acid/100 mgm fresh tissue
* Difference significant from group I below 5 per cent level
† Difference significant from group II below 5 per cent level
Each value is a mean of 3 observations, each observation was on cartilage pooled from 4–12 rats

quantitative determination of these amino-acids Duplicate values at three concentration-levels were obtained and only those in which there was good agreement within 5 per cent selected

Results shown in Table 1 revealed that administration of vitamin D affected the concentration of some free amino-acids in the rachitic cartilage While serine and glycine showed a gradual increase during healing, glutamic acid, glutamine and threonine showed an initial decrease by 48 hr followed by a rise to the pre-treatment level at 96 hr These different trends observed preclude the possibility that other simultaneous changes in the cartilage, for example, changes in the concentration of calcium and phosphorus[6], are the cause of the observed changes in the concentration of the free amino-acids

Thus there appears unmistakable evidence that protein metabolism in the rachitic epiphyseal cartilage altered under the influence of vitamin D

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Failure of the Purified Growth Hormone to restore the Size of the Kidneys in Hypophysectomized Rats

IN previous work the effect of hypophysectomy on the size of the remaining kidney after unilateral nephrectomy was investigated in dogs and rats[1] After hypophysectomy rats, unlike dogs, lose weight or fail to grow normally It is, therefore, difficult to assess whether the failure of the remaining kidney to hypertrophy is a non-specific effect or the result of a deficiency of (a) particular hormone(s) Bearing this in mind, it seemed necessary to study the effect of growth hormone

Young male rats, weighing between 35 and 120 gm , were hypophysectomized by the parapharyngeal approach Of the hypophysectomized animals, one group (six rats) received growth hormone while another group (ten) was untreated After the operation the animals were given drinking water containing glucose and, in addition to the ordinary rat diet, brown bread, milk and glucose were given

Purified growth hormone was dissolved in faintly alkaline isotonic solution of sodium chloride and was injected beneath the skin twice a day with a 7-hr interval between the two injections, in doses indicated in Table 1

Table 1 EFFECT OF SUBSTITUTING GROWTH HORMONE ON THE WEIGHT OF THE KIDNEY OF HYPOPHYSECTOMIZED RATS

Body-weight (gm)		Weight of both kidneys (mgm)	Remarks
Initial	Final		
46	102	676	Injected with 2 mgm growth hormone for 20 days
54	182	831	
35	86	823	
84	124	843	Injected with 4 mgm growth hormone for 20 days
115	192	1,350	
72	148	1,035	

Grant from N H I (United States), N I H -*GH-B*2 (bovine growth hormone), growth potency, 1 53 × U S P reference standard, contamination LH, 0 01, follicle stimulating hormone, 0 009 U S P , prolactin, 0 33 I U , adrenocorticotrophic hormone, 0 25 U S P , thyroid stimulating hormone, 0 009 U S P

The animals were killed with ether on the twenty-first day after hypophysectomy The sella turcica was carefully examined for any remnants If the pituitary was completely removed, the sella turcica was seen to be covered with a smooth glistening layer of the meninges, beneath which a central depression in the bone, representing the site of the drill hole, was seen

From Table 1 it would appear that the growth hormone was effective in promoting growth in the hypophysectomized rats and the rate of growth in the treated animals was quite comparable with that of normal animals The adrenals and testes, however were found to be lighter in weight than in the normal controls In contrast to these findings, the kidneys of hypophysectomized rats receiving growth hormone were found to be the same size as those from the untreated hypophysectomized animals This conclusion was reached as a result of expressing kidney size as the ratio of kidney weight to body-weight Statistical analysis of the results showed that there was no significant difference in kidney size between the hypophysectomized group and the group which was treated with the hormone, but that there was a significant difference between these two groups and the control normal group (Table 2) It is interesting that the stomach weight and gastric mucosa were found restored completely to normal in the hypophysectomized animals and those treated with growth hormone (Crean, G P , personal communication) It can be seen that injections of the purified growth hormone can maintain the growth of the hypophysectomized rat, but are unable to prevent the relative shrinkage of the kidney that occurs after this operation This is contrary to results with the crude extract of the adenohypophysis[1]

Depression of kidney functions and blood-flow are known to occur after hypophysectomy[2,3] Unlike adenohypophysial crude extract, gonadotrophic hor-

Table 2 EFFECT OF GROWTH HORMONE ON KIDNEYS OF HYPOPHYSECTOMIZED MALE RATS

No of rats	Condition of rat	Kidney weight in mgm /body-weight in gm Mean (± *S D*)	Statistical significance
13	Normal	9 533 (± 2 14)	*P* 0 01
10	Hypophysectomized	7 461 (+ 0 84)	None
6	Hypophysectomized, growth hormone	6 931 (± 1 59)	*P* 0 01

mone, thyroid stimulating hormone and adrenocorticotrophic hormone, when given individually, are unable to restore kidney function completely to normal.

From these results it seems unlikely that an explanation in terms of hæmodynamic changes[4] of the failure of the kidney to grow is correct, for the kidney remained small in hypophysectomized rats even when the animals grew at a normal or slightly greater than normal rate. It is more likely that there is a deficiency of a pituitary hormone without which the kidney cannot well maintain its size and growth in the hypophysectomized animals. It would appear, from this work, that growth hormone is unlikely to be the only deficient hormone.

The following acknowledgments are made: Dr. J. A. Loraine for his criticism and facilities for work, Dr. A. E. Wilhelmi for supplying the growth hormone, Dr. K. E. Kirkham for work on statistics, Dr. E. B. Roberts for reading the manuscript, and Mr. J. Lowe for animal care-taking.

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Effect of Urea Ingestion on Parotid Salivation in Sheep

Urea is the most common source of non-protein nitrogen used for the partial replacement of protein in the diet of ruminants. In association with readily available carbohydrate such as molasses, urea is used to supplement the low-quality roughage diets which are often consumed by ruminants in Africa, the United States and Australia.

This form of supplementation results in an increase in the rate of cellulose digestion in the rumen[1]. Food intakes are increased by approximately 30–40 per cent, live-weight losses are decreased, and wool production is increased[2–5].

Balch and Johnson[6] found a correlation between the fluidity of the rumen contents and the rate of cellulose digestion. It is therefore important to know if the volume of saliva secreted is altered when urea and molasses are added to a roughage diet.

The volumes of parotid saliva secreted by two Merino wethers were measured by means of permanent unilateral parotid fitulæ. Each sheep weighed approximately 40 kgm. and they were kept in electrolyte balance by allowing them to drink their own saliva.

The basic diet consisted of oaten straw containing 2 per cent crude protein. The supplement of urea (25 gm.), molasses (200 gm.) and water (200 gm.) was intimately mixed with straw (600 gm.) to make up the daily ration. This supplement raised the nitrogen content of the straw to the equivalent of 11 per cent crude protein.

One sheep received straw only, while the other received straw plus the supplement. After two weeks of recordings, the diets were reversed. The unsupplemented straw was offered without stint and the daily intake per sheep was approximately 500 gm. The intake of straw when fed with the supplement was restricted by offering only 600 gm. of straw plus the supplement. On every occasion this ration was completely consumed.

The volumes of saliva secreted did not alter significantly with the addition of the supplement. The average volumes for each animal were 2,160 and 2,560 ml. without the supplement, and 2,250 and 2,380 ml., respectively, with the supplement.

The mean total nitrogen of the saliva from each animal rose from 2.9 and 4.2 mgm. nitrogen/100 ml. to 12.1 and 13.3 mgm. nitrogen/100 ml. respectively, when the supplement was added to the straw.

It is concluded that the increased rate of passage of food through the alimentary tract, and the increased rate of cellulose digestion in the rumen, which are brought about by urea feeding, are not associated with quantitative changes in the pattern of parotid salivation. In the present work the urea supplement produced an increase of 20 per cent in the consumption of straw, but this was not accompanied by any increase in parotid salivation.

A reasonable estimate for the total volume of saliva secreted by each sheep would be 10 l. a day[7]. Also, it has been shown that the nitrogen content of this total salivary secretion, excluding mucoprotein, is similar to that of parotid saliva[8]. Since the total nitrogen content of parotid saliva was raised by 10 mgm./100 ml. on feeding urea, the increase in saliva nitrogen represents an overall increase in nitrogen economy to the sheep of only about 1 gm. nitrogen a day.

Therefore, the influence of urea supplementation on the parotid secretion does not seem to be particularly important from either the qualitative or quantitative point of view.

I thank the Australian Dairy Produce Board for the financial assistance that made this work possible.

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HISTOCHEMISTRY

Relationship between Tissue Eosinophils and Peroxidase Activity

Four different peroxidase enzymes have been found in mammalian organisms: lactoperoxidase, myeloperoxidase, hepatic tryptophan peroxidase and labile peroxidase of the thyroid gland. There is insufficient knowledge of the physiological significance of these enzymes for animal organisms. This applies especially to the myeloperoxidase found in the granules of leucocytes[1,2].

It has been shown histochemically that the peroxidase activity possessed by eosinophils in the rat differs from other granulocytes[3]. This enzyme is very resistant in eosinophils to the effect of, say, formalin, ethyl and methyl alcohol and other agents of similar type and it is therefore possible to demonstrate it specifically by histochemical methods. It can, however, be asked whether this enzyme is a myeloperoxidase at all.

The highest peroxidase concentrations of the extramedullary organs of the rat have been established in the small intestine, uterus, large intestine, spleen, lungs and stomach[3] It is in these same organs that the relatively highest extra-medullary eosinophil counts have also been established in the rat[2] It has also been observed that both the peroxidase concentration[4] and the number of eosinophils[2] increase clearly in the rat uterus under the influence of œstrogen stimulation It is thus possible that the high peroxidase content of the organs mentioned derives from eosinophilic granulocytes

The observation that the peroxidase enzyme in rat uterus is in its behaviour like lactoperoxidase[4] —and not like myeloperoxidase—agrees well with the histochemical differences existing between eosinophils and other granulocytes

The views cited are supported by the fact that physiological, intense eosinophilia appears in lactating lactiferous gland[5]

The high peroxidase concentration established *in vitro* in the gastrointestinal tract, uterus, spleen and lungs is probably caused by the eosinophil granulocytes of tissues The enzyme contained in the eosinophils resembles lactoperoxidase

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PATHOLOGY

Acid Extract of Leucocytes and *Staphylococcus pyogenes*

SEVERAL workers[1-6] have demonstrated recently the inhibitory action of acid extracts of leucocytes towards microbes

Amano[7] *et al* found a cell-free acid extract of guinea pig leucocytes to be lethal to coagulase-negative *Staphylococci*, and to be slightly inhibitory to coagulase-positive *Staphylococci* This active principle they call leucozyme *A* and show that although lysozyme is present it is not the agent responsible for the destruction of the *Staphylococci*

Hirsch[8] has also reported on the destructive activity of an acid extract (phagocytin) of leucocytes obtained from several species This extract is prepared free of lysozyme I have confirmed both these observations using *Staphylococcus albus* and *aureus* and extracts made from guinea pig, rabbit and human leucocytes My work has been extended to compare the activity of human and rabbit phagocytin towards *Staphylococcus pyogenes* obtained from a variety of lesions which exhibit qualitative and quantitative differences in toxin production, I also compared the action of an extract made from leucocytes obtained from patients with acute osteomyelitis with that from healthy donors

It seemed reasonable to expect that *Staphylococcus pyogenes* which have survived phagocytosis and proliferate in the bone marrow would show a greater resistance to phagocytin The results, shown in Table 2, do not support this No significant difference is found in the action of either of the extracts towards *Staphylococci*.

Table 1 REDUCTION IN STAPHYLOCOCCAL COLONY COUNTS BY PHAGOCYTIN

No of strains staphylococcoid *pyogenes*		Human phagocytin extract Col count reduced to		Rabbit phagocytin extract Reduction of from 140 to 10 or less per cu cm
		10 or less per cu cm	10/20 per cu cm	
Nasal	4	4	0	4
Boils	3	1	2	3
Osteomyelitis	30	26	4	30
Miscellaneous	23	16	7	23
Total	60	47	13	60

Average count for untreated controls, 130–140 colonies per cu cm

Table 2

No of strains		Normal human white blood cells extract Col count		Osteomyelitic white blood cells extract	
		10 or less	10–20	10 or less	10–20
Nasal	2	2		2	
Osteomyelitis	8	6	2	6	2
Total	10	8	2	8	2

Average count for untreated controls, 130–140 colonies per cu cm.

The extracts have been made by Hirsch's methods, incubated for 2 hr. with a 4-hr shaken culture of the test organism in broth standardized to give a reading of 1 0 on an EEL colorimeter using an orange filter After incubation this standard extract and suspension is finally diluted to 1/50,000 and pour plates made with 5 ml agar in standard 2-in Petri dishes Controls are set up using distilled water instead of the extract The colonies are counted per cubic centimetre, for the controls the average count is between 130 and 140 per c c

In view of the Japanese report that their acid extract was more inhibitory towards coagulase-negative *Staphylococci*, an attempt was made to see whether a partially purified coagulase preparation gave any protection No difference was noted when this was used The *p*H at which tests were made fell to *p*H 5 6–6 0 (The coagulase was prepared by the late Dr Edward Duthie of Southampton)

Coagulase, α toxin, staphylokinase, leucocidin (some) and hyaluronidase production of the *Staphylococci* were noted, and there is no correlation between the toxigenicity of the *Staphylococci* tested and their susceptibility to phagocytin, or the nature of the lesion from which the organism was originally isolated, the two strains showing greater resistance to the osteomyelitic extract were different from those two relatively resistant to the normal extract Neither is there any apparent relationship between phagocytin susceptibility and the ability to provoke antibody response in the patient to the various antigens of the coccus The morphology of the *Staphylococci* surviving incubation with extracts is normal, as might be expected from the appearance of the colonies

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Lathyrogenic Activity *in vitro*

REASONS have already been given for the view that the basic aortic lesion in experimental lathyrism is mainly a failure of formation of elastic tissue[1]. An attempt has since been made to investigate, *in vitro*, the effect of various lathyrogenic substances on the production of elastic fibres.

Explants were taken from the ventricles of chick embryo heart (the great vessels being carefully removed so that the only elastic tissue in the initial explants was the small amount present in the coronary vessels) and were cultured by a modification of the method described by Bloom[2] using Waymouth's medium[3] with 10 per cent calf serum for 10–12 days at 39° C as the fluid phase. Whether the explants were grown in fibrin clot or directly on glass, in stoppered glass tubes or in Petri dishes in an atmosphere with 5 per cent carbon dioxide did not affect the results. Some additional results obtained from experiments with mouse embryo hearts do not differ markedly from those with chick embryo hearts and are not reported in detail here.

A preliminary experiment showed that fibres similar to those described by Bloom[2] first appeared after 7 days incubation, and were well formed after 10 days. Subsequent cultures were therefore examined after this time. The fibres stain with Weigert's method for elastica, with orcein, and with Gomori's aldehyde fuchsin, providing strong presumptive evidence that they are indeed elastica; further investigations are being made into this matter. The fibres usually run parallel to each other to form a circle or arc of a circle around the explant at the junction of the original tissue and the monolayer extending from it. They appear to have formed immediately under the surface layer of cells at the farthest point from the glass. In a few cases, particularly in small explants, the fibres form a disorganized network, or may show some radial orientation. In the presence of lathyrogenic substances these fibres were not found.

For assay of lathyrogenic activity each experiment comprised multiple cultures and graded concentrations of test substances. Only those experiments in which one or more control cultures produced elastica were considered. In successful cultures 40 out of 51 (78 per cent) of the controls produced elastica. When β-aminopropionitrile hydrochloride—known to be lathyrogenic *in vivo*—was added to the cultures it was found that a concentration of 2 mgm/ml killed the majority of the explants, and in the few cases where the cells survived elastic fibres were never formed. With lower concentrations down to 80 μgm/ml, although the explants were healthy as frequently as in the control cultures, elastic fibres were never formed. With still lower concentrations the percentages of healthy cultures producing elastic fibres were as follows: 16 μgm/ml, 10 per cent; 3·2 μgm/ml, 12·5 per cent; 0·64 μgm/ml, 66 per cent. Two other compounds active *in vivo*, β-mercaptoethylamine[4] and semicarbazide[5], inhibited the formation of elastic fibres at concentrations of 3·4 μgm/ml and 0·66 μgm/ml respectively. Sodium propionate and *n*-propylamine, both inactive *in vivo*, were also inactive *in vitro* at concentrations of up to 364 μgm/ml and 0·16 per cent respectively; higher concentrations caused cell death. Assuming uniform distribution of lathyrogenic substances throughout animal tissue, the doses of β-aminopropionitrile hydrochloride, β-mercaptoethylamine and semicarbazide which inhibit the formation of elastica *in vitro* correspond to the concentrations calculated as being present in the tissues of mice treated with these compounds and showing rupture of the aorta. The results demonstrate that lathyrogenic substances act at a cellular level, and the parallelism between the *in vivo* and *in vitro* findings supports the original hypothesis that the aortic rupture in experimental lathyrism is secondary to a failure of formation of normal elastic tissue.

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An Early Test for Possible Skin Carcinogens

IN previous papers[1–4] it has been shown that it is possible by a tetrazolium reduction method to estimate changes in the rate of formazan deposition in the epidermis of hairless mice after different skin irritants.

A characteristic reaction was observed after the application of some carcinogenic substances and after local irradiation with X-rays. The topical application of carcinogens produced a short initial rise in formazan deposition, followed by a depression of longer duration. An initial decrease in formazan deposition was observed after the application of some non-carcinogenic compounds. It was suggested that an initial rise in formazan deposition might be specific for skin irritants with carcinogenic potency.

Examining the validity of this hypothesis, a blind-test with 20 different compounds was performed. The substances were dissolved in benzene and the different samples were indicated by *A*, *B*, *C*, ... etc.

Hairless mice, 8 males and 8 females for each test substance, were given 0·005 ml. of the solution as a single, topical application to a circumscribed area of the skin. The formazan deposition per mgm dry epidermis per hour measured on the first and the second day after the application was recorded. It was postulated that mean relative values of 1·100 and above should indicate a compound with skin carcinogenic potency.

The results of the blind test are given in Table 1. The values are arranged in order from the lowest to the highest. To the right is presented the list of answers, including an indication of the carcinogenic potency of the substances. In this communication only those substances are listed as complete carcinogens which each alone are known to produce epidermoid carcinomas in mouse skin after topical application.

It appears that there is a correlation between such complete carcinogenic activity and initial rise in formazan deposition. The above-mentioned hypothesis is thus given some further support and the method may probably be used as an early test for possible skin carcinogens. It must, however, be kept in mind that correlation never proves specificity.

Table 1

Blind test			List of answers	
Compound	Mean	S D of the mean	Compound	Skin carcinogenic potency
H	0.682	0.07	1,2 benzanthracene	–
L	0.754	0.11	Pyrene	–
K	0.770	0.07	*d*-Limonene	–
F	0.825	0.12	1 stearyl 2,3 polyethylene monoglyceride	–
D	0.827	0.12	Naphthalene	–
J	0.838	0.09	3 methyl 1,2 benzanthracene	–
S	0.848	0.06	Oil of turpentine	–
G	0.880	0.13	Fluoranthene	–
T	0.881	0.09	Cholesterol	–
I	0.897	0.09	Phenanthrene	–
E	0.908	0.07	Ethyl carbamate	–
B	0.909	0.08	Anthracene	–
Q	0.970	0.05	1,2,6,7 dibenzanthracene	–
P	1.010	0.08	1,2,3,4 dibenzanthracene	–
O	1.307	0.12	4 nitroquinoline N oxide	+
C	1.337	0.11	Croton oil	+
N	1.405	0.12	9,10 dimethyl 1,2 benzanthracene	+
A	1.408	0.08	3,4 benzpyrene	+
R	1.410	0.09	3 methylcholanthrene	+
M	1.634	0.12	1,2,5,6 dibenzanthracene	+

The substance *P* (1,2,3.4 dibenzanthracene), for which a borderline value (1.010) was observed, is the substance that represents an exception in the interesting observations of Heidelberger[5] concerning binding of carcinogens to skin proteins.

There is no general agreement as to what tetrazolium really measures. It acts as an electron acceptor and is thereby reduced to formazan. The amount of formazan deposited per mgm dry epidermis per hour may probably be considered as an estimate of the activity of the energy-generating processes in the cell, mainly the mitochondrial enzyme systems. Increased values may mean some injury to the mitochondria, for example increased permeability of the mitochondrial membrane.

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PALÆONTOLOGY

A Mammalian Fauna from the Kimmeridgian of Portugal

A MAMMALIAN fauna, consisting of 14 specimens, has been collected from the small Guimarota coal pit, near Leira. The age has been determined as Kimmeridgian by means of the ostracods and characeans. The pit is still in operation and it seems possible to obtain a good fauna, qualitatively as well as quantitatively. A typical coal swamp fauna has been encountered. Lamellibranchs, gastropods, and ostracods are frequently found, the holostean genus *Lepidotus* is well represented by scales. Carapace fragments of turtles are less frequently found. Crocodiles predominate numerically, with many shed teeth and scutes. Many lizard jaws have been collected, as well as a few shed teeth of dinosaurs.

The fauna contains the earliest known multituberculates (two good jaws without molars and one skull without teeth) and the oldest pantotherian upper dentition. The latter has a tooth formula of P^4/M^5 (P^{2-4} have been lost, P^4 prior to fossilization). There is also a good pantotherian lower jaw which has a tooth formula of $I/C_1/P_4/M_8$. All the molars are unbasined, the trigonid being open lingually. The talonid is covered by the paraconid of the tooth behind, it is not worn by the opposing lingual cusp of the upper molars. The lower molars have their longitudinal axis oblique to the axis of the jaw.

The molars are shorter than those of *Amphitherium* and the talonid is much smaller than in the latter tooth. The metaconid almost equals the protoconid in height and is higher than the paraconid. This is a feature which is found also in the dryolestid genera *Dryolestes*, *Laolestes*, *Phascolestes* and *Peramuspalax* as well as in the paurodont genera *Archaeotrigon* and *Tathiodon*. The tooth formula of the present jaw is now diagnostic in respect to differentiation between Amphitheriidae and Dryolestidae, but it excludes paurodont relationship.

The right lower, M_3, and right upper, M^3, of the pantothere are shown in Figs 1 and 2. There is no suggestion that these upper and lower jaws belong to the same genus. The tooth shown in Fig 2 is typical as regards number of cusps on all teeth. M^{1-3} are longer than broad, M^4 and M^5 are broader than long. The tooth is similar in many respects to *Herpetairus arcuatus* from the Morrison formation. Both have the same number of cusps of equal relative height. A large basin is formed by five of six cusps, two buccally, two posteriorly and one, the highest, lingually. The sixth cusp, at the anterobuccal corner, stands outside

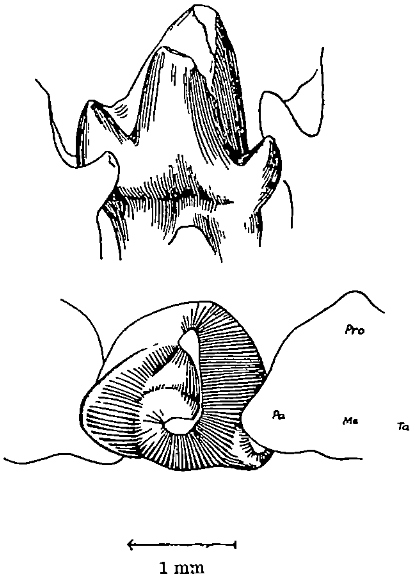

Fig 1 Right lower M_3 of Kimmeridgian pantothere

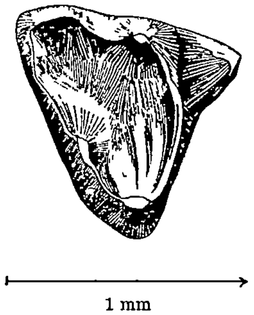

Fig 2 Right upper M^3 of Kimmeridgian pantothere

the triangular basin and dorsal to it The function of this cusp is to buttress the posterobuccal corner of the tooth in front , similarly the talonid cusp buttresses the paroconid The second highest cusp, as in *Melanodon, Herpetairus, Amblotherium* and *Kurtodon,* stands at the anterobuccal corner of the basin

Primitive features of the lower jaw and maxilla, seeing that they are older than Purbeckian, are: (1) in the jaw there is an open, not a closed trigonid (the Purbeckian paurodontids with an open trigonid are similar to the Bathonian *Amphitherium* in this respect), it is agreed that in *Amphitherium*, the earliest known pantothere, the open trigonid is a primitive character which the paurodonts retain, (2) it has been postulated that in pantotherian upper molars of pre-Purbeckian age the length would equal the width in all teeth In the Kimmeridgian upper dentition now available, M^{1-3} are longer than broad, whereas in the Purbeckian only M^1 has such dimensions

It has been customary to discuss the origin of the Pantotheria whenever a new mammalian fauna in the Mesozoic is discovered Such procedure is inevitable, taking into consideration the few faunas known and the interest that the Pantotheria arouse as ancestors of the placentals and marsupials I do not agree with a derivation of Pantotheria from Rhætic symmetrodonts, as Butler[1], Kermack and Mussett[2], and Patterson[3] do I consider a derivation from Rhætic triconodonts more likely The new maxillary dentition does not provide any new item of symmetrodont affinity The second largest cusp (Butler's buccal cusp, Patterson's stylocone) stands right in the middle of the buccal side as in Purbeckian teeth, forming a tricuspid front with the cusp at the anterobuccal corner (Butler's anterior cusp, Patterson's parastyle) and the cusp at the postero-buccal corner (Butler's posterior cusp, Patterson's "metastyle") Patterson's cusp on the anterior crest of some Purbeckian dryolestids, said to indicate symmetrodont affinity, is not present on any of the molars of the Kimmeridgian maxilla The central buccal cusp has in the new maxilla the same relative height as in the Purbeckian teeth, while if it were to be a homologue of the anterobuccal cusp of the Symmetrodonta, one would expect it to be less high

The morphological break between upper premolar and molar is considerable in the dryolestid upper dentition Only the aberrant *Docodon* has a P^4 which is so similar to the M^1 that the nature of the paracone and protocone in the molars of this genus has never been debated The paracone stands in the middle of the buccal row of cusps, flanked by two smaller cusps, the protocone is a lingual neomorph, added to an original triconodont pattern

I believe that *Docodon* and all other Pantotheria have—as Simpson[4] originally claimed—the same cusp pattern and the same origin. A history of all cusps of the Albian therian from Texas, of the Purbeckian maxilla, of the new Kimmeridgian maxilla and the Rhætic triconodonts or symmetrodonts can be written irrespective of whether one begins with a triconodont or a symmetrodont With a triconodont at the base a much simpler history of all dental elements concerned is possible Moreover, this would solve the *Docodon* paradox, namely, that one genus which is patently neither triconodont nor symmetrodont has a different origin from all (other) Pantotheria

As regards jaws as well as teeth, the Purbeckian *Docodon* was a living fossil, giving a hint of what happened to the still unknown triconodonts which, in the Upper Liassic, embarked on the road to the Pantotheria

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Chitinozoa from the Ordovician Nod Glas Formation of Merioneth

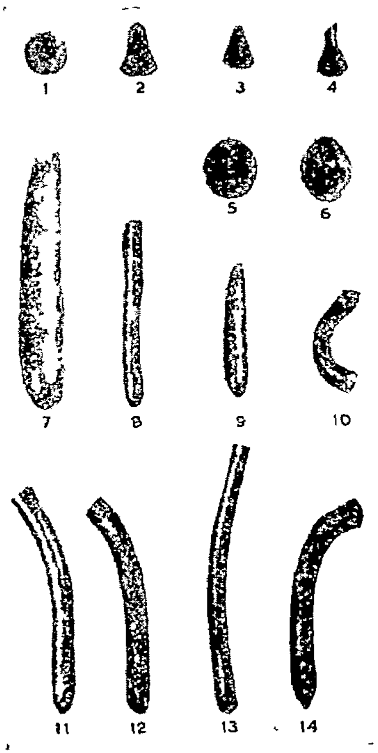
CHITINOZOA[1] are microscopic, flask-like fossils of problematical affinities They are generally black, structureless and opaque, being composed of 'pseudochitin', and ranging from 0 03 mm to 1 5 mm in length They are bilaterally symmetrical and may be club-like, rod-like, or globular in form, the test being thin, hollow and open at one end The surface of the test is usually smooth, but is sometimes tuberculate Spines may be present and aggregates of up to six individuals have been recorded[2].

The zoological affinities of the Chitinozoa have been discussed by Collinson and Schwalb[2], who conclude that they represent an extinct order of rhizopod protozoans

Chitinozoa are known from strata ranging from Ordovician to Devonian in age from Germany, France, Czechoslovakia, the United States, Canada and Brazil The only previous record of Chitinozoa from Britain is that of Lewis[3], who described two species of *Conochitina* (C cf *C filifera* Eisenack and *C cactacea* Eisenack) in thin sections of the Upper Carodocian "Phosphate band" of Pen-y-garnedd, Montgomeryshire

The present specimens were collected from crags on the face of the Graig Tynant (M R SH/898218), about two miles north of Llanmawddy, Merionethshire[4] They occur in limestone nodules 15 ft above the base of the Nod Glas Formation[4], and were extracted by digestion of the limestone in acetic acid.

The Nod Glas chitinozoa fall into three well-defined morphological groups The most common are elongated tubular forms, with the oral end open and the aboral end roundly closed Most of these (for example, Fig 8) are straight and agree broadly with Eisenack's description of *Rhabdochitina magna* Eisenack, although they show rather more variation in the form of the distal end than is recorded by Eisenack Numerous other specimens show, however that there is a complete gradation between such forms and those with a gently or strongly curved axis (Figs. 11–14) and also between forms which have a uniform diameter along their entire length (that is, 'tubular' forms) and those in which the aboral diameter is rather larger than the oral (that is, 'club-shaped' forms) Such forms as the latter have been referred to the genus *Conochitina* Eisenack, but the present specimens suggest that it is difficult to separate these from 'typical' members of the genus *Rhabdochitina* The axial curvature does not seem sufficient to exclude most of these forms from the species *R magna* A precedent for the evaluation of such variation as infra-specific is provided by Eisenack[5],



Figs 1–14 Chitinozoa from the Ordovician Nod Glas Formation of Merioneth (× c 44)

1–4, *Illichitina* cf *I campanulaeformis* (Eisenack) 1, oral view of specimen, showing cross-section of cylindrical neck and basal outline of chamber, 2–4, oblique lateral views of specimens showing general form of chamber

5–6, *Lagenochitina*? sp Lateral views showing general form 7 and 9, Club-shaped forms of *Rhabdochitina* (*Conochitina* of some authors)

8, 11–14, *Rhabdochitina* spp lateral views of specimens showing variation in general form of chamber

10, fragmentary specimen Gen et sp indet

who included various forms showing comparable variation within the species *Rhabdochitina taenia* Eisenack

The second group of chitinozoa are bell-shaped forms (Figs 1–4) which closely resemble the species *Illichitina campanulaeformis* (Eisenack) The base is often perforated, and they appear to show morphological variation which partly bridges the gap between that species and *I crotalum* Collinson and Schwalb

The third group are globular forms (Figs 5 and 6), which may represent the genus *Lagenochitina* Eisenack Some have an invaginated opening, but all appear to lack the tubular neck which is characteristic of most members of the genus

I am most grateful to Prof Harry B Whittington, of Harvard University, who accompanied me in the field, and to Mrs Shirley Osborn, who has given me considerable help in the extraction of specimens

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BIOLOGY

Growth-stimulating Activity of *Trans*-Caffeic Acid isolated from *Coleus rhenaltianus*

In our previous work[1] on the abscission of debladed petioles of *Coleus rhenaltianus*, we met with an auxin that was not an indole derivative, in ether extracts from the leaves

On paper chromatograms of ethanolic leaf and stem extracts, developed with isopropanol/water (5 1 v/v), we now find two blue fluorescent spots (R_F 0 84 and 0 79), the eluates from which both enhance the elongation growth of *Avena* coleoptile sections (Table 1)

Table 1 Growth-rate of 3 mm *Avena* Coleoptile Sections in a Solution of 1 per cent Glucose and the Eluate from a Chromatogram (Spots at R_F 0 79 and 0 84) of an Ethanolic Leaf and Stem Extract of *Coleus* Growth recorded after 24 hr Number of Sections between Brackets

Increase in length (eye-piece micrometer units)			
Spots at R_F 0 79		Spots at R_F 0 84	
Control	Diluted eluate	Control	Diluted eluate
16 8 ± 1 59 (15)	26 8 ± 1 50 (14)	13 0 ± 0 72 (15)	19 4 ± 0 76 (17)
16 6 ± 1 34 (13)	26 2 ± 1 73 (14)	8 4 ± 0 89 (10)	20 4 ± 1 04 (10)
8 4 ± 0 89 (10)	23 9 ± 1 86 (9)	5 0 ± 0 45 (10)	10 8 ± 0 58 (10)

The eluates show practically the same ultra-violet absorption spectrum as the auxin found earlier in ether extracts of leaves The substance at R_F 0 84 was identified by us as *trans*-caffeic acid Most probably the substance at R_F 0 79 is a closely related one, since it gives several identical colour reactions and, after treatment with diluted sulphuric acid, is transformed into *trans*-caffeic acid, with R_F 0 84 It is neither chlorogenic nor *cis*-caffeic acid Both compounds are precipitated with neutral lead acetate, after removal of the lead from the yellow precipitate, growth activity in the coleoptile section test is unimpaired.

Generally the cinnamic acid derivatives caffeic and ferulic acid are considered to be growth inhibitors[2] Other reports mention a synergistic action of these substances with the growth regulators indole-3- and α-naphthyl-acetic acid[3,4] Caffeic acid alone is thought not to exert any true auxin activity We find, however, that commercial *trans*-caffeic acid, at a concentration of about 1 μgm /ml or less, greatly enhances the elongation growth of coleoptile sections (Table 2), about as much as does indole-3-acetic acid in these circumstances.

Table 2 Growth-rate of 2 mm *Avena* Coleoptile Sections in Solutions of *trans*-Caffeic Acid and Indole-3-acetic Acid in Distilled Water Both Substances were added 6 hr after cutting the Sections Growth has been recorded after 18 hr Number of Sections between Brackets

Increase in length (eye-piece micrometer units)					
trans-Caffeic acid			Indole-3-acetic acid		
Control	1 μgm /ml	0 5 μgm /ml	Control	1 μgm /ml	0 5 μgm /ml
3 8±0 45 (16)	5 7±0 35 (21)	4 3±0 34 (18)	3 0±0 48 (17)	5 0±0 82 (17)	4 8±0 60 (10)
1 2±0 28 (14)	5 0±0 34 (14)	3 7±0 27 (18)	1 5±0 54 (10)	3 0±0 59 (10)	3 2±0 46 (11)
2 2±0 42 (15)	5 6±0 34 (15)	4 4±0 44 (13)	2 4±0 34 (14)	5 5±0 54 (14)	4 6±0 58 (14)

The rather general occurrence[5] of caffeic acid in plant tissues thus suggests that this substance may be a very important natural growth regulator, not less important than indole-3-acetic acid

Of both stereo-isomeric cinnamic acids, only the *cis*-isomer is physiologically active in growth[6] Our own experiments have confirmed once more the inactivity of *trans*-cinnamic acid The *cis* configuration has been supposed to be generally important in growth regulation It is believed, moreover[7], that the introduction into the lipophilic aromatic nucleus of ydrophilic substituents results in the formation of nactive or weakly active compounds Taking into ccount these structural requirements, we should not t all expect auxin activity in *trans*-caffeic acid, which is 3′,4′-dihydroxy cinnamic acid The extreme difference in growth activity between *trans*-cinnamic and *trans*-caffeic acid on the contrary seems to indicate a special importance of the ortho-diphenol configuration in growth regulation

Our results stress the importance of a thorough study of the physiological function of cinnamic acid derivatives in growth and metabolism

Support from the Nationaal Fonds voor Wetenschappelijk Onderzoek (Belgium) is gratefully acknowledged

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High Root-Pressures in Palms

Despite some impressive demonstrations of root-pressure[1-3], 'pumping' or positive pressure is generally thought to be of little importance for the ascent of sap in trees This is probably true for dicotyledons, but in ten species of palms at Calcutta I was able to detect and measure positive root-pressures throughout the year, sometimes enough to lift sap to heights exceeding those of the trees

A palm has rather uniform adventitious roots growing from the bole, or stem base The number at bearing stage varies from a few hundred to more than 13,000 as in *Cocos nucifera* Such a root, generally about 0·7 cm in diameter, is cut about a metre from the bole The proximal end, attached to the tree, is connected by a long rubber tube to a glass tube of 3 mm bore dipped in water, and connexions sealed with paraffin The distal end, severed from the tree, is connected by a short rubber tube either to a vertical glass capillary tube or a mercury manometer

The maximum suction, or negative pressure, is usually reached within 10 hr, and may persist for as long as four months Its value fluctuates rapidly with atmospheric changes, as would be expected if transpiration were an important cause of it The positive pressure usually starts developing after about 24 hr, and reaches a maximum after 10–20 days, the time varying between species and between roots of the same tree After a day or two it begins to decline, and disappears after about two months There are slight diurnal fluctuations, the pressure being higher by day. Table 1 gives data on 51 palm and 2 banana (*Musa sapientum*) trees The roots of *Phoenix sylvestris* are not suitable for the operation described, and the low values obtained are probably due to leakage In seven of the eleven species, the positive pressure exceeds the negative pressure considerably It often exceeds the plant's needs. Thus a seedling of *Borassus flabellifer*, a little under a metre high, had a root which developed a pressure of 5·24 m of sap Again, in *Musa sapientum*, the only monocotyledon other than a palm which I studied, and which behaves just like the palms, the suckers die before reaching a height of 4 m

In order to demonstrate that my results are due to a physiological property of palms, and not to differences of method, I used just the same methods on

Table 1

Species of trees studied	Number	No of roots connected for		No of roots showing		Maximum height (cm) of water column	
		Suction pressure	Pumping pressure	Suction	Pumping	Sucked	Pumped
Cocos nucifera	11*	20	39	20	34	20 1	1,250
Borassus flabellifer	10	29	36	27	24	51 0	524
Areca catechu	6	25	25	23	19	27 5	550
Livistonia chinensis	6	16	24	16	15	42 0	100
Phoenix sylvestris	5	13	20	12	1	18 2	34
Ptychosperma macarthuri	4	19	19	17	13	34 3	650
Oreodoxa regia	3	5	12	4	6	25 9	650
Chrysalidocarpus lutescens	3	9	15	8	7	39 9	310
Livistonia rotundifolia	2†	5	5	4	4	24 0	40
Caryota urens	1‡	3	3	3	1	62 0	70
Total for the 10 species	51	144	198	134	124	62 0§	1,250§
Musa sapientum	2	11	11	10	9	25 1	670

* Two of these palms were studied at Kayangulam, Kerala State
† Connexions damaged due to heavy rains at an early stage
‡ The palm was at its last stage of flowering
§ Denotes the maximum for all the palms studied

Table 2

Species of trees studied	Number	No of roots connected for		No of roots showing		Maximum height (cm) of water column	
		Suction pressure	Pumping pressure	Suction	Pumping	Sucked	Pumped
Dalbergia sissoo	1	6	6	6	nil	54 5	0
Polyalthia longifolia	1	5	5	5	nil	59 0	0
Sterculia urens	1	5	5	4	nil	27 6	0
Psidium guajava	1	5	5	5	nil	61 2	0
Ficus religiosa	1	4	4	4	nil	27 5	0
Total	5	25	25	24	nil		
Cycas circinalis	1	7	7	5	nil	35 7	0

five dicotyledonous trees and one gymnosperm The results are given in Table 2, and require no comment

It seems likely that monocotyledonous plants, having no secondary growth, behave very differently from dicotyledons As M H Zimmermann (private communication) says "because most monocots do not form new vascular tissue in the lower part of the trunk as dicots do, a cohesion mechanism alone would run into difficulties" The method is clearly important as others[4-6] have failed to demonstrate root pressure in palms

A few simple experiments suggest that the observed root pressures suffice to drive sap up the stem about as easily as up a capillary tube The distal part of a cut root of a fruited banana plant was seen to develop pressure The plant was decapitated at 2 25 m above ground-level, and the cut root reconnected with a rubber tube Other agencies such as transpiration are probably less important than root pressure.

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Probable Mechanism of Resistance of Varieties of Cocoa to Black Pod Disease caused by *Phytophthora palmivora* (Butl.) Butl.

Isolates of *Phytophthora palmivora* showed different degrees of pathogenic activity (measured by rate of spread of lesions at 25° C) when wound-inoculated into surface-sterilized pods of particular varieties of cocoa (*Theobroma cacao*, L) One isolate, *WA* 8, failed to attack any variety Isolates also showed varied rates of spread in different varieties of cocoa The variety *SCA* 6 was consistently more resistant than the variety *ICS* 1. Holliday[1] has shown that the variety *SCA* 6 is more resistant to artificial inoculation of intact pods with a zoospore suspension than is the variety *ICS* 1

Rotted cocoa pod tissue showed no evidence of pectolytic enzyme activity when tested by Brown's maceration method[2], and by the polygalacturonase agar plate method[3] The same tests, however, showed that pectolytic enzymes were produced by *Phytophthora palmivora* on autoclaved potato blocks

Aqueous extracts of host tissue, prepared by homogenization of tissue in distilled water (during which browning occurred), when added to pectolytic enzymes of *P palmivora* from invaded autoclaved potato blocks caused inhibition of the pectolytic enzyme activity The inactivating ability of such extracts was removed by pre-treatment with 0 5 per cent aqueous solution of gelatin If the homogenization was carried out in 0 1 per cent aqueous sodium dithionite solution browning did not occur and the extracts did not cause inhibition of pectolytic enzyme activity These results suggested that the inhibition was due to high molecular weight tannins produced by polymerization of the products of oxidation of host polyphenols by polyphenol oxidase[4-7]

Tests of macerating activity of pectolytic enzyme of *P palmivora* on cocoa pod tissue showed tha tissue disks were more resistant to maceration whe allowed to brown after cutting than if browning wer prevented by immersion in a 0 1 per cent sodiu dithionite solution Moreover, disks of the mor resistant variety *SCA* 6 browned more rapidly tha those of the susceptible variety *ICS* 1 and showe correspondingly greater resistance to maceration

If the resistance of cocoa varieties to rotting b isolate *WA* 8 were due to the inactivation of pectolyti enzymes of the fungus by action of the host polyphenol oxidase system, then reduction of the activity of the latter system by low oxygen tension would be expected to break down this resistance Some of these effects have been demonstrated Cocoa pods o variety *IMC* 67 were wound-inoculated with the avirulent isolate *WA* 8, placed at 60, 21 and 2 per cent oxygen tension and incubated at 25° C Lesions were produced at 2 per cent oxygen tension but were very light brown in colour, whereas at oxygen tensions of 21 and 60 per cent no lesions were produced, the only reaction being a narrow dark margin around the point of inoculation

Pods inoculated with isolate *WA* 8 and incubated at 15° C produced lesions, whereas no attack occurred at 25° C or 30° C Low temperature also might be expected to reduce the polyphenol oxidase activity and this reduction in host resistance would explain the better growth of the fungus in the host tissue at low temperatures than at temperatures nearer the optimum for growth of the fungus on agar culture

A virulent isolate produced lesions at 60, 21 and 4 per cent oxygen tensions; size of lesions increased from an average diameter of 2 1 cm at 60 per cent, to 3 3 cm at 21 per cent and 4 5 cm at 4 per cent after three days incubation at 30° C The lesions varied from dark brown at 60 per cent, to brown at 21 per cent and lighter brown at 4 per cent, indicating a correlation between rate of attack and browning of host tissues

Tests of growth-rates of *P palmivora* at various temperatures and oxygen tensions showed that the results could not have been solely due to direct effects on the growth-rate of the parasite Other host factors would be affected by alteration of oxygen tension and temperature, but an alteration of the balance between the pectolytic enzyme systems of the parasite and the polyphenol oxidase system of the host offers the simplest explanation of promotion of attack by low oxygen tension and low temperature

If resistance is correlated with polyphenol oxidase activity of cocoa pod tissue it may be possible to select varieties with high activity of the polyphenol oxidase system, and if, as would be expected, these show some degree of resistance, to use them in breeding resistant varieties

It is hoped that a fuller account of this work will be submitted for publication shortly

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Canadian Salmon caught off Greenland

On October 10, 1960, an Atlantic salmon 71 cm total length, weighing 3 7 kgm (8 2 lb), and carrying Fisheries Research Board of Canada tag No 1,616, was caught in the sea off Tassiussaq (65° 06′ N , 52° 08′ W), near Napossok to the south of Sukkertoppen, Greenland The fish was tagged on May 22, 1959, in the estuary of the Miramichi River near Chatham, New Brunswick (47° 04′ N , 65° 28′ W), as a smolt 17 5 cm in total length It was one of 3,500 smolts tagged there in May and June, 1959, with tags patterned after those used by Dr Borje Carlin[1] in Sweden, but had printed laminated plastic pendants supplied by a commercial firm

Scales from the recaptured fish show three years of river life typical of Miramichi River smolts, followed by a first sea year of moderate growth and a relatively wide winter band The second sea year includes a period of fast growth followed by a summer check, then a period of fast growth continuing to the time of capture in October

This recapture represents the longest migration yet recorded, about 1,500 miles, for Canadian Atlantic salmon the river of origin of which is known through fin-clipping or tagging Many fin-clipped salmon from Quebec, New Brunswick, and Nova Scotia rivers have been recaptured, however, in sea fisheries as far away as Newfoundland and Labrador The recapture also lends some support to the suggestion[2] that salmon from both sides of the North Atlantic share common feeding grounds The first direct evidence of Scottish salmon reaching Greenland waters was provided by the recapture in October 1956 near Sukkertoppen, Greenland, of a fish tagged as a kelt in November 1955 at Loch na Croic, Ross-shire[3]

We are indebted to Dr Jørgen Nielsen, of the Ministeriet for Grønland, for details of this recapture accompanied by a scale sample, and to Dr P F Elson, of the Biological Station, St. Andrews, New Brunswick, for reading the scales

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Penetration of Spines from a Marine Diatom into the Gill Tissue of Lingcod (*Ophiodon elongatus*)

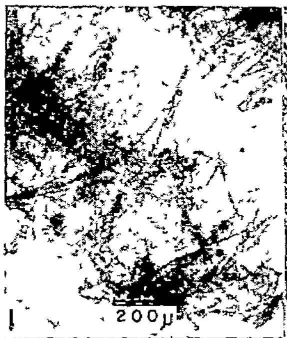

Fig 1 *Chaetoceros* cells and setæ on surface of lingcod gill filaments Specimen had died in live-well within 8 hr of sampling

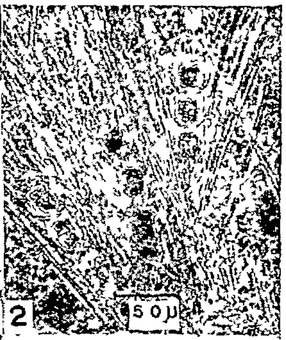

Fig 2 Phase contrast view of *Chaetoceros* sp and setæ on gill mucus Same specimen as in Fig 1

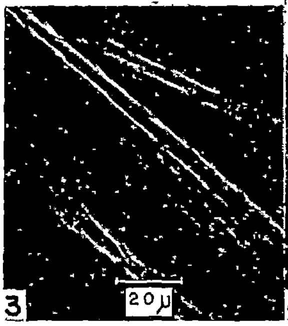

Fig 3 Dark field picture of *Chaetoceros* setæ showing apically directed barbs

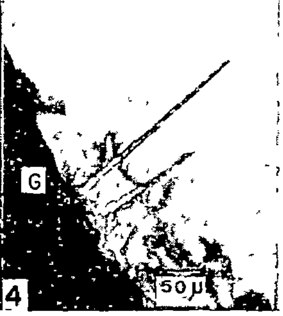

Fig 4 Setæ embedded in gill of dead lingcod which had been exposed for 24 hr at 11° C to 1–5 × 10^5 *Chaetoceros* cells/l *G* indicates gill tissue

Fishermen from the Porlier Pass area of Vancouver Island were experiencing unusually high mortalities among lingcod (*Ophiodon elongatus*), which according to common practice were being held in surface live-wells prior to the weekly slaughtering and transport to market Examination of dead and dying fish showed only that all specimens had discoloured gills with considerable detritus embedded in the gill mucus (Fig 1) Much of this detritus was composed of *Chaetoceros convolutus*, one of a common group of marine plankton diatoms[1], and their siliceous spines (Fig 2) The spines or setæ with their apically directed barbs are shown in Fig 3 These findings suggested the possibility that the setæ might bring about tissue damage and be a direct or indirect cause of death. Gill tissue is particularly vulnerable to injury from chemical and biotic agents because of its intimate contact with the external environment and because of its delicate structure It was therefore decided to expose apparently healthy lingcod (18 in) to 3–4 times the natural concentration (that is, 1–5 × 10^5 cells/l) of *Chaetoceros* while the fish were held in aquaria. Control fish were kept in natural sea water

Only one of the three treated fish died, from undetermined causes, and although the final results were inconclusive, it is of considerable interest that setæ were found embedded in the gill tissue of the dead fish (Fig 4) It was established that the spines had actually penetrated the tissue and were not merely trapped in the mucus The incidence and extent of the penetration were not determined, but four embedded setæ were seen in an area of a few square millimetres The only comparable record appears to be that of Wood and Yasutake[2], who found that ingested 'hairs' from the lepidopterous larva *Halisidota argentata* penetrated the stomach and visceral organs of fingerling salmon by working their way inwards and thus causing extensive damage to the tissues The insect hairs, like the algal setæ, have apically directed barbs and therefore both shafts must enter butt first for maximum penetration This direction of entry into lingcod gill tissue is shown in Fig 4 Setæ which are entrapped on or embedded in the gills might cause death by capillary hæmorrhage, by anoxia from the stimulation of heavy mucus secretion or by debilitation of the tissue allowing microbial invasion

It is not yet possible to say whether this penetration is only a laboratory oddity or whether it is also a significant natural phenomenon, but the subject appears to warrant further investigation by marine biologists

I am very grateful to Dr T. Parsons of the Pacific Oceanographic Group for his valuable advice and assistance

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An Intra-Pericardial Hepatic Intrusion in the Common Dogfish

MISS BARBARA STEVENS, of Brooklands Technical College, has been kind enough to send me a dogfish (*Scyliorhinus canicula*) with an intra-pericardial hepatic intrusion Only the anterior end of the dogfish is available, but the length from the tip of the snout to the anterior insertion of the pectoral fin is 9 cm, and the anterior ends of the testes present a mature appearance Thus it may be inferred that the dogfish had practically reached full size with this abnormality when it was caught

The septum transversum is perforated on the right side only, and the hepatic intrusion is remarkable for its extent (Fig 1) On the right side the liver extends through the pericardial cavity for a distance of 18 mm, almost reaching the anterior end of the conus arteriosus Laterally, the liver extends 23 mm, reaching across almost the entire width of the pericardial cavity at its posterior end The heart appears to be normal, except that the ventricle is displaced slightly forward and to the left

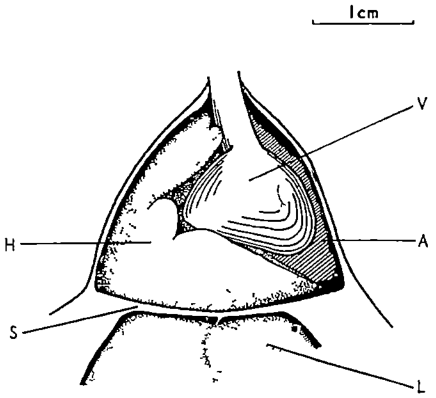

Fig 1 Hepatic intrusion in the pericardial cavity of a dogfish *A*, Auricle, *H*, hepatic intrusion, *L*, liver, *S*, septum transversum, *V*, ventricle

The volume of the intra-pericardial intrusion was estimated by making a 'Plasticine' replica and measuring the volume of this by displacement This was found to be 2 7 c c Similarly, the volume of the heart was found to be 2 2 c c Thus the hepatic intrusion occupied rather more of the pericardial cavity than the heart The volumes of the hearts of a small number of dogfish under class dissection were also estimated by making 'Plasticine' models, and then the volumes of the actual hearts were determined by displacement after excision The method, as performed by students, was found subject to an error of not more than 20 per cent The volumes of the pericardial cavities of the normal animals were also found by making 'Plasticine' casts of the cavities after excision of the hearts From these results it appears that the volumes of the heart and of the pericardial cavity of the abnormal animal are within the range to be expected, and that often the heart occupies only about half the volume of the pericardial cavity

In the development of craniates, the pericardial cavity is first delineated from the perivisceral cœlum by the mesocardia At this stage the pericardial cœlom is still in contact with the perivisceral cœlom by the dorsal and ventral parietal recesses of His Afterwards the primary septum transversum is formed by proliferation of connective tissue on the anterior face of the developing liver[1] It must be presumed that in this animal the liver rudiment initially developed asymmetrically in such a way that the aperture ventral to the right mesocardium was not occluded by connective tissue on the anterior face of the liver rudiment Thus the abnormality represents a patent right ventral parietal recess of His Goodrich[2] has shown that in *Scyliorhinus canicula* the dorsal apertures remain as a forked pericardio-peritoneal canal The anterior end of this canal was clearly visible in the abnormal animal, although the forked posterior ends could not be traced

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Shoaling Behaviour in a Mysid

Mysidium columbiae (Zimmer) is common in shallow water in Kingston Harbour, Jamaica It is found during the day in shoals close to the mangroves, usually but not exclusively in the shade of the trees The shoals can be seen swimming horizontally a few centimetres below the surface of the water in a manner similar to shoals of small fish, for which indeed they were at first mistaken The behaviour of *Mysidium* proves to be a combination of fixed responses to external stimuli and social responses by which the shoals are built up and maintained

A number of *Mysidium* placed in a large vessel of sea-water will form a shoal in a few minutes and swim around with their bodies oriented parallel with each other at a constant distance apart Small shoals tend to join larger ones and *Mysidium* separated from the main shoal by a sheet of glass attempt to swim through the glass to join the shoal provided the latter is close enough for them to see it The critical distance for shoaling by visual orientation is about 4 cm at 150 ft-candles illumination *Mysidium* placed in a circular dish 18 cm in diameter illuminated from above swim in one direction around it, keeping station with each other so that all those along a given radius tend to complete a circuit in the same time, those near the circumference swimming several times faster than those near the centre The speed of swimming is photokinetically controlled, being proportional to the logarithm of the light intensity over a range of at least two logarithmic units of illumination At very high intensities swimming tend to be inhibited and the mysids sink to the bottom, while in very dim light the shoal breaks up Removal of both eyes abolishes both the photokinetic response and the ability to swim in a shoal Some astonishingly high swimming speeds have been recorded in these experiments up to 15 4 cm /sec , which is equivalent to about twenty times the animal's length in a second

Light-adapted *Mysidium* are positively phototactic and swim towards a source of light Dark-adapted ones, however, always swim to the bottom of the tank when illuminated irrespective of the position of the light After removal of the pair of statocysts situated in the uropods they always swim away from a light source, to the surface when illuminated from below and to the bottom when illuminated from above

The behaviour of *Mysidium columbiae* is remarkable in several respects Unlike most mysids it is a 'bright-light' animal living close to the water surface under the noonday tropical sun, while in its shoaling behaviour it shows a precision and co-ordination of locomotion comparable with that seen in fish In some respects, however, the social responses of *Mysidium* are not so well developed as in shoaling species of fish Two or more fish will form a shoal instantly but small numbers of mysids will not It seems that critical density or number of animals is required before they will form shoals, which then build up rather slowly, taking from 30 sec to 5 min to become well developed Also a proportion estimated at about 5 per cent do not join the shoals, but swim freely in other directions or remain motionless on the bottom *Mysidium* is more suitable than fish in some ways for an experimental study of shoaling, since it shows no apparent capacity to modify its behaviour as a result of experience

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Evidence for a Sexual Phase in the Life-Cycle of an Amœba

THE amœba have generally been thought to be devoid of a sexual phase in their life-history[1], though one earlier report[2], and a recent one[3], indicate a cycle involving fusion of flagellated gametes This communication concerns evidence for sexuality in a diphasic amœba

A flagellate ascribable to the genus *Heteramœba* and very near *H globosa* Skuja[4] is a widespread, though by no means common, inhabitant of brackish supra-littoral rock pools This organism, referred to here as *Heterochromonas clara* nom prov , differs from *H globosa* in being larger (30µ diam) and in not having contractile vacuoles, but, like *H globosa*, it has no stigma It was abundant in August 1960 in the polluted pools at the base of the cliff below the large gannetry on Ailsa Craig, where it was feeding voraciously on green flagellates Two dozen bacteria-free clones were isolated from this source using a strain of *Brachiomonas submarina* for food One clone was set aside for nutritional work and the remainder were maintained in composite culture for reference The amœbæ first appeared in the latter culture They were unexpected since *Heterochomonas* is not recorded as being the stage in the life-cycle of an amœba, moreover, an isolate of *H clara* from a similar habitat in Finland had been kept for ten years without sign of amœbæ

Several of the amœbæ were isolated and washed singly and used to start cultures Without exception every one of these cultures quickly gave rise to flagellates Twenty flagellated cells were similarly isolated from one of the amœba isolates (145), the ensuing clones being designated 145/*a*–*t* Amœbæ appeared in several of these within a few days, but others have given rise to no amœbæ after four months of maintenance The latter, six in number, were then crossed with each other, that is, mixed in subculture two at a time in all combinations, for it seemed that the flagellate/amœba transformation might, in contrast to other diphasic amœbæ, be under genetic rather than environmental control So indeed it proved, for with certain combinations amœbæ appeared within 24 hr. of the operation, while with others, and in the six parent clones, the populations remained uniformly flagellate (Table 1) Furthermore, the pattern of amœba-formation is consistent with the existence of two mating types among the clones, all of which, it will be recalled, are descended from the same amœba cell This result is taken as indirect, though conclusive proof of a sexual phase in the life-cycle of this organism

Table 1 PATTERN OF AMŒBA-FORMATION IN BI-CLONAL CULTURES

Clone	145*d*	145*k*	145*r*	145*f*	145*i*	145*n*
145*d*	—	—	—	*A*	*A*	*A*
145*k*	—	—	—	*A*	*A*	*A*
145*r*	—	—	—	*A*	*A*	*A*
145*f*	*A*	*A*	*A*	—	—	—
145*i*	*A*	*A*	*A*	—	—	—
145*n*	*A*	*A*	*A*	—	—	—

A, Formation of amœbæ within 24 hr

The amœbæ are at first minute (7 × 30µ) and typically *limax*-like but later reach a size of 40 × 70µ; they have been observed to multiply by binary fission Cysts (up to 25 × 30µ, smooth-walled) are found only in amœba cultures, they germinate to produce single amœbæ Possible isogametic fusions between minute flagellates can be observed in mixed cultures, but this is open to an alternative interpretation since *H clara* is phagotrophic and able to devour cells of its own size or larger and is not above resorting to cannibalism

The following hypothetical conclusions may be drawn from these preliminary observations (1) the amœbæ are diploid, (2) flagellated cells may be diploid or haploid, (3) only diploid cells can encyst, (4) haploid cells are of two mating types; (5) multiplication can occur both in the diploid and haploid states and can continue indefinitely in the latter

Multiplication of flagellated cells of diphasic amœbæ is not unknown, it has been reported, for example, for *Tetramitus rostratus*[5], together with the suggestion of a sexual phase It is not yet established that amœbæ of *H clara* can multiply for an indefinite period without reversion to flagellates, nevertheless, considerable fluidity appears to exist in the balance between haploid and diploid phases of the life-cycle, the cycle being primitive on that count as compared with other sexual Sarcodina

A cytological investigation of *H clara* is being undertaken as an aid to a proper diagnosis of the organism and to a more detailed description of the life-history

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Influence of Larval Environment on Adult Size and Fecundity in the Moth *Panaxia dominula* L.

THE scarlet tiger moth, *Panaxia dominula*, is to-day found only in southern parts of the British Isles Since it was at one time a little more widespread[1] the present distribution may be due to the action of environmental limiting factors rather than to the lack of opportunity to colonize more northern parts of the country Evidence is presented here which supports this assumption

The wing-span of moths caught in several English colonies was measured from the tip of the right wing to the centre of the thorax on pinned specimens There is considerable variation in size between different colonies, insects from Devon being significantly larger than those from Berkshire (Table 1*a*) (For the difference between Axmouth and Cothill· males, $t = 10\ 37$, $P < 0\ 001$, females, $t = 10\ 89$, $P < 0\ 001$). When larvæ were collected late in development and reared to maturity under similar conditions it was not manifested (Table 1*b*) For the males, $t = 0\ 23$, $P > 0\ 80$, for females, $t = 2\ 11$, $P = 0\ 05$–$0\ 025$ It seems likely, therefore, that the differences in size between wild-caught insects are determined primarily by environmental dissimilarities between the colonies rather than by the genetic constitution of the individuals in them

The number of eggs produced by bred females was counted and their wing-span measured When productivity is plotted against wing-span it is seen that there is a significant positive correlation between them in a sample of 22 from Cothill ($r = 0\ 604$, $P < 0\ 01$), and in a sample of 6 from Kingsdown, Kent ($r = 0\ 852$, $P < 0\ 05$) The correlation is just below the 5 per cent level for 28 specimens from Axmouth ($r = 0\ 347$ With 28 points the 5 per cent value is 0 374) The data are shown in the graph (Fig 1) It may be that in Nature environmental conditions do not affect the mean number of eggs to the same extent, but the range in size in the experimental régime was considerably less than that between naturally emerging insects from the Devon and Berkshire colonies, so that the results strongly suggest that size differences in them are accompanied by a corresponding variation in egg production Similar data from other insect species are discussed by Klomp[2] In this example the smallest individuals come from colonies near the edge of the present range They probably lay fewer eggs than those from south-west England, so that reduced productivity may be one of the limiting factors

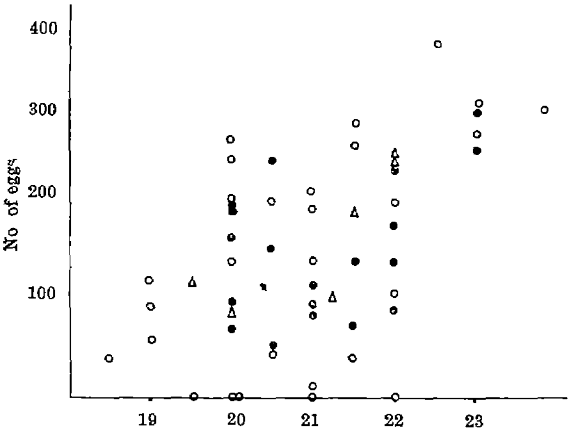

Fig 1 Regression of number of eggs on wing-span of female parent ○, Cothill, ●, Axmouth, Δ, Kingsdown In this series the size was measured from wing-tip to the join of nervures 1 and 2

Table 1 *a*, SIZE IN MM (TO NEAREST HALF MM) FROM TIP OF WIN TO CENTRE OF THORAX IN INSECTS CAUGHT WILD

	No.	Mean and standard deviation	Range
Cothill (Berks) ♂♂	27	23 30 ± 1 15	21 0–26
Cothill ♀♀	25	22 78 ± 0 98	21 0–24
Sheepstead Hurst (Berks)	15	24 10 ± 1 15	22 5–25
Bradfield (Berks)	4	25 75 ± 0 65	25 0–26
Newbury (Berks)	4	25 88 ± 1 32	24 0–27
Burgate (Hants)	8	25 81 ± 1 12	23 5–28
Axmouth (Devon) ♂♂	68	25 96 ± 1 07	23 5–28
Axmouth ♀♀	42	25 54 ± 1 04	23 0–27

Table 1 *b*, MEASUREMENTS (TO THE NEAREST HALF MM) ON ADULT BRED FROM FOURTH INSTAR LARVÆ

	No	Mean and standard deviation	Range
Cothill ♂♂	16	24 81 ± 1 48	21 5–26
Cothill ♀♀	22	23 82 ± 1 66	22 0–27
Axmouth ♂♂	20	24 70 ± 1 42	22 5–27
Axmouth ♀♀	31	24 71 ± 1 28	21 5–27

This work was carried out at the University o: Oxford during the tenure of a grant from the Nature Conservancy

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Localization of Esterase in the Cuticle of the Nematode *Ascaris lumbricoides*

THE cuticle of nematodes, because of its obvious skeletal functions and its importance as a barrier to nematocides, has recently been the subject of much research Bird and Deutsch[1] and Bird[2] have greatly increased our knowledge of the structure of the cuticle of *Ascaris lumbricoides* and various other workers have added to our knowledge of the chemical composition of the cuticle[3-5] Although Fairbairn[6] states "it would seem wise to regard the integument as a fully integrated living tissue, rather than as an inert horny sheath" there has been no evidence to show that the cuticle of nematodes is metabolically active

Frozen sections (50µ) of *Ascaris lumbricoides* (from pigs), fixed in 10 per cent neutral formol saline at 4° C for 24 hr , were tested for non-specific esterase activity by two methods as described by Pearse[7] (1), the naphthol *AS* acetate - fast red *TR* salt and (2), the 5-bromo-indoxyl acetate methods

A positive result was obtained, with both methods, in the homogeneous, or matrix, layer of the cuticle but not in any of the other layers The colour was most intense at the anterior and posterior ends of the nematode In transverse sections there is a narrow gap in the deposit of stain in the homogeneous layer at the lateral lines

There was complete inhibition of colour formation after incubation with 10^{-2} *M* silver nitrate, 90–100 per cent inhibition after 10^{-4} *M* *E*600 (diethyl *p*-nitrophenyl phosphate); 50 per cent inhibition after 10^{-3} *M* cupric sulphate and 90–100 per cent inhibition after boiling for 15 min , but 10^{-4} *M* eserine appeared to have no inhibitory effect

Lipids are known to be present in the cuticle of *Ascaris* and certain other nematodes[6,8,9]. Contrary to the findings of Bird[10], a weakly positive result for lipid was obtained with oil red *O* in the homogeneous layer of the cuticle Thus the distribution of non-specific esterase coincides with the distribution of lipid in the homogeneous layer of the cuticle of

Ascaris These findings provide strong evidence in favour of the view expressed by Fairbairn[6] and by Bird[10] that the cuticle of *Ascaris lumbricoides* is a metabolically active structure The presence of at least one enzyme in the cuticle would indicate that more are present and it is possible that these enzymes are concerned with the laying down of more cuticle as the nematode increases in size after the final moult.

Esterase, inhibited by 10^{-4} *M E*600, has also been localized in the œsophageal glands, in the intestine, in the rectal glands, in the cœlomocytes, in the nervous system including the innervation processes of the body wall muscles, in the gonads, and as 'caps' of enzyme on fat globules in the hypodermis, in the dorsal, ventral and lateral lines, and in the muscles The cells of the intestine have been found to be merocrine in that they discharge esterase through the striated border without undergoing any marked changes The presence of esterase in the innervation processes of the body wall muscles, but not in the non-contractile part of the muscle other than on fat globules, would suggest that this esterase is a cholinesterase or an esterase serving a similar function in nerve transmission

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ENTOMOLOGY

Effect of Chlorinated Hydrocarbon Insecticides on Insect Choline Acetylase, Condensing Enzyme and Acetylkinase

THE role of acetylcholine in the transmission of nerve impulses is by no means as well established in insects[1,2] as it is in mammals, but the presence in insect nervous tissue of large quantities of acetylcholine[3] and of enzymes capable of its rapid synthesis[4] and hydrolysis[5] is clearly established

It has also been shown that chlorinated hydrocarbon insecticides have an effect on nerve conduction in insects[6,7] and on their acetylcholine-levels[8]

That these insecticides kill insects as a direct or indirect consequence of their effect on nerve conduction has not been clearly demonstrated but it still remains the most likely explanation of their mode of action

The work reported here was carried out in an attempt to determine what effect these insecticides have on the synthesis of acetylcholine by insects, being part of a re-investigation of the effects of chlorinated hydrocarbon insecticides on the synthesis, hydrolysis and action of acetylcholine in insects

Dieldrin-susceptible house-flies *Musca domestica* L were reared at 25° C on a larval diet of 120 gm. powdered whole milk, 120 gm powdered dried yeast and 24 gm agar powder made up to 1,200 ml with water The adults were fed on glucose and on a 5 per cent aqueous glucose solution Tissue homogenates were prepared using a small Waring blender 300 adult *M domestica*, 5–10 days old, were homogenized at 0° C in 10 ml 0 25 *M* sucrose for 5 min Alternatively, the flies were decapitated, after anæsthesia by cooling, and the heads only were homogenized (300 heads/10 ml 0 25 *M* sucrose solution) as above In both cases the resultant homogenates were filtered at the pump through muslin to remove the larger pieces of cuticle, and made up to 10 ml

To determine the normal rate of synthesis of acetylcholine using either sodium acetate or sodium citrate as acetyl donor, reaction mixtures were prepared as follows and incubated in a water-bath at 30° C 2 0 ml homogenate, 0 2 ml coenzyme *A* (1 mgm /ml Light and Co , Ltd), 0 2 ml 6 6 × 10^{-2} *M* adenosine triphosphate, 0 2 ml 0 1 *M* L-cysteine, 0 4 ml 16 × 10^{-3} *M* eserine sulphate, or preferably, 0 4 ml 0 01 *M* tetraethylpyrophosphate (TEPP) (both cholinesterase inhibitors), 0 4 ml 0 4 *M* choline chloride, 0 4 ml 0 1 *M* phosphate buffer *p*H 7 0, 0 2 ml sodium fluoride (10 mgm /ml phosphatase inhibitor)

To test the effect of insecticide, sufficient was dissolved in 0 2 ml acetone to give a final concentration in the reaction mixture of 10^{-3} *M* Control experiments were carried out using 0 2 ml acetone added to the reaction mixture 0 4-ml aliquots were removed at intervals and their acetylcholine content measured using Hestrin's method[9]

The activity of the fly preparations, with and without acetone[10] and irrespective of whether acetate or citrate was the actual donor, was 0 7 μgm acetylcholine synthesized/head/hr and 1 3 μgm acetylcholine whole fly/hr The activity when expressed per gram of fresh tissue was 567 μgm acetylcholine/gm / hr for fly heads and 120 μgm acetylcholine/gm /hr for whole flies On the addition of the chlorinated hydrocarbon insecticides *pp'*DDT (dichlor diphenyl trichlorethane), dieldrin, heptachlor and lindane in 0 2 ml acetone and at a final concentration of 10^{-3} *M* the activity of the preparations remained unaltered

Smallman[4], in his demonstration of acetylcholine synthesis in the blowfly *L sericata*, found that the rate of acetylcholine synthesis from acetate by acetylkinase was considerably greater than that from citrate by the condensing enzyme working in reverse He also demonstrated that choline acetylase is much more active than either condensing enzyme or acetylkinase and that these latter enzymes appeared to control the rate of synthesis of acetylcholine

Our results on *M domestica*, in which preparations of similar activity were used, show that as in *L sericata* both pathways for the synthesis of acetyl-coenzyme *A* are present, but that in *M domestica* the rate of synthesis from acetate or citrate was the same This indicates that either choline acetylase is less active than the other two enzymes and thus acts in a rate-controlling capacity, or that in the house-fly as in the guinea pig both pathways are equally available

Lewis *et al* [8] have shown that in cockroaches poisoned and prostrated by *pp'*DDT and in cockroaches physically stimulated to prostration there is a rise in their acetylcholine-levels He postulates that this is due to the release of acetylcholine from a bound form at abnormal sites in the nervous system since his results also showed that cholinesterase was not inhibited by the above treatments Our finding that the rate of synthesis of acetylcholine in *M. domestica* is not affected by the chlorinated hydrocarbon insecticides *pp'*DDT, dieldrin, heptachlor and lindane supports Lewis's suggestion since it excludes the possibility that the increased acetylcholine-levels could be due to an increased rate of production of acetylcholine due to chemical or physical stimulation

We wish to thank the Colonial Office Pesticides Committee for financial support and Dr C T Lewis for helpful discussion and advice

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Substances controlling the Rate of Beating of the Heart of *Periplaneta*

A PAPER now in the press[1] shows that the heart accelerating factor from the corpus cardiacum of the cockroach *Periplaneta americana* described by Cameron[2,3] acts by stimulating the pericardial cells to produce a second substance It is this pericardial cell factor which is responsible for the direct stimulation of the heart This communication describes the properties of the two substances

The factor from the corpus cardiacum appears to be a peptide or protein Heating extracts (prepared by grinding corpora cardiaca in insect Ringer or in distilled water) on a boiling water-bath for 5 min results in a slight increase in activity as measured on the isolated cockroach heart More prolonged heating has no further effect on the activity The activity of extracts is unaffected by evaporation to dryness at room temperature and resuspension in distilled water The dried residue can be extracted with *n*-butanol, acetone or diethyl ether without loss of activity The active factor appears to be soluble in 80 per cent ethanol, but treatment of the dried residue with absolute ethanol destroys the activity Incubation of extracts with trypsin at pH 8 or pepsin at pH 3 results in complete inactivation within 60 min at 37° C

However, Cameron[2,3] has suggested that the active substance from the corpus cardiacum is an *o*-diphenol Since Cameron's extraction technique involves the use of absolute ethanol as a solvent for the active principle, his material must be different from the material under study here Further confirmation of the non-identity of the two materials is obtained from a consideration of the behaviour on paper chromatograms of distilled water extracts of corpora cardiaca and extracts prepared according to the method of Cameron Using *n*-butanol/acetic acid/water (4 1 5) as a solvent, the activity from corpora cardiaca extracted in distilled water is associated with a ninhydrin-positive spot at R_F 0 23 Similar chromatograms using Cameron's technique have yielded highly variable results, it is sufficient to say here that no activity was ever recovered from the area around R_F 0 23 Further, a number of spots, some sensitive to ninhydrin, others to ferric ferricyanide, appeared at higher R_F values None of these spots could be detected on chromatograms developed from distilled water extracts Using phenol as a solvent, it has not been possible to detect a spot on chromatograms developed from distilled water extracts which turns pink after treatment with 0 44 *M* potassium ferricyanide On the other hand, such spots appear on chromatograms in which Cameron s technique was used to extract the corpora cardiaca It is worth noting that Cameron preferred to extract the *o*-diphenol from whole larvæ of *Tenebrio*, as the extraction from the corpora cardiaca of *Periplaneta* was difficult and unreliable[2]

The material from the pericardial cells appears to be an indolalkylamine The isolated cockroach heart is very sensitive to serotonin, the threshold for stimulation is below 10^{-7} *M* Extracts of pericardial cells activated by the factor from the corpus cardiacum have a slight and transient melanophorotropic effect on the melanocytes of the skin of the frog *Rana temporaria*, a property shared by the indolalkylamines[4] The action of extracts of the corpus cardiacum on the isolated heart of *Periplaneta* can be potentiated by first exposing the heart to a concentration of 100 μgm /ml of iproniazid, an inhibitor of the enzyme monoamine oxidase Further, 2-bromolysergic acid diethylamide, an antagonist of indolalkylamines, markedly reduced the effect of extracts of corpus cardiacum when applied at a concentration of 10^{-5} *M* These results are taken as evidence that an indolalkylamine is involved in the action of the corpus cardiacum on the heart

If activated pericardial cells are extracted by a technique designed to separate amines[5], a pharmacologically potent extract results which possesses a single ultra-violet absorption peak at 266 mμ Similar extracts of inactive pericardial cells, on the other hand, are pharmacologically inert, and possess an absorption peak at 275 mμ, with a shoulder at 286 mμ This probably represents an inactive precursor of the active factor

Extracts of active pericardial cells have been run on paper chromatograms using *n*-butanol/acetic acid/water (4 1 5) as a solvent The pharmacological activity on such chromatograms is located in the vicinity of R_F 0 5 After treatment with ninhydrin-acetic[6], a spot exhibiting blue-green fluorescence in ultra-violet light appears at R_F 0 5 This spot is absent from chromatograms of extracts of inactive pericardial cells The test is diagnostic for derivatives of tryptamine It is worth noting that the active factor from the opaque accessory glands of male insects[7] can be extracted by the same technique and shows a strong absorption peak at 266 mμ

Finally, active and inactive pericardial cells have been examined histologically Active cells are considerably larger and contain more secretion droplets A greater proportion of the active cells exhibit argentaffin granules, a characteristic feature of cells which produce indolalkylamines

I am indebted to Prof V B Wigglesworth for his continued interest and for the provision of facilities

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CYTOLOGY

Chromosomes of the Orang-Utan (*Pongo pygmaeus*)

Apart from the data on the chromosome number of chimpanzees[1,2] there are no other data available concerning the number and the morphology of the chromosomes of other anthropoids While engaged on a programme of research on the phylogenetic relationships of primates, I had the opportunity of studying the chromosomes of two orang-utans (male and female, *R* 33 and *R* 34) in the Zoological Gardens of Rome

Since it is planned to publish at a later date morphometric comparative data for most species of primates, in this communication only a description of the chromosomes is given

The usual techniques for the study of chromosomes in tissue cultures were followed[3] The chromosome counts are summarized in Table 1

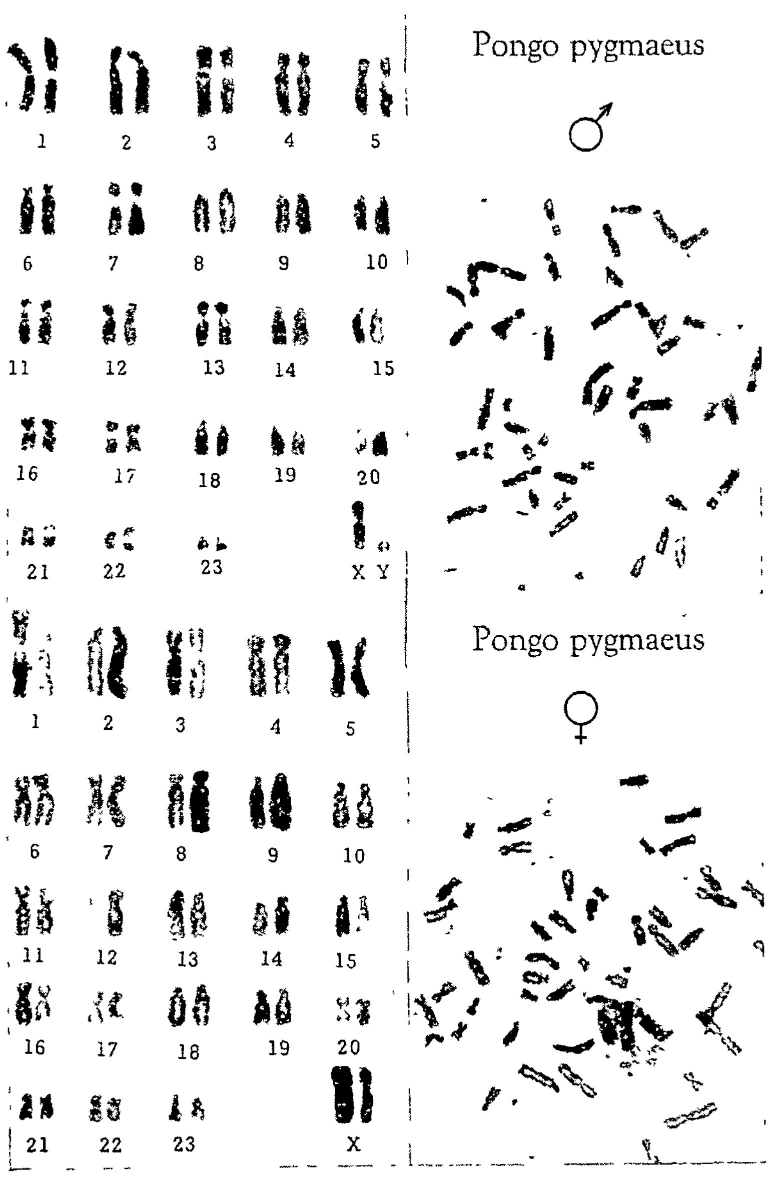

Fig 1

Table 1

2*n* =	46	47	48	49
R 33	1	2	16	1
R 34	—	3	21	—

The diploid number of the chromosomes of *Pongo pygmaeus* can thus be considered $2n = 48$, the same as that of the chimpanzee

Fig 1 shows the reconstruction, in pairs of male and female chromosomes They are arranged in a preliminary order, a more satisfactory grouping will become possible when more data become available

In Table 2 the autosomes are classified according to the position of the centromeres

Table 2

Metacentric	Submetacentric	Telocentric
1, 17, 20, 21, 22	3, 4, 5, 7, 11, 12, 13, 16, 23	2, 6, 8, 9, 10, 14, 15, 18, 19

The *X*-chromosome is metacentric, the *Y*-chromosome has a large achromatic zone

The orang-utan's karyotype shows certain similarities to the human one Chromosomes 1, 4, 5, 6 and 7 closely resemble chromosomes 1, 4, 5, 6 and 7 of man, according to the nomenclature of the Denver human chromosomes study group The three pairs of telocentric chromosomes of man (13, 14, 15) correspond morphologically and dimensionally with 10, 14 and 15 of the orang-utan The pair 23, moreover, looks like chromosome 22 of man, having one of the two arms heterochromatic As regards the sex chromosomes, the *X*-chromosome of the orang-utan shows a strong resemblance to the human one, while the *Y*-chromosome differs by having a large achromatic portion

A more careful study by means of metric analysis[3] will be required, however, before establishing such similarities

My thanks are due to the director of the Rome Zoological Gardens, Prof E Bronzini, for enabling me to study these animals

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[See also p 225 of this issue]

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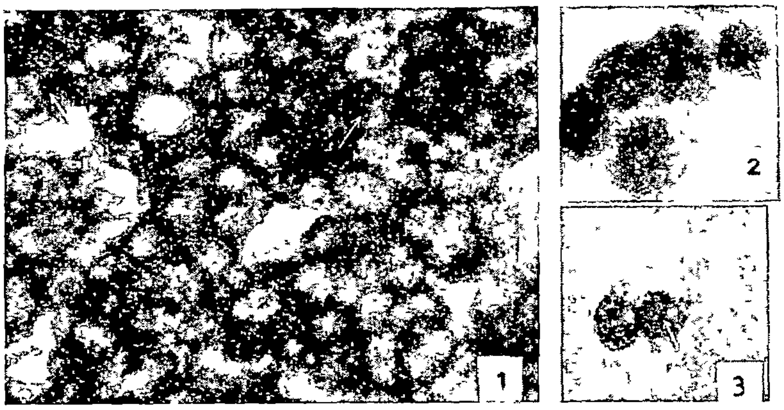
Nucleolar Succinic Dehydrogenase in Mouse Mammary Carcinoma Cells

The oxidative enzyme succinic dehydrogenase is principally localized[1] in the mitochondria and absent in the nuclei of all mammalian cells However, the enzyme had been localized in the nuclei of bird



Figs. 1 and 2. Arrows indicate the nucleoli of malignant cells in histological section and whole-cell suspension from mouse mammary carcinoma, respectively (pyronin methyl green). Fig. 3. Arrow indicates the nucleolar formazan deposition in the same type of malignant cells in suspension. (All × c. 400)

erythrocytes which according to Brachet[2] may be due to absence of mitochondria in these cells.

Nucleolar succinic dehydrogenase has been shown to be present, in normal rat liver cells[3] and also in the malignant cells[4] from epidermoid carcinoma of human cervix. Now nucleolar localization of this enzyme has been confirmed in malignant cells of a different biological material (mouse mammary carcinoma).

A suspension of intact whole cells in 0·1 *M* ice-cold phosphate buffer of *p*H 7·6 was prepared from spontaneous solid mammary carcinoma arising in *DBA* mice, which removed the endogenous substrates for succinic dehydrogenase. A portion of this suspension was smeared on glass slides for staining with pyronin methyl green and cellular sediment from the remaining portion was stained for succinic dehydrogenase[5]. A small piece of tissue from the tumour was fixed in carnoy, sectioned at 5μ thickness and stained by pyronin methyl green.

Photomicrographs show a close similarity as regards nucleolar shape, size and position of the pyronin methyl green preparations and formazan-stained nucleolar bodies (Figs. 1, 2 and 3). More than a thousand malignant cells were investigated on each slide.

Thanks are due to Dr. S. K. Brahma for active co-operation and to N. Saha for the photomicrography.

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Chromosomal Lesion associated with Carcinogen-induced Tumours in Mice

PRIMARY tumours induced by polycyclic hydrocarbons have been examined by several investigators to evaluate the importance of karyotype modification in carcinogenesis[1-3]. A significant number of such tumours has been characterized by cells with aneuploid chromosome numbers. In a study of 14 fibrosarcomas induced by methylcholanthrene in a variety of F_1 hybrids of inbred mouse strains, Hellstrom[2] reported that only 4 tumours contained predominantly diploid cells. The cells comprising the remaining ten tumours were either hyperdiploid, triploid, or hypertetraploid. Marked variability in chromosomal pattern was observed from one tumour to another as well as within any one tumour.

Studies in our laboratory are under way to determine whether the cytogenetic variability observed in carcinogen-induced tumours might be correlated with the inducing hydrocarbon, sex and strain differences in tumour incidence, or the latent period, antigenicity, or growth behaviour of individual tumours. The results of these investigations will be reported in detail elsewhere.

Tumours were induced, in mice of a variety of strains, by subcutaneous injection of either 0·1 mgm. 20-methylcholanthrene or 3,4-9,10-dibenzpyrene dissolved in sesame oil (Table 1). Primary tumours or tumours maintained in isologous passage by trocar pieces for various periods were examined. Cells were prepared for chromosome studies either after brief *in vitro* culture or by direct examination of solid tumours. For tissue culture, tumour fragments were trypsinized and the recovered cells incubated in Eagle's minimum essential medium containing 10 per cent fœtal bovine serum. Variability in time of attachment and initiation of cell division, usually 24–48 hr., determined the interval in culture prior to examination. Squash preparations of metaphase cells were obtained by standard colchicine and hypotonic citrate pretreatment, aceto-orcein fixation and staining. For direct examination of solid tumours, mice were injected, 1 hr. prior to death, with colchicine. Tumour fragments were minced in hypotonic citrate solution and fixed and stained with aceto-orcein. Short-term growth in tissue culture offered distinct advantages over the use of material derived directly from the mouse since larger numbers of dividing cells, as well as chromosomal preparations of superior morphological detail, were obtained. The cytogenetic variability manifested by these carcinogen-induced tumours is illustrated in Table 1. No distinction between tumours induced by methylcholanthrene or dibenzpyrene is apparent. Similar to Hellstrom's findings, those tumours represented by a true diploid modal chromosome number were seen to be composed of some aneuploid cells.

The presence of chromatid breaks, occurring regularly in a certain proportion of the cells of 20-methylcholanthrene- and 3,4-9,10-dibenzpyrene-induced tumours, has been observed in preparations directly from solid tumours as well as from cultured material (Fig. 1). Table 2 indicates the frequency of cells with such breakage for several tumours of each carcinogen group. Only those cells selected as suitable for accurate chromosome counts were scored for breakage. Characteristic breaks were noted in cultured material which was not subjected to colchicine. Similar lesions have not been seen in other murine cells,

Table 1 DISTRIBUTION OF CHROMOSOME NUMBERS OF METAPHASE CELLS IN CARCINOGEN-INDUCED TUMOURS

	Tumour no	Host strain	Sex	Passage generation	Chromosome numbers																																				Total no cells	Tumour characterization
					38	39	40	41	42	43	44	45	46	47	48	49–69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89–97	98	99	100	101	102	103	104	105–177		
20-Methylcholanthrene	*M*-I	*BALB/c*	♀	6			4*	19	12	6																	2	1	1	3		1												*162*	50	Hyperdiploid
	M-II	*BALB/c*	♀	2			1																				2	2	2	1	2	1	1	1										*163, 168*	15	Hypertetraploid
	M-IV	$(I \times C3H)F_1$	♀	Primary		9	17	1	1	1																2																			31	Diploid
	M-V	*BALB/c*	♀	3			12	18	7																				1		1			1											40	Hyperdiploid
	M-XII	*BALB/c*	♀	1	2	1	3	18	9	2	1	1															3																		40	Hyperdiploid
	M-XIV	*BALB/c*	♂	Primary		3	5	5	2							*52*† *60*	1										2	1				1						1						*136*	24	Diploid-hyperdiploid
3,4-9,10-Dibenzpyrene	*D*-I	$(C3H \times BALB/c)F_1$	♂	Primary			19	0	1	1																	1	1	1																30	Diploid
	D-II	*BALB/c*	♀	Primary	1		3	5	5	3	1																																		18	Hyperdiploid
	D-V	*BALB/c*	♀	Primary			2	5							1	*57*						1		1	2	1																		*119, 154, 155*	17	Hyperdiploid-hypotetraploid
	D-VI	*BALB/c*	♂	Primary				1								*60, 61, 69*		2		2	3	2	1	3	3	2	3	5	1					1		*92, 95*	2	3		3	2	2	1	*109, 125*	49	Near-tetraploid
	D-VII	$(I \times C3H)F_1$	♂	Primary															1	1	2				1	3	4	1		4	1	3	3		1									*157*	26	Hypertetraploid
	D-X	$(C3H \times BALB/c)F_1$	♀	Primary					2		1		1			*57*		1	4	1	1	1	1	1											1	*92, 99*								*177*	19	Hypotetraploid

* Figures refer to number of cells counted † Numbers in italics represent actual chromosome number per cell

Table 2 FREQUENCY OF CHROMATID BREAKAGE IN METAPHASE CELLS OF CARCINOGEN-INDUCED TUMOURS

	Tumour No	No of cells examined	No of cells with breaks	Frequency (per cent)
20-Methylcholanthrene	*M*-I	50	25	50
	M-II	15	9	60
	M-IV	31	0	—
	M-V	40	6	15
	M-XII	40	4	10
	M-XIV	24	10	42
3,4-9,10-Dibenzpyrene	*D*-I	30	6	20
	D-II	18	7	39
	D-V	17	4	24
	D-VI	49	7	14
	D-VII	26	14	54
	D-X	19	8	37

from normal or tumour tissues, cultured in our laboratory

Although the chromosomal discontinuity appears to be 'complete' most frequently, a thin interconnecting thread is occasionally seen Rarely the break in the chromatid arm is only partial In more than half the total number of metaphase cells with this chromatid lesion, only one break per cell was found Higher numbers of breaks, up to 5 per cell, occurred with decreasing frequency The number of breaks in each cell is apparently not related to ploidy Breaks were present with a relatively high frequency in tumour *M*-I, which was originally examined in its 6th transplant generation This tumour was again studied in its 11th passage generation and the breakage frequency was found to be similar

At this time, we feel that no preferential breakage sites characterize these tumours We have noted that for any one tumour there is occasional evidence of breaks occurring at comparable sites on the chromosomes of different cells Investigations are under way to clarify this point, elucidation, however, will be made difficult by the lack of individuality of the mouse chromosome, in addition to the well-known occurrence of cryptic structural changes

Whether or not the portion of the chromatid arm distal to the break remains attached through the next cell-division cycle is not known Such a process could occur by means of a non-visualized chromosome matrix, or by a process of re-healing That the chromatid fragment is at least sometimes lost was apparent A portion of a metaphase chromatid was occasionally missing or a chromatid fragment was sometimes found distant from a deleted chromosome At times the fragment was seen 'paired' to the sister chromatid on the side opposite the proximal portion

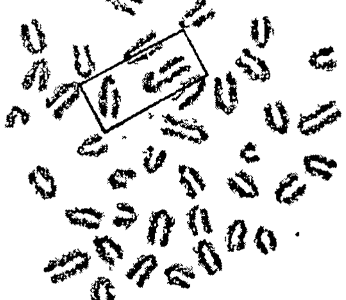

Fig 1 Metaphase cell from tumour *M*-I, containing 43 chromosomes Two chromatid breaks are demonstrated in area outlined (× 1,760)

of the broken chromatid. The possible contribution of the observed chromatid breakage to structural changes within the chromosomal complement will require further analysis.

The work reported in this communication was supported in part by a grant from the American Cancer Society and in part by Contract *SA*-43-*ph*-2445 from the Cancer Chemotherapy National Service Center, National Cancer Institute, Public Health Service, Bethesda, Md.

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GENETICS

Effect of Kinetin on the Fertility of some Strains of *Neurospora crassa*

In experiments with *Neurospora crassa*, some strains when crossed are highly infertile and, therefore further genetic analysis is difficult. This communication reports the effects of an addition of kinetin (6-furfurylamino-purine)[1] on the fertility of some strains of *Neurospora crassa* which are normally highly infertile.

The strains used were all derived from the Abbot and/or Lindegren wild-types[2] and had become infertile as a result of either ultra-violet irradiation (designated *A* in Table 1) or of selection for ascospore size (Lee, B. T. O., unpublished results) (designated *B* in Table 1).

Crosses were set up between various strains of the *A* and *B* series on a medium favouring sexual reproduction with kinetin (0–10 p.p.m.) added. The fertility was assessed after incubation for 15 days at 25° C. Each treatment was replicated five times. The fertility was measured by counting the number of perithecia per slope and ascospore production assessed on an arbitrary scale 0–10, in which 0 is equivalent to a complete lack of ascospore production and 10 corresponds to normal wild-type production of ascospores. The results are given in Table 1. It is evident that the perithecial production in eight of the nine crosses used is greatly increased by the presence of kinetin. Estimates of ascospore production follow a similar trend. It is notable that kinetin had no effect on the cross *B* 21 × *B* 14.

Table 1. Perithecial and Ascospore Production with Added Kinetin

Cross		Kinetin (p.p.m.)					
		0.0	0.5	1.0	2.0	4.0	10.0
A 13 × *A* 18	P*	64	122	128	154	146	152
	A†	2	2	3	3	4	3
A 12 × *A* 13	P	60	104	116	102	122	106
	A	2	4	4	4	4	3
A 19 × *A* 14	P	12	96	154	252	206	212
	A	1	3	4	6	5	5
A 21 × *A* 16	P	26	80	72	104	92	88
	A	1	4	4	5	4	4
B 17 × *B* 19	P	4	82	104	84	158	196
	A	0	2	2	2	4	4
B 21 × *B* 14	P	8	16	24	14	22	18
	A	1	1	1	1	1	1
B 1 × *B* 3	P	20	34	106	66	74	86
	A	1	2	3	1	2	3
B 1 × *B* 7	P	5	76	94	106	118	126
	A	0	2	3	3	4	3
B 16 × *B* 8	P	8	76	108	92	106	122
	A	0	2	3	2	4	4

* P = No. of perithecia produced per slope (mean of 5 replicates).
† A = Estimated ascospore production per slope (mean of 5 replicates).

It is thought that this effect of kinetin on the crossing behaviour of *Neurospora crassa* might assist in the study of many strains the infertility of which on crossing has hitherto prevented a genetic analysis.

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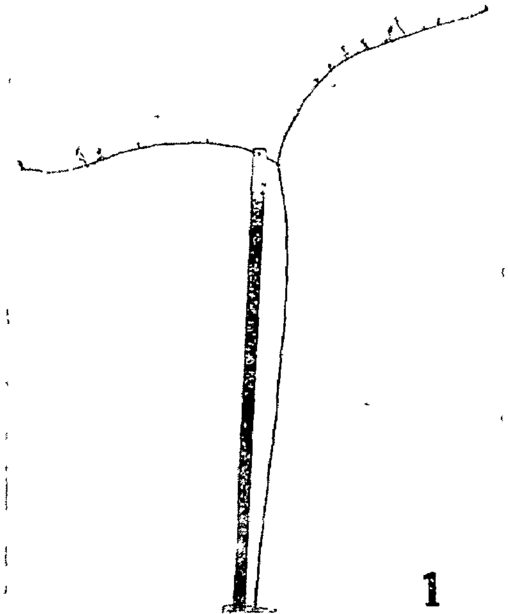
Haploidy in Jute (*Corchorus olitorius* L.)

Haploidy was first reported to occur in *Datura stramonium* by Blakeslee *et al.*[1] Since then, haploids occurring spontaneously or induced under artificial conditions have been recorded in many flowering plants[2]. As far as we are aware, haploidy in *Corchorus* has not yet been reported. Datta[3] reported a case of



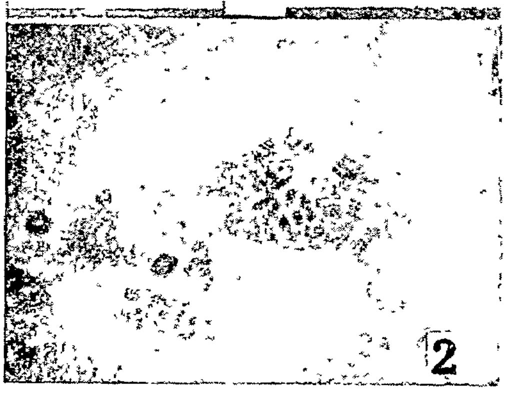


Fig. 1. The haploid *Corchorus olitorius* (*JRO*-632) plant (× 1/14)
Fig. 2. Seven somatic chromosomes in the root-tip cell (× 1,700)

polyembryony in *Corchorus* without, however, any cytological analysis of the twin seedlings

From a selection of X-ray-irradiated progenies of 70,000 r treatment of the X_3 generation of *Corchorus olitorius* (*JRO*-632) in 1960, seven variants were found from a culture of healthy, vigorously growing normal plants These plants with comparatively slender stem and small leaves were very late-flowering and the pod was small and stumpy Among these variants, one plant was characterized by the presence of a very short and slender stem (Fig 1) (length 97 5 cm , thickness 5 2 mm) and bearing a few flowers, from which only nine mature pods developed, measuring 1–1 5 cm in length and 4–5 mm in breadth Six of the pods were empty In the remaining three pods, one to three full seeds and two to three shrivelled seeds were obtained

In most of the anthers, unseparated tetrads with collapsed walls were found The anthers of the flowers did not dehisce, pollen sacs were empty with collapsed walls, though two full grains, taking stain in iodine, were found in a flower

Of the seven full seeds, which are similar morphologically to the seeds of normal plants, three were germinated, from these, chromosome investigations were carried out In the orcein squash of root-tip meristem of all the three seeds, seven chromosomes were counted from the metaphase plates (Fig 2)

The seeds having haploid number of chromosomes may have arisen by parthenogenesis of a cell from the female gamete[4]

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VIROLOGY

Transmission of Lapinized Rinderpest Virus by Contact between Rabbits

Rabbits are readily infected with lapinized rinderpest virus administered parenterally, but contact infections are alleged not to occur[1,2] Protocols, however, have not been published yet

Eight groups of twenty rabbits were randomly paired and each pair was closely confined in a cage measuring 45 × 45 × 45 cm One rabbit of each pair was given 1,000 ID_{50} of lapinized rinderpest virus intravenously Rectal temperatures were recorded daily In the first trial insufficient attention was paid to the risk of transmission by clinical thermometers for we have found since that lapinized rinderpest virus can be recovered from the fæces of infected rabbits and that intrarectal instillation of the virus readily infects In subsequent trials different thermometers were used to record the temperatures of inoculated and uninoculated rabbits

Close contact was maintained until the inoculated rabbit died Thereafter the uninoculated rabbits were observed for a further period of 10 days, after which they were killed and examined for signs of rinderpest In the final trial the period of observation was extended to 30 days Uninoculated rabbits which developed a fever were killed and examined. Tissues from those having suggestive lesions were collected, emulsified and inoculated into other rabbits

All eighty inoculated rabbits were infected and all died In the first trial one of the in-contact rabbits contracted rinderpest and we now believe that transmission occurred rectally via the thermometer In the subsequent trials two in-contact rabbits had suggestive lesions but virus was not recovered from their tissues and infection was not considered to have occurred

Our findings have confirmed the earlier statements that contact transmission of lapinized rinderpest virus does not occur between sick and healthy rabbits Direct contact, nevertheless, is the primary mode of transmission of wild strains of rinderpest and all authenticated outbreaks in previously disease-free countries have been linked to the importation of live animals from endemic areas[5] Lapinized rinderpest virus has undergone many serial passages in rabbits and in the process has become attenuated for cattle and, at the same time, highly virulent for rabbits It has also lost its ability to transmit by contact In this respect it simulates the Kabete *O* strain of rinderpest virus which has been 'needle-passaged' in cattle for many years and which does not spread readily between sick and healthy cattle although it is highly virulent for cattle[3] Presumably the route of infection with both strains has favoured the selection of mutants deficient in natural invasiveness Both strains still, however, induce gross changes in the alimentary tract[4]

Recognition of the possibility of selecting mutants of virulent strains of virus which do not spread by contact should stimulate the search for a selection of similar mutants of attenuated strains intended as live virus vaccines

We wish to thank Mr H R Binns, director of the East African Veterinary Research Organization, for his advice and encouragement and permission to publish this communication

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Photo-Reactivation of Tobacco Mosaic Virus *in vivo*

Tobacco mosaic virus, inactivated *in vitro* by ultra-violet irradiation, could not be photo-reactivated by exposing plants, inoculated with the inactivated virus, to light[1,2] However, ribonucleic acid preparations from tobacco mosaic virus could be photo-reactivated in this manner, following inactivation by ultra-violet light[1,3] Siegel *et al* [4] investigated the effects of ultra-violet light on tobacco mosaic virus and tobacco mosaic virus nucleic acid, at different times after inoculation of *Nicotiana glutinosa* plants, on which local necrotic lesions are formed They interpreted their results to suggest that the nucleic acid of tobacco mosaic virus is freed from its protein moiety during a lag period of approximately 2 5 hr following inoculation and before the infective centre begins to increase in resistance to ultra-violet light If this model is correct, and the nucleic acid of tobacco mosaic virus separates from the protein, then it should be possible to photo-reactivate the nucleic acid during the period when the separation occurs.

Bawden and Kleczkowski[5] reported experiments to test this hypothesis, but concluded that the photo-reactivation effects observed in their experiments could always be attributed to the effects of ultra-violet light on the 'capacity' of *N glutinosa* leaves, capacity being defined as the ability of a leaf to support the multiplication of tobacco mosaic virus to the extent of forming local lesions The effect of ultra-violet light on capacity can be determined by irradiating leaves prior to inoculation, and in experiments designed to study the direct inactivation of tobacco mosaic virus in leaves, the dosage of ultra-violet light must be such that the capacity remains unimpaired

Table 1 EFFECT OF ULTRA-VIOLET LIGHT ON THE CAPACITY OF LEAVES OF *N glutinosa*

Dose (min)	Experiment No 1	2	3
2	0 97	1 00	
3	1 00	0 99	
4	0 91	0 99	
5	0 75	0 79	
6			0 56
8			0 49
10			0 24

Each figure represents the fraction of local necrotic lesions developed on detached half-leaves of *N glutinosa* irradiated before inoculation with the *U*2 strain of tobacco mosaic virus as compared with un-irradiated half-leaves

Table 1 shows the effect of ultra-violet light from a Philips *TUV*30 germicidal lamp (90 per cent transmission at 2537 Å) on the capacity of detached leaves of *N glutinosa* irradiated at 30 cm from the lamp and immediately inoculated with the *U*2, or mild, strain of tobacco mosaic virus Clearly, capacity is affected by prolonged irradiation prior to inoculation, and in subsequent experiments no period of irradiation longer than 90 sec was employed The *U*2 strain of tobacco mosaic virus was used as it is 5 5 times more sensitive to ultra-violet light than the *U*1, or common strain Suitable killing fractions for infective centres of *U*1 tobacco mosaic virus could not be obtained without impairing capacity *N glutinosa* plants were inoculated by means of a paint brush with an inoculum containing 10^{-3} mgm /ml of ultracentrifugally purified *U*2 tobacco mosaic virus in *M*/15 phosphate buffer, *p*H 7, containing 4 per cent 'Celite' as abrasive In all experiments glasshouse grown plants were placed in a growth room 16–20 hr before inoculation, at a temperature of 25° ± 1° C , and continuous fluorescent light of 800 ft -candles intensity Inoculations were carried out under these conditions, while irradiation was done under the ultra-violet lamp in the same room Leaves were detached prior to irradiation, placed on cotton wool in shallow trays and irradiated For each treatment 3 leaves from each of 8 plants were used, one half of each leaf was covered to serve as the unirradiated control Leaves were retained in the light, or dark, in the growth room for 24 hr following irradiation and then transferred to continuous light of 800 ft -candles intensity and a temperature of 25° ± 5° C , for a further 2 days, until lesions were counted For each treatment the lesion totals from the irradiated halves of each leaf were compared with the totals from the unirradiated halves, and since infective centres lead to the formation of local lesions the fraction of infective centres surviving irradiation was calculated

Table 2 PHOTO-REACTIVATION OF TOBACCO MOSAIC VIRUS A DIFFERENT TIMES AFTER INOCULATION

Exposure after inoculation	Time after inoculation (hr) 2	2 5	3 5	3 75	4 0			4 25	6 0	
Light	0 24	0 25	0 42	0 47*	0 45	0 46	0 45	0 57*	0 61*	0 5
Dark	0 23	0 24	0 40	0 41*	0 39	0 40	0 32	0 47*	0 59*	0 5

Figures represent the fraction of infective centres of *U*2 tobacco mosaic virus on *N glutinosa*, surviving a dose of 90 sec ultra-violet light

* Experiments carried out at a dose equivalent to 75 sec ultra-violet light

Table 2 shows the results from photo-reactivation experiments carried out at different times after inoculation In the period from 2 to 3 5 hr after inoculation no photo-reactivation could be demonstrated It is clearly evident that from 3 75 to 5 hr after inoculation photo-reactivation, as measured by the difference in the fraction of infective centres surviving irradiation in leaves exposed to light, as compared with dark, has been demonstrated This period of photo-reactivation corresponds to the period of rapid change in sensitivity to ultra-violet irradiation shown by Siegel and Wildman[2] to commence 2 5 hr after inoculation with the *U*2 strain of tobacco mosaic virus Siegel and Wildman retained plants at a lower temperature, 20° C , and a higher light intensity, 1,000 ft -candles, than those used in the present experiments However, it is evident from the fraction of infective centres surviving when leaves are exposed to light following inoculation (Table 2) that conditions used in the experiments reported here resulted in a change in sensitivity commencing at approximately 2 5 hr after inoculation, the same time at which Siegel and Wildman reported a change

The demonstration of photo-reactivation supports the hypothesis that nucleic acid, free from the protein moiety, is present in the infective centre for a period of time following the inoculation of tobacco mosaic virus Siegel and Wildman[4] suggested that the separation of protein and nucleic acid occurs during the lag period of 2 5 hr before the infective centre begins to increase in resistance However, in a later publication, Wildman[6] considered that the process of protein removal from the nucleic acid may occupy a period of up to 5 hr after inoculation Since it has not been possible to demonstrate photo-reactivation of tobacco mosaic virus until 3 75 hr after inoculation it would appear that free nucleic acid is not present in the infective centre until this time period has elapsed Thus it seems more realistic to propose that the separation of the protein moiety and the nucleic acid of tobacco mosaic virus in an infective centre on a leaf of *N glutinosa* takes place following the lag period and when the infective centre is already undergoing an increase in resistance to ultra-violet irradiation

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk * are open to the public*)

Monday, October 23

University of London (in the Physiology Theatre, University College, Gower Street, London, W C 1) at 5 p m —Prof J N Davidson "The Biosynthesis of Polynucleotides" * (Further lecture on October 30)

Institution of Electrical Engineers (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Is Automation Making Satisfactory Progress ?"—opened by Prof A Tustin

University of London (at King's College, Strand, London, W C 2), at 5 30 p m —Dr F A E Pirani and Dr G F Elliott "Ballistic Missiles and Nuclear Weapons—Missile Ballistics" * (Second of seven lectures in War Studies Further lectures on October 30, November 6, 13, 20 and 27)

Society of Chemical Industry, Plastics and Polymer Group (at 14 Belgrave Square, London, S W 1), at 6 30 p m —Mr W Reddish "Chemical Structure and Electrical Properties of High Polymers"

Tuesday, October 24

Institution of Electrical Engineers, Measurement and Control Section (at Savoy Place, London, W C 2), at 5 30 p m —Mr R W Hockney and Dr T O Jeffries "The Use of Analogue Computers in Predicting the Space–Time Behaviour of Nuclear Reactors"

Royal Institution of Naval Architects (at the Institute of Marine Engineers, 76 Mark Lane, London, E C 3), at 5 30 p m —Dr J E Richards "High Temperature Gas-cooled Reactors"

University of London (at King's College, Strand, London, W C 2), at 5 30 p m —Père Roland de Vaux (Jerusalem, Jordan) "Tell el-Far'ah – Tirzah—Excavating a Biblical City" *

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Dr L Brent "Some Current Problems in Transplantation Immunity" * (Third of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

University of London (at the School of Oriental and African Studies, London, W C 1), at 5 30 p m —Dr L Dumont (Paris) "The Conception of Kingship in Ancient India, and Anthropologists' View" * (Further lecture on October 25)

Wednesday,' October 25

Polarographic Society (at the School of Pharmacy, 29/39 Brunswick Square, London W C 1), at 2 45 p m —Prof M von Stackelberg (Bonn University) "Some Special Problems in the Polarography of Indium and Tellurium"

Institute of Navigation (at the Royal Geographical Society, 1 Kensington Gore, London, S W 7), at 4 p m —Annual General Meeting Dr G E R Deacon, F R S "Navigation and the Science of the Sea" (Duke of Edinburgh's Lecture)

Bone and Tooth Society (at the Institute of Orthopædics, 234 Great Portland Street, London, W 1), at 4 30 p m —Symposium on "Fluorides"

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W C 2), at 5 30 p m —Mr R J Halsey "Global Communication" (Chairman's Address)

British Institution of Radio Engineers, Computer Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Symposium on "Digital Differential Analysers".

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Sir Kenneth Hague "Coordination in Engineering Research and the Conservation of Scientific and Technical Manpower" (Presidential Address)

Society of Environmental Engineers (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S W 7), at 6 p m —Mr A M P Brookes "Structures and Components at Elevated Temperatures"

Society of Chemical Industry, Food Group—Nutrition Panel (at 14 Belgrave Square, London, S W 1), at 6 15 p m —Meeting on "Foods Low in Salt"

Society of Instrument Technology, Data Processing Section (at Manson House, 26 Portland Place, London, W 1), at 7 p m —Mr W H Baker and Mr P Huggins "Mechanising Factory Shop Floor Form Filling".

British Psychological Society, Medical Section (at the Royal Society of Medicine, 1 Wimpole Street, London, W 1), at 8 30 p m —Mr Emanuel Miller "Personal and Social Approaches to Adolescents' Problems"

Thursday, October 26

Royal Society (at Burlington House, Piccadilly, London, W 1), at 10 30 a m —Symposium on "Biochemistry and Nutrition" under the leadership of Prof R A Morton, F R S , to celebrate the Centenary of the Birth of a former President of the Society, Sir Frederick Gowland Hopkins, O M , 1861–1947

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1 15 p m —Dr A S G Curtis "The Capabilities of a Living Cell" *

Institution of Electrical Engineers (joint meeting with the British Section of the Société des Ingénieurs Civils de France, at Savoy Place, London, W C 2), at 5 30 p m —Mr P M J Ailleret "A Comparison between Generation and Transmission Problems in Great Britain and France"

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London W C 1), at 5 30 p m —Dr Henry Harris "The Transfer of Information from Nucleus to Cytoplasm" * (Fourth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

Friday, October 27

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W 12), at 4 p m —Prof L S Penrose, F R S "Mongolism" *

Royal Astronomical Society (in association with the Geological Society, at the Geological Society, Burlington House, Piccadilly, London, W 1), at 5 p m —Geophysical Discussion on "Interpretation of Geophysical Surveys" Chairman Dr W Bullerwell Speakers will include Prof J M Bruckshaw, Dr M H P Bott, Mr G Tait and Mr A N Thomas

Royal Institution (at 21 Albemarle Street, London, W 1), at 9 p m —Prof M A Ellison "Cosmic Rays from the Sun"

Friday, October 27—Saturday, October 28

Institute of Biology (in the Lecture Hall of the Royal Geographical Society, 1 Kensington Gore, London, S W 7), at 10 a.m daily—Symposium on "The Better Use of the World's Fauna for Food"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Research Assistant (Animal Behaviour) (graduate in psychology or zoology) in the Department of Psychology—The Registrar The University, Leicester (October 27)

Scientific Officer (with a good honours degree in chemistry or botany) in the Weeds Section, to carry out research on the adsorption, mobility and persistence of herbicides in soils—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (October 28)

Assistant Lecturer (with a science qualification and preferably a medical qualification) in Physiology—The Secretary, St Mary's Hospital Medical School (University of London), Paddington, W 2 (October 30)

Senior Lecturer or Lecturer (with medical qualifications registrable in this country) in Virology, and a Senior Lecturer or Lecturer (with medical qualifications registrable in this country) in Immunology—The Registrar, The University, Manchester 13 (October 30)

Analyst (with at least a second-class degree in chemistry or biochemistry or considerable experience in analytical chemistry) in the Department of Chemical Pathology—The Registrar, The University, Leeds 2 (October 31)

Assistant Lecturer (with special qualifications in experimental physics, aeronomy or astronomy) in Applied Mathematics—The Secretary, Queen's University, Belfast, N Ireland (October 31)

Lecturer in Biology—The Principal, Bede College, Durham (October 31)

Lecturer (with a major interest in elasticity or the theory of plasticity) in Mechanical Engineering—The Registrar, Manchester College of Science and Technology, Manchester 1 (October 31)

Research Assistant (graduate in physics or electrical engineering) in the Department of Physics, to investigate microwave radiation from plasmas—The Registrar, University College of Wales, Aberystwyth (October 31)

Senior Lecturer (with a good grounding in biochemistry and clinical medicine or clinical pathology, and preferably experience of a hospital biochemical laboratory) in Chemical Pathology—The Registrar, The University, Leeds 2 (October 31)

Chair of Geology in the University of Malaya, Kuala Lumpur —The Secretary Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Malaya and London, November 1)

Demonstrator (graduate who intends to read for a higher degree) in Pharmacognosy—The Registrar, The University, Nottingham (November 1)

Assistant Lecturer in Physics—The Registrar, Manchester College of Science and Technology, Manchester 1 (November 4)

Assistant (with a degree in science and/or agriculture (preferably honours), and interest in grassland husbandry and an aptitude for investigational and research work) to the Advisory Officer in Grassland Husbandry—The Secretary, West of Scotland Agricultural College, Blythswood Square, Glasgow, C 2 (November 7)

Senior Lecturer or Lecturer (qualified in applied mathematics) in Mathematics)—The Registrar, University College of North Staffordshire, Keele, Staffs (November 7)

Lecturer or Assistant Lecturer (with special interests in the geography of the Americas) in Geography—The Registrar, The University, Southampton (November 11)

Vokes Research Fellow (preferably with a special interest in fluids and elastic theory) in the Department of Mechanical Engineering—The Secretary and Registrar, The University, Southampton (November 11).

Experimental Officer or Senior Experimental Officer (with H N C, a degree or similar qualification, and preferably experience with transistor circuits and pulse equipment) in the Department of Physics, to develop and construct special electronic devices required for experimental work on a tandem accelerator—The Registrar, The University, Manchester 13 (November 13)

Scientific Officer or Senior Scientific Officer (honours graduate with appropriate training and research experience), to work as a member of a team undertaking research on hill sheep nutrition—The Secretary, Hill Farming Research Organization, 48 Palmerston Place, Edinburgh 12 (November 13)

Lecturer (preferably with qualifications and/or experience in palæontology or stratigraphy) in Geology at the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (November 18)

Reader in Fluid Mechanics at Imperial College—The Academic Registrar University of London, Senate House, London, W C 1 (November 20)

Senior Research Assistant to the Immunopathology and Fluorescence Microscopy Research Group, to work in the University Department of Pathology on immunological and histological methods—The Secretary, The University, Aberdeen (November 20)

Senior Lecturer in Physiology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, November 25)

Lecturer in Zoology at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia, November 30)

Senior Lecturers/Lecturers in the School of Physiology within the Faculty of Medicine, University of New South Wales, Australia—The Agent-General for New South Wales, 56–57 Strand, London, W C 2, and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (November 30)

Assistant Professor (Poultry Genetics) (with a Ph D or equivalent in poultry genetics), to develop a research programme in poultry genetics and to carry some teaching responsibility—Head, Department of Animal Science, University of Alberta, Edmonton, Alberta, Canada (January 1)

Agricultural Chemist (male, under 35, with a degree in agriculture with chemistry as a major subject, or a science degree with postgraduate experience in soil or agricultural chemistry) in the Department of Land Utilization, Swaziland, to develop an agricultural chemistry section at Malkerns Research Station, organize routine analysis of soil and plant material, and to conduct an investigation into fertility problems in soils, particularly leached types of tropical soils—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S W 1, quoting RC 213/141/01/T

Assistant Experimental Officer (graduate, or holding H N C, or an equivalent qualification, or, if under 22, G C E "A" level, O N C, etc, or equivalent, and interested in both chemical analysis and working with animals) in the Chemistry Department, to assist in studies of mineral metabolism in cattle—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref 61/15

Assistant Professor in Pharmacology—Prof M F Murnaghan, University of Ottawa, Ottawa, Ontario, Canada

Biochemist or Chemist (with a research degree and experience in the chemical industry involving research on new products and processes and their technical or commercial development, and preferably experience in the virology and antibiotics fields), for work concerned with the development and commercial exploitation of inventions in the public interest—The Director of Administration, National Research Development Corporation, 1 Tilney Street, London, W 1

Chief Laboratory Technician—Head of the Department of Applied Physics, Northampton College of Advanced Technology, St John Street, London, E C 1

Fisheries Officer (with a university degree in science with experience in up to date fishing methods, and the storage, marketing and distribution of fish) in the Department of Agriculture and Fisheries, Bermuda, to undertake the development of all aspects of the fishing industry in Bermuda—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S W 1, quoting RC 289/13/01/T

Head (with an honours degree in mathematics of a British university and at least three years experience of teaching) of the Mathematics Department—The Headmaster, Pocklington School, West Green, Pocklington, Yorks

Lecturer (with some knowledge of logical design) in Computing—The Secretary, Northampton College of Advanced Technology, St John Street, London, E C 1

Physicist, Basic grade (with a first- or second-class honours degree), for routine and development work in hospital physics—The Secretary, Lambeth Hospital, Brook Drive, London, S E 11

Postgraduate Student (recent graduate with a good degree in physics or physical chemistry) in the Department of Physics, to research under the supervision of Dr J G Powles on some aspect of magnetic resonance—The Registrar, Queen Mary College (University of London), Mile End Road, London, E 1

Research Assistant (honours graduate) in Physics—Clerk to the Governors, Woolwich Polytechnic, London, S E 18

Research Assistant (with a good honours degree in physics) in Physics, for work in the field of vacuum physics—Head of the Department of Physics, The Polytechnic, Regent Street, London, W 1

Research Assistant (with graduate or equivalent qualifications and appropriate experience in electronic instrument design and construction), to take part in a space research project under Dr T R Kaiser The work will involve the development of instruments for installation in rockets and satellites—The Registrar, The University, Sheffield

Research Fellow in Physical Chemistry, for work with Prof M C R Symons on the study of unstable intermediates including spectrophotometric and electron spin resonance techniques—The Registrar The University, Leicester

Research Scholar, and a Research Fellow in the Department of Chemistry, for work on aluminium–nitrogen and aluminium–phosphorus polymers—The Registrar, The University, Leicester

Senior Physicist (graduate in physics, preferably with honours, and normally with five years experience of hospital physics or with radio-elements) in the Radiotherapy Department—The Secretary, Lambeth Hospital, Brook Drive, London, S E 11

Senior Research Fellowship at the University of Melbourne, available in any one of the academic subjects taught there—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1, or Dean of Graduate Studies, University of Melbourne, Parkville, N 2, Victoria, Australia

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Colonial Development and Welfare Act Return of Schemes made and of Loans approved under the Colonial Development and Welfare Act in the period from 1st April, 1960, to 31st March, 1961 Pp 58 (House of Commons Paper No 240) (London H M Stationery Office, 1961) 3*s* 6*d* net [258

Colonial Development Corporation, Annual Report and Statement of Accounts for the year ended 31st December 1960 Pp vi+82 (London H M Stationery Office, 1961) 5*s* net [258

Vertebrate Locomotion Proceedings of a Symposium held at The Zoological Society of London, Regent's Park, London, N W 1, on 9th November, 1960 Edited by J E Harris (Symposia of The Zoological Society of London, No 5) Pp v+132+7 plates (London The Zoological Society of London, 1961) 32*s* 6*d* [258

British Association for Commercial and Industrial Education Education and Training in France Report of a Visit made by the BACIE West Midlands Group in May 1960 Pp v+51 (London British Association for Commercial and Industrial Education, 1961) 10*s* [258

Other Countries

The Carlsberg Foundation's Oceanographical Expedition Round the World 1928–30, and previous "Dana" Expeditions "Dana" Report No 53, A Contribution to the Life History, Biology and Geographical Distribution of the Bonefish, *Albula vulpes* (Linnaeus) By Elizabeth C Alexander Pp 51 (Copenhagen Andr Fred Høst & Son, 1961) 15 Danish kr [158

United States Department of the Interior Geological Survey Water-Supply Paper 1475–D Geology and Occurrence of Ground Water at Jewel Cave National Monument, South Dakota By C F Dyer Pp iii+139–157 15 cents Water-Supply Paper 1499–A Water Resources of the Providence Area, Rhode Island By H N Halberg, C E Knox and F H Pauszek Pp iv+50+1 plate Water-Supply Paper 1536–B Water Levels near a Well Discharging from an Unconfined Aquifer By Irwin Remson, S S McNeary and J R Randolph Pp iii+27–39 15 cents Water-Supply Paper 1536–C A Simple Method for Determining Specific Yield from Pumping Tests By L E Ramsahoye and S M Lang Pp iii+41–46 15 cents Water-Supply Paper 1539–F Aquifers in Melt-Water Channels along the Southwest Flank of the Des Moines Lobe, Lyon County, Minnesota By Robert Schneider and Harry G Rodis Pp iii+11 15 cents (Washington, D C Government Printing Office, 1961) [258

United States Department of the Interior Geological Survey Water-Supply Paper 1539–I Evaluation of Bank Storage Along the Columbia River between Richland and China Bar, Washington By R C Newcomb and S G Brown Pp iii+13 15 cents Water-Supply Paper 1540–B Concentration Method for the Spectrochemical Determination of Minor Elements in Water By William D Silvey Pp iii+11–22 Water-Supply Paper 1592–A Resistance Coefficients and Velocity Distribution, Smooth Rectangular Channel By H J Tracy and C M Lester Pp iv+18 (Washington, D C Government Printing Office, 1961) [258

National Academy of Sciences—National Research Council Nuclear Science Series, Report No 36 A Cross-References Index of Radiochemical Teaching Experiments Applicable to Chemistry By Gregory R Choppin Pp v+50 (Publication 905) (Washington, D C Office of Technical Services, Department of Commerce, 1961) 1 dollar [258

Annals of the New York Academy of Sciences Vol 92, Article 3 Pavlovian Conference on Higher Nervous Activity By Nathan S Kline, Gregory Razran and 23 other authors Pp 813–1198 (New York Academy of Sciences, 1961) [258

Smithsonian Contributions to Astrophysics, Vol 5, No 10 Sampling Dust from the Stratosphere By Paul W Hodge Pp ii+145–152 (Washington, D C Government Printing Office, 1961) 20 cents [258

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO, LTD
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number. Whitehall 8831 Telegrams Phusis Lesquare London

Advertisements only should be addressed to
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SCIENTIFIC MAN-POWER IN BRITAIN

DURING the latter part of 1960, the Statistics Committee of the Advisory Council on Scientific Policy made a fresh survey of the long-term demand for scientific man-power in Britain, supplementing that previously made by the Council's Committee on Scientific Manpower in preparation for its survey of scientific and engineering man-power in 1956 This survey is intended to relate the subsequent expansion of educational institutions to the Council's plans for training scientific and technological man-power during the next decade and to the demands for employment which might be expected to arise by about 1970 This survey has now been published as an appendix to a memorandum by the Committee on Scientific Manpower* Although the Statistics Committee did not regard the nature of the evidence at its disposal, particularly in relation to future demands for employment, as sufficiently precise for detailed conclusions to be drawn, the report should none the less form a useful guide to the Council in determining its general policy in training scientific man-power

The Committee on Scientific Manpower points out that much might happen to invalidate the estimates made by the Statistics Committee, and the assumptions made will not necessarily be fulfilled It is believed, however, that the present shortage of scientific man-power will continue for a long time in the educational profession, and in those disciplines which involve a high degree of mathematical skill While the Statistics Committee has calculated the balance of supply and demand from its own assessment of the soundest of the alternatives before it, the report provides the material for a range of differing assumptions to be applied should further evidence become available

In its terms of reference, the Statistics Committee was asked to assume that the university population would be raised to 170,000 by 1971, and that the projected increases in the output of qualified man-power for colleges of advanced technology and similar institutions would also be achieved Its estimates of supply accordingly assume that suitable school-leavers will wish to enter such institutions in sufficient numbers to fill all scientific and technological places, that the present pattern of university output will continue (including lengths of courses and proportions of overseas students), that technological education will develop on lines at present foreseen (including present standards of professional qualification), and that the present pattern of migration, and loss by death, retirement, marriage, etc , will continue The Committee on Scientific Manpower believes that in so far as these assumptions are closely linked with the physical limits of expansion, the report gives a substantially accurate picture of the numbers likely to qualify during the next decade, though changes in the pattern of output from the universities and in the lengths of courses are to be expected It is assumed that the Committee's estimate in its earlier report of a total 'qualified' population of 72,200 scientists and 100,800 engineers in 1959 was reasonably comprehensive

On these assumptions the qualified man-power available is expected to rise to 77,400 scientists and 100,800 technologists at the beginning of the 1960–64 period, 107,700 scientists and 147,300 technologists at the beginning of 1965–69, 149,300 scientists and 196,600 technologists at the beginning of 1970–74, and 206,000 scientists and 257,500 technologists in 1975. The numbers qualifying in each year are estimated as rising from 6,490 in science and 9,055 in technology in 1959–60, of whom 8,345 will be from universities and 7,200 from technical colleges, etc , to 8,660 in science and 11,620 in technology in 1964–65, 11,810 in science and 14,770 in technology in 1969–70, and 14,200 in science and 16,470 in technology in 1975–76, when 17,370 will come from the universities and 13,300 from technical colleges, etc These figures are believed to provide a sound basis from which to consider possible demand, and stress is laid on the relatively low figure for wastage, due in part to the comparatively low average age of scientists and technologists in employment

The assessment of demand has been accepted as essentially more hazardous than the calculation of supply, and in this review a more detailed assessment of each sector of employment has been used than in 1956 and of the main industrial groups separately Moreover, the Statistics Committee has avoided restrictive assumptions and endeavoured to avoid significantly under-estimating the demand for employment or drawing too fine a balance between supply and demand An exclusively vocational approach has also been avoided, and account taken of the tendency, which may well accelerate, for scientists and technologists progressively to invade fields of employment outside their disciplines, such as management, public services and elsewhere

The estimates of demand depend on assumptions as to the programmes of research in the Civil Departments (particularly the research councils) and the expenditure on defence, the use of qualified man-power in each group in private industry, the ratio of scientists to technologists, and the proportion of the working population in 1970 in each industry, the requirements for teaching staff implicit in filling all planned and projected places in further education and in schools, and the form of the curve of demand between 1959 and 1970 As regards industrial employment, the most important assumption, made on the basis of inquiries, is that the speed of absorption of qualified man-power in technologically advanced industries tends to decline when a density is reached at which the technologies concerned are fully manned Thereafter, the rate of absorption tends to be governed by the size of the industry and by replacement, until far-reaching but unknown technological changes create an entirely new industrial situation, but in a large industry there is reason for believing that technological 'break-throughs' as such do not normally involve taking on significant additional resources of man-power The most advanced firms in certain leading industries in both the United Kingdom and the United States already appear to be exhibiting these tendencies, and the Committee assumes therefore that, for one economic reason or another, the density of employment of scientific man-power within these industries will rise to the level already characterizing the best firms in that group

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* *The Long-Term Demand for Scientific Manpower* Pp ix+26 (Cmnd 1490) (London H M Stationery Office, 1961) 1s 9d net

On these assumptions, and subject to the reservations indicated, the Statistics Committee concludes that the overall supply and demand for qualified man-power will not be much out of balance at the end of 1965 If anything, a slight shortage of technologists will be balanced by a slight surplus of scientists, and any possible divergence of supply and demand falls in the second five years of the 1960–70 decade The survey suggests that the number of qualified persons per thousand in the working population can be expected to rise from 7 to 13 during the next ten years, and in many engineering industries as well as in the chemical industry, this doubling in a decade will be exceeded over the whole of the industry

In presenting the report, the Committee on Scientific Manpower describes this doubling, with some justification, as in itself a potential technological revolution, and the report endorses the assumption that by 1970 there will be substantial improvements in pupil–teacher ratios and in the proportion of graduate teachers in the schools in Britain, notwithstanding big increases in the numbers of pupils in the higher forms and a doubling of the number of postgraduate students in the universities Overall, the Committee's assumptions imply almost the same rate of growth of scientific employment during the next decade as has occurred in the years 1956–59, and the methods by which the estimates have been built up should command much readier acceptance of the conclusions, particularly in the present circumstances, than would have followed a simple projection of an existing trend Indeed, it might be argued that this continued growth in scientific employment is an essential factor if Britain is to hold her place in the world's markets, independently of whether or not she enters the European Common Market, and not to decline, with all that that implies for her economic future and the standard of living of her citizens.

The conclusion that by 1965 the supply and demand of scientific man-power should be in balance and that, thereafter, a surplus may exist contrasts almost startlingly with the crippling shortages of scientific man-power that have been experienced since the Second World War and with the findings of previous reports on this subject As the Manpower Committee points out, however, something can now be seen of the fruits of the efforts which have led to such a striking increase in our annual output of qualified scientific man-power since the War from 10,000 in 1955 to 16,500 in 1961 and a prospective 20,000 by 1965 and 30,000 in 1973 It is true that the balance is a statistical one, and that supply and demand in individual disciplines, of which mathematics may be instanced as the most important example, cannot be so easily equated Nevertheless, the Statistics Committee found no adequate basis for making separate estimates of the supply or demand for particular kinds of scientist or technologist, and the Manpower Committee recognizes that there may well be important differences in the factors which govern the prospects of employment between the specialized technologist and the engineer with a general training, and between technologists as a group and scientists, and that these problems remain to be examined

In the view of the Committee on Scientific Manpower, the possibility of a surplus of scientists over immediate demands for employment should be welcomed It should make possible a rational—as opposed to an emergency—use of the scientific disciplines, and it should mean that at long last Britain would have a supply of qualified man-power with a scientific training for management, administration and the professions generally in addition to those who up to the present have been drawn inevitably into vocational employment The Committee does not doubt that scientific education will adapt itself to this new prospect, and that, just as only a proportion of those trained in the classics and history have expected to find employment in their own fields of study, an increasing proportion of those trained in specialized scientific disciplines will obtain employment outside them

The Committee considers that both science and the nation will benefit from this adjustment and it believes that the universities are already thinking along these lines If the Committee's confidence is to prove well founded here, it is certainly essential that the universities should not only think constructively but also be ready to act vigorously before the expected surplus arises Modifications in the training of chemists and physicists such as have already been suggested from within the universities or by professional institutions could go far to correct the narrowness and rigidity of training which limit the employability of a scientist or technologist trained in a particular discipline; and too much care cannot be taken to ensure that neither the scientist nor the technologist is too narrowly trained Colleges of technology, as well as the universities, may well have to resist very firmly pressure to give training in techniques which should properly be acquired in industrial or professional practice, and in this context the growing stress being laid on a broad general education is to be welcomed

The report, therefore, should administer a wholesome check to the tendency to excessive or premature specialization; but if waste of highly skilled man-power is to be avoided, there are other matters that require attention The Manpower Committee is fully aware of the danger of complacency, and that the balancing of supply and demand is a relative, not an absolute, situation The size of the reserves of ability on which Britain can draw, the problem of quality and the appropriate claims of disciplines in arts as well as science have to be considered, moreover, the calculations in the report do not cover medical, dental, agricultural or veterinary qualifications, the specialized technologies or the social sciences All these must be considered in any national review, and will involve questions of transfer which may not be easy to forecast Fully as important, however, are major questions concerning the relations, both in industry and in research, of qualified man-power and the technicians and other skilled man-power on which efficient use of professional man-power depends

A report from the University Grants Committee, issued almost simultaneously with the report of the Committee on Scientific Manpower, directs attention to the low ratios of technicians to research workers in the universities, which is regarded as below that necessary to ensure that the time of research workers is used most economically The Committee on Scientific Manpower hopes to examine this last issue more closely in the near future, but it is obvious that these questions of the conditions of service will not become any the less important if the nation experiences a surplus in place of a shortage of scientific man-power Further, while the adaptability of the scientist and technologist becomes more important, whether in fact he or she is used in a wider range of occupations depends equally on the willingness of the employer to experiment The scarcity of scientists and technologists in administrative appointments, for example, is not solely due to their reluctance to take up such posts It is just as much, and possibly more, due to the unwillingness of departments or firms to give such appointments to the scientist or technologist, to the persistent belief that a person trained in a scientific discipline is inherently unfit for such responsibilities, though on this point it should be noted that the estimated demand of 12,400 scientists in the central Government by 1970 depends on the assumption that they will be increasingly employed outside the scientific field No improvement in the general training of the scientist or technologist, or in the provision of opportunities for training for management, will avail while this prejudice persists

The clear warning this report gives of a probable surplus of scientists and technologists within the next decade should stimulate fresh endeavours to deal with this and numerous other factors which hinder the wise, widespread and efficient use of science and technology in the ordering of British national affairs.

VISION, WITH AND WITHOUT TEARS

The Eye and Its Function

By Dr R A Weale Pp vii + 209 (London. The Hatton Press, Ltd , 1960) 55*s* net

Anatomy and Histology of the Eye and Orbit in Domestic Animals

By Prof Jack H. Prince, Prof Charles D Diesem, Prof Irma Eglitis and Gordon L Ruskell Pp xi + 307. (Springfield, Ill , Charles C Thomas; Oxford: Blackwell Scientific Publications, 1960) 126*s*

The Structure of the Eye

Proceedings of the Symposium held April 11–13 during the Seventh International Congress of Anatomists, New York Edited by George K Smelser Pp xv + 570 (New York : Academic Press, Inc ; London: Academic Press, Inc (London), Ltd , 1961) 107*s* 6*d*

THREE additions have recently been made to the already long list of books on the eye, and these all to a remarkable degree accentuate the structural aspects, but from very different points of view.

The first, *The Eye and its Function*, is addressed to "those who use the eye and wish to read about its function" Since all who are not blind use the eye for various purposes, the book is apparently intended for all those who wish to read about the eye This category may obviously include all classes, from the film star interested in the practical use of the ocular muscles and the lacrimal glands, to the medical student, the research physiologist, the experimental psychologist, and even the greatest expert in ophthalmology Naturally enough, the book reflects this lack of decision as to who are its potential readers and is a curious mixture of the commonplace, the significant and the abstruse For the completely uninitiated there is probably too much technical information and knowledge required for the text to be easily comprehensible For the expert in any one branch of the subject, the treatment is often inadequate and sacrifices of accuracy have sometimes been made in the interests of brevity or of an apparent, though possibly deceptive, simplicity. There is, however, a wealth of general information about the eye and its function to be gleaned from perusal of the contents Both general biologists and medical students should find the book helpful and stimulating In places, the English could be improved (for example, the legend for Fig. 9 3) and references to original sources are sometimes inadequate

The second book, *Anatomy and Histology of the Eye and Orbit in Domestic Animals*, is clearly addressed to the veterinary profession, though the first five chapters, which give a good general picture of the eye and its associated structures, are based largely on human studies The emphasis of the book is essentially on structure rather than on function, and special attention is paid to information on those features which are peculiar to the particular species investigated The gross anatomy is, in general, better treated than the histology and cytology Retinal structure. for example, is somewhat superficially treated.

The third, *The Structure of the Eye*, is a book for the specialists in cytology and vision It incorporates the proceedings of a symposium held in New York in April 1960, and, unlike many books published after similar symposia, is very informative and has both depth and breadth. Structure here includes mostly the molecular, cytological and histological, gross anatomy being scarcely mentioned. The book is an amazing compendium of the main new advances made in a very fertile field of research during the past decade or so. It includes excellent chapters on the microstructure, properties and functions of the photoreceptors, on the pigment epithelium, on the development, formation and properties of the lens, the vitreous body, the ciliary bodies and on the cornea. It is a book which is full of recently acquired information on all these topics and is thus of the greatest importance to all those who study the physiology of the eye and its visual functions, whatever their particular interest may be. Moreover, for cytologists in general, the problems of cellular structure and function which are discussed in this book must have an appeal far beyond the visual orbit. Indeed the structural basis for much general physiology is analysed within the pages of this book. The symposium was clearly very well organized and the fruit of it is well blended into a very digestible compôte. The book is very well produced and illustrated; it forms a valuable contribution to the literature on vision. E. N. WILLMER

LIGHT AND LIFE

A Symposium on Light and Life

Edited by William D. McElroy and Bentley Glass. (Sponsored by the McCollum-Pratt Institute of the Johns Hopkins University with support from the National Science Foundation.) Pp. xii+924. (Baltimore, Md.: The Johns Hopkins Press; London: Oxford University Press, 1961.) 120s. net.

THIS volume is the most recent addition to a series of symposia which have received acclaim for the authoritative character of the contributions and the breadth of the treatment which successive topics have received. The debt which biochemists and biologists owe them is evinced by the frequency with which they are quoted in the research literature, for they have provided both an accurate and comprehensive historical record of major fields of active research and a stimulus to further work and thought. A reviewer is compelled to appraise this recent addition to the series by the standards which its predecessors have set.

Against such a record, this volume appears somewhat disappointing. Difficulty is encountered at the outset in that the subject appears to have no natural unity. Three groups of biological phenomena are discussed: the emission of light in bioluminescence, the utilization of light in photosynthesis, and the photosensitization of certain processes, including phototropism, photoperiodism and vision. Some correlation between these diverse activities might be expected in the physicochemical character of their primary reactions and the molecular changes associated with light absorption or emission, but at this stage the gap between our knowledge of the biological and chemical features is so wide that the two groups become almost autonomous, and individual papers even within sections can appear little related to any other. Our ignorance is too great; our knowledge cannot provide the cement to hold them together, and imagination has yet to come to our aid.

The arrangement of the contributions is rational enough. The response of molecules to photo-excitation is discussed first, with treatment of the excited states of simple molecules, the nature and importance of the biologically interesting $n \rightarrow \pi^*$ transition, and the triplet state. The general conclusions are then applied to the specific properties of biologically important molecules, including proteins, and pyridine and flavin nucleotide enzymes. This section, however, deals with the application of spectral data to structural and kinetic problems, rather than the biochemical consequences of the excitation of these molecules.

Chemical and biological aspects are wedded most effectively in the section on bioluminescence. Here, the chemistry of luminescence in the porphyrin-catalysed decomposition of peroxides and of the luminol reaction is described and discussed before proceeding to the various bioluminescence phenomena, ranging from the firefly and *Cypridina* to fungi, the dinoflagellates and the sea anemone. The comprehensive paper on firefly luminescence by McElroy and Seliger is particularly valuable.

Extended discussions on photosynthesis have now become so frequent that contributors must experience difficulty in sensing positive achievement year by year; the same arguments can be rehearsed too often before the same public. Certainly, much that appears here has already been published. Three long articles—on the spectral character of chloroplast pigments, the immediate consequences of chlorophyll excitation, and the biochemistry of generation of adenosine triphosphate in chloroplasts and bacterial chromatophores—dominate this section, which gains interest from the papers discussing electron spin resonance studies, and the likelihood and consequences of more than one primary photochemical reaction sensitized by different pigments. Some of the other contributions are rather slender and appear to deal with reactions too remote from the primary action of light to justify inclusion in a symposium under this title.

The final section deals with photosensitized phenomena in biology, in which both the initial activation and the amplification necessary to achieve the response, have to be considered. Phototropic curvatures and photoperiodic responses have still not progressed far beyond the descriptive stage, and critical experiments which contribute to an appreciation of their mechanisms have yet to be carried out. The contributions by Rushton and by Wald on the molecular character of the excitation process in vision and its consequences set a pattern for the treatment; two interesting but inconclusive papers on colour vision in insects and goldfish conclude the symposium.

The discussions after lectures, with their mixture of information, wit, boorishness and irrelevancy, have been included, though it is regrettable that the comments on some papers have been recorded before their delivery (or publication). The whole symposium is summarized *in extenso* by Bentley Glass, usually accurately and informatively, so that those not minded to read each paper can obtain the gist with less effort.

Even though the organization and content of the papers in this volume may be subject to some criticism, any who read this volume thoroughly cannot fail to be impressed by the range and variety of the information and to be stimulated to integrate it each after his own taste. V. S. BUTT

SELECTED STUDIES IN CHEMICAL KINETICS

Selected Studies in Chemical Kinetics
By Prof Farrington Daniels (Thirty-fifth Annual Priestley Lectures) Pp vi+123 (University Park, Penn. Phi Lamda Upsilon and the Department of Chemistry, Pennsylvania State University, 1961) 2 75 dollars

WHEN considering a new plant for the chemical process industries the three important questions that must be answered are how big, how much and when In approaching the problem of how big, knowledge of the rates of reaction or kinetics and the conditions of equilibrium are two vital tools of design Prof Daniels, in the 35th Priestley Lectures, sets out to give us an indication of the progress made and the general techniques available in handling the problem of kinetics

The general arrangement of these lectures is to give initially an outline of the principles of kinetics and the relation of this subject to other sciences This is followed up with a number of illustrations of work in this field primarily conducted by Prof Daniels and his associates It is by these examples that Prof Daniels succeeds in indicating the methods of attack that are possible and at the same time makes it clear how difficult this subject really is Kinetics, as understood here, deals primarily with the time and mechanism of a chemical reaction It includes such subjects as catalysis, photochemistry and spectroscopy It is important to realize that when two or more compounds react they give rise to several different products but the one with the largest yield is that produced by the fastest reaction It is becoming both a science and an art to adjust the temperature, concentration, catalyst and solvent so as to favour the desired reaction to the maximum extent For industrial purposes all reactions must be made to proceed fast enough to be economically feasible, and this is often a vital problem At the same time, although kinetics is essential in industrial design it also offers an intellectual challenge, as, in the words of Prof Daniels, "We have to make the molecules do our bidding"

The first main illustrations cover the work on the decomposition of nitrogen pentoxide Here it is interesting to read how the mechanism in this reaction has proved a considerable puzzle and the sorting out of this complex reaction has provided many opportunities for developing new techniques in experimental work The second illustration refers to the oxidation of nitric oxide, again a reaction of enormous chemical importance where the slowness of the reaction has presented problems in engineering design This is a particularly interesting reaction as it is not only termolecular, which is relatively rare, but also has a negative temperature coefficient

Other reactions discussed in some detail are the decomposition of nitric oxide and the Wisconsin process for fixation of atmospheric nitrogen The account of the work is interesting both in terms of physical chemistry and chemical engineering, where the application of sudden chilling of the gases by pebble beds is relatively new and remarkably effective

In the final lecture, Prof Daniels refers to the kinetics of reactions in solution and in a solvent He offers some useful information on some of the latest techniques used in studying these reactions, and his chapters on photo-kinetics and photo-chemistry are very interesting reading

This set of lectures is welcome as it provides an extremely valuable account of the importance of kinetics in tackling industrial problems where design of reactors has proved more difficult than the design of much of the equipment used for physical separation The lectures are of value to chemists and to chemical engineers J M COULSON

MATHEMATICAL THEORY OF NUTRITIONAL RELATIONSHIPS OF FISHES

Experimental Ecology of the Feeding of Fishes
By Prof V S Ivlev Translated from the Russian by Douglas Scott Pp viii+302 (New Haven, Conn, and London Yale University Press, 1961.) 7 50 dollars, 60s

THIS book, by a leading Soviet experimental ecologist, constitutes an important contribution to the nutritional ecology of fishes Many factors govern the rate of feeding of a fish, and also its choice of particular foods in selective feeding They include the total density of food, its aggregation, the partial densities of its separate components, the protection afforded to the prey by refuges or by mobility, the amount of contact with other fishes, the formation of habits of grazing on particular foods These factors influence the fish simultaneously, so that the analysis of any one of them under natural conditions is practically impossible In a long series of carefully controlled laboratory experiments, Prof Ivlev has been able to isolate the factors one by one, and to investigate the influence exerted by each acting alone Then, by means of a graded series of recombinations of the factors, he builds up a system of formal equations which represent not only the quantitative changes in the diet of the fish but the qualitative ones as well, very closely

Ivlev's approach is essentially biophysical He has modified the classical approach of Lotka and Volterra to the question of the effects of the concentration of the food on the rate of feeding, by introducing a factor which inhibits unrestricted increase in the rate of feeding at higher food concentrations This leads him to apply the monomolecular or inverse exponential equation This equation satisfies the experimental data very closely

A large part of the book is concerned with the expression of selective feeding Here Ivlev adopts a new type of coefficient of selectivity, which works well in a variety of situations It can also be applied to data suitably collected under natural conditions

Ivlev is the first to have published such an experimental analysis of these problems, I believe that the publication of the work in English will stimulate many new investigations into outstanding problems in this field It may be noted in passing that all the experimental animals used were freshwater fishes, as regards marine animals, the field is wide open

Considering the importance of the book, the standard of translation is regrettably poor The Yale edition contains the most shocking mistranslations, there are misleading blunders on every page, while the text has scarcely been rendered into good English. The author's style is laconic and it is difficult sometimes to find apt ways of rendering it, this is no excuse for writing the first thing that comes to mind Editing plays no small part in producing a work of this nature, manifestly, this translation has not been edited at any stage For the garbled result, the blame rests equally on Mr Scott and the Yale University Press It certainly does no credit to either

In view of these latter considerations it might be thought that the stipulated price is inordinately high L BIRKETT

PROGRESS IN CANCER RESEARCH

Cancer Progress, 1960

Edited by Ronald W Raven Pp xv+242+16 (London Butterworth and Co (Publishers), Ltd, 1960) 60*s*.

ONE of the problems of the organization of research, particularly in the cancer field, is to steer between the Scylla of lack of co-ordination and the Charybdis of too much When the ship sails too near the latter, senior workers find themselves spending almost all their time attending committees and meetings and writing review articles, while junior workers, who are in any event forced to specialize too early, rarely find the time to read review articles outside their own subject Regrettably, it is with increasing frequency that we see the same data presented in more than one paper, and worse still, the same fields of investigation reviewed more than once by the same individual

Against this background the editor, Mr R W Raven, and his advisers, are most certainly to be congratulated both on their choice of well-defined subjects and on their selection of authors The brevity of most of the contributions, and the fact that many of them are presented as sequels to chapters in the parent book, *Cancer*, gives this volume an entirely different form and function from *Advances in Cancer Research* or the *Year Book of Cancer*

As one would expect, the standard of contributions varies considerably Articles on tumour viruses (R J C Harris), bone marrow replacement (H E M Kay and P C Koller), circulating cancer cells (J W Stewart), carcinomatous neuropathies (D S Russell), carcinoid tumours (P J D Snow), industrial causes of cancer of lung, nose and paranasal sinuses (P L Bidstrup), the effects of radiation on the normal lung (T J Deeley) and the effect of oxygen in radiotherapy (I Churchill-Davidson) are among the many interesting and useful contributions

Russian authors (N I Lazarev and L F Larionov) contributed three of the five chapters in the section on chemotherapy In one of these it is cogently, though somewhat naïvely, argued that both the carcinogenic and the anti-tumour effects of hormones rest on their physiological activities The last chapter in this section (D A G Galton) corrects any impression given by the previous four, that chemotherapy almost holds the key to the cure of cancer

In an interesting article on cancer in the South African Bantu, A G Oettlé describes the enormous difficulties in obtaining a clear picture of the incidence of cancer in a primitive society This is a timely warning to those who criticize civilized practice on the grounds that cancer is infrequent among communities living a more 'natural' life With regard to treating a member of such a community, he writes, "His fears and prejudices, his misunderstandings and his phobias have all to be considered in the therapy of the whole man". Such is equally true treatment of less-primitive men

Among the least pleasing features of the book are the fairly small size of the type and the narrow width of the margins it is hoped that this form of economy will be dispensed with in future volumes

F J C ROE

MEDICINE AND INSECT PARASITES

Medical Entomology

By Dr William B Herms and Dr Maurice T James Fifth Edition Pp xi+616 (New York and London The Macmillan Co, New York, 1961) 87*s* 6*d*

THE fourth edition, by the senior author only, was published in 1950 This fifth edition is the work of Prof M T James, who has clearly, with much care and endeavour and with considerable success, produced a book several pages shorter than its predecessor yet combining a good deal of its original format and material with new matter This includes both new text, which brings the book more up to date in many aspects of the subject, and new illustrations, which enhance the pictorial presentation of its topics As noted in the preface, veterinary entomology has been pruned considerably and the sub-title of the fourth edition, implying this aspect, has been dropped Nevertheless, sufficient remains to serve the medical man who might well, in rural communities, have to think broadly, especially of mites and ticks, œstrids and biting flies

In general, trends in control are well revised regarding contact insecticides and repellents Malaria is discussed, with leading references, in terms of control and world eradication—for which the book observes a cautious optimism The mathematical approach to the epidemiology of malaria, as analysed by Macdonald, is indicated usefully and briefly Due elaboration is given to more recent discoveries on arthropod-borne virus diseases transmitted by mosquitoes and by ticks Revision of the housefly and its allies is commendable and, although the section on tsetse is barely altered, the reader is led to the newer important general works on this subject Nomenclature has usually been brought into line with recent authoritative works There are, of course, points of criticism Garnham is spelt Garman, there is no indication of the use of dieldrin for triatomid bugs; the section on loiasis makes no mention of the important ecological studies of this disease in the past decade, nor is the association of *Simulium* larvæ with crabs noted, there could be more about *Mansonia* mosquitoes

Of the several keys, some new, those referring only to American fauna (for example, mosquitoes, *Culicoides*) must have limited interest, but others (for example, on fleas, ticks and mites) will have wider utility It is difficult to see how, in a general work, such selection can be avoided

Many sections are simply reprinted, but much new information is included in the re-arrangement of chapters and within chapters, the new format providing much clearer printing of sectional headings The reader is assisted to main discussions of particular topics by bold-face page references in the index

This is a worthy successor to the four previous editions and should prove an informative and useful general work for the student, and teacher, of medical entomology D S BERTRAM

ADVANCES IN THE GENERATION AND USE OF VERY HIGH, CONTINUOUS MAGNETIC FIELDS

By Dr HENRY H KOLM

National Magnet Laboratory*, Massachusetts Institute of Technology, Cambridge, Massachusetts

A MAGNETIC field exerts four effects which make it useful as a research environment it produces a torque on a magnetic dipole, it exerts a force on a moving charge, a space-varying field exerts a force on a magnetic dipole, and finally a magnetic field varying with time exerts a force on a stationary charge

When a magnetic field is applied to the microscopic dipoles in various solids, such as paramagnetic salts for example, the dipoles do not simply become aligned with the field, for two reasons ordering is opposed by thermal motion which tends to preserve random orientation, in the second place, these magnetic dipoles also possess angular momentum and therefore tend to behave like gyroscopes, instead of aligning themselves with the direction of field, they precess around it at their precession frequency The alignment of dipoles against thermal agitation produces reversible heating, a mechanism which is utilized in achieving the lowest possible temperatures The precession frequency of gyroscopic dipoles is observable as absorption in the microwave or infra-red frequency-range, and provides valuable information concerning electronic band structure, crystalline magnetic fields, etc

The second effect, the force exerted on moving charges, forms the basis of all particle accelerators in which a magnetic field is used to confine the accelerated particles within circular orbits This confining force is loss-less, since a magnetic force always acts perpendicularly to the direction of motion Magnetic fields can therefore also be used to confine very hot plasma, or ionized gases, by preventing the energetic particles from ever giving up their energy to the material walls of a physical container They are also a valuable tool in producing a measurable curvature in the trajectory of high-energy particles. Space-varying fields, or fields with a high gradient, are used to measure magnetic susceptibilities, and also to analyse beams in experiments with atomic beams

Finally, the force produced on a stationary charge by a field varying with time simply represents the law of electromagnetic induction, which forms the basis of electric motors and generators, as well as radio propagation The effect is also used experimentally for accelerating particles in circular accelerators and for heating plasmas in fusion reactors. Thus we see that a magnetic field is required for producing the lowest as well as the highest attainable temperatures Both these applications require magnetic fields of reasonably large volume, while solid-state research requires primarily fields of high intensity but modest volume

Laboratory magnetic fields are conventionally produced by iron electromagnets which consist of an iron frame, one or two legs of which are surrounded by coils The field induced in the iron is approximately 1,000 times as intense as the field which would be produced by the same coils in the absence of iron, and it is made accessible for experimentation by introducing a gap or break in the iron frame This method fails if a long or prolate field volume is required, because an excessively long gap in the iron core eliminates the effect of the iron, the method also fails if the required field intensity is higher than the value at which iron becomes saturated magnetically In both these cases it is then necessary to generate the field without the use of iron, or with only incidental assistance from iron The elimination of iron requires approximately 1,000 times as much current, which makes the generation of very high continuous fields a difficult and costly undertaking Although thermodynamically it should require no expenditure of power to maintain a magnetic field, it is an ironical fact that this is the only process we perform with zero efficiency It is necessary to remove all the power supplied to a magnetic coil in the form of heat Recent progress in the development of superconductors capable of withstanding substantial currents and magnetic fields suggests that we may actually be on the verge of eliminating this thermodynamic incongruity

It is much simpler to produce high fields for very short periods of time by using stored energy and relying on thermal inertia to keep the coils from melting Work with pulsed fields was pioneered at the University of Cambridge in 1927 by Kapitza[1], who achieved 500 kilogauss for periods of milliseconds by using energy stored in batteries and in rotating generators More recently, the technique has been advanced into the megagauss range by the use of capacitor energy-storage and improved coil design[2,3] and by harnessing explosive energy[4,5]

The earliest effort at generating very high continuous fields was undertaken at the Massachusetts Institute of Technology in 1936 by Bitter[6] It was stimulated by the need for more resolution in optical measurements of the Zeeman effect during the pioneering days of spectroscopy, and by the need for achieving lower temperatures by adiabatic demagnetization The magnets developed by Bitter are helices formed by stacking copper disks with a radial cut, interleaved with sheets of insulation Neighbouring copper disks overlap at their radial cuts so as to establish pressure contact when the entire stack is compressed The copper is cooled by direct contact with water, which flows through some six hundred small holes passing through the entire stack in the axial direction. Bitter succeeded in generating 100 kilogauss in a helix of this sort having a 1-in bore and an outside diameter of 16 in, by supplying it with 1 7 MW (10,000 amp at 170 V.) of power and 800 gallons per min. of cooling water Similar installations and magnets have been built since in several laboratories

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* Supported by the Air Force through the Air Force Office of Scientific Research

throughout the world, but for more than twenty years no effort was made to advance beyond the 100-kilogauss level This is due largely to the fact that interest in obtaining higher continuous fields was relatively mild and de-centralized among so many widely separated scientists that no concerted effort of the required magnitude was made

The situation changed quite suddenly during recent years with the advent of several vital experiments which use magnetic fields as a tool for investigating the nature of charge carriers and their energy-band structure in solids. The most prominent among these is the observation of 'cyclotron resonance', a phenomenon based on the circumstance that all particles (or holes) having the same charge-to-mass ratio will orbit around the flux lines of an applied, continuous magnetic field at the same frequency, regardless of their individual energy faster particles will simply describe larger orbits than slower ones This coherent behaviour of statistically distributed but identical particles manifests itself in resonance absorption at a frequency varying with the applied magnetic field and situated in the short microwave region when the magnetic field is in the range 20–30 kilogauss These experiments[7] could only be performed in crystals of great perfection (originally germanium and silicon) in which the mean free path of charge carriers is large compared with the cyclotron orbits, because otherwise scattering by collision occurs so frequently as to mask the resonance In order to observe cyclotron resonance in materials of less perfection, it was clearly necessary to increase the frequency from the shortest usable microwave to at least the longest usable infra-red Unfortunately, this made it necessary to increase the magnetic field from the 30 kilogauss range, readily available in iron electromagnets, to beyond the 100-kilogauss range The first attempt to do so was made by the Lincoln Laboratory group in 1955 (refs 8 and 9), using a pulsed magnet capable of up to 750-kilogauss pulses of 120 μsec duration, in a hole 3/16 in. in diameter It was energized by an underdamped (that is, oscillatory) capacitor discharge so as to minimize resistance losses and permit the use of coils made of alloys having greater strength but also higher resistance than copper Problems involved in the rapid detection of changes in infra-red transmission or reflexion presented some difficulties and limited the accuracy of measurements, but valuable exploratory results were obtained for InSb, InAs and Bi Quite incidentally, certain oscillatory effects were also observed which were eventually resolved and studied in continuous fields.

This early experience emphasized both the importance of working in higher fields and the inadequacy of pulsed fields, particularly in the farther infra-red region, where the decline in both source intensity and detector sensitivity requires long measurements It appeared imperative for a number of other good reasons as well that a new attempt be made to advance the art of generating very high continuous fields beyond the point at which it had remained for such a long time

It was in this spirit that, with several other members of the Lincoln Laboratory Solid State Division at the Massachusetts Institute of Technology, notably Benjamin Lax, Donald Stevenson and D Bruce Montgomery, I joined forces with Francis Bitter for a renewed attack on the old problem Three efforts were launched at about the same time first, the problem of designing high-performance solenoids was re-examined in the light of all available information; secondly, a proposal was made and presented to a number of Government agencies and leading scientists suggesting that a new laboratory be built for the purpose of making generally available for research the highest continuous magnetic fields which can be generated at the present state of the art, thirdly, communication was established among scientists concerned with the generation and use of such fields I propose to report briefly the progress made along these three lines during the first three years

The problem of designing solenoid magnets is best characterized in terms of a relation discussed extensively by Bitter, namely

$$H = G\,(W\lambda/r\rho)^{1/2}$$

where H is the field at the centre in gauss, W the power dissipated in watts, λ the 'space factor' or fraction of solenoid volume occupied by conductor (as compared with insulator and cooling channels), ρ is the resistivity at operating temperature, r the inner radius in cm, and G is a geometry factor (the 'Fabry factor') which characterizes the current distribution; this factor is 0·179 for a uniformly wound solenoid of optimum dimensions and about 0·209 for a helix such as the Bitter magnets, in which the current distribution is highest near the centre and falls off inversely with the radius When generating fields up to about 200 kilogauss, it is possible to maximize G by selecting the best realizable current distribution but beyond this point it is necessary to depart from the optimum distribution in order to prevent mechanical failure of the windings near the centre The space factor and resistivity must be considered together, since they are both governed by cooling, the former by the amount of cooling area required and the latter by operating temperature. Operation at cryogenic temperatures might appear attractive at first sight, but unfortunately as the temperature is lowered the required cooling area increases much more rapidly than the resistivity decreases, so that magnets become progressively inefficient Only if fields of very large volume are required or if operation is to be brief and infrequent do the economic factors favour cryogenic cooling Water offers considerable advantages even under conservative transfer-rates, say, 200 W/cm^2, if local boiling in sub-cooled water is permitted, it has been found possible to transfer as much as 5,000 W/cm^2, a rate which exceeds the rate of heat transfer at the surface of the Sun After verifying experimentally that transfer-rates of this order of magnitude are indeed feasible, and that they can be maintained with stability in very small channels (about a quarter millimetre thick), we undertook our first concrete task, that of building a magnet which would produce a higher field than the old Bitter magnets with the same amount of power It was intended not only as a research tool for immediate use but also primarily as a means for exploring new solenoid structures and new levels of performance

Since the helical structure used in the Bitter magnets does not lend itself to reduction of size without loss of heat transfer area, we selected a tape-wound spiral structure with funnelled ends for optimized current distribution Successive windings are separated by ribs which are aligned along radii like spokes of a wheel so as to transmit the radial outward forces to a strong confining envelope Cooling is achieved by axial flow through the channels between these

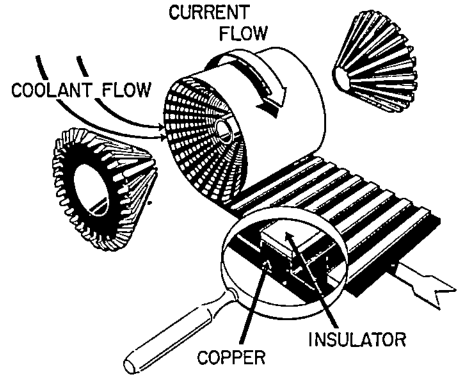

Fig. 1. Principle of the Kolm solenoid

ribs, and the ribs themselves consist largely of conductor with a minimum of insulation so as to reduce the loss of conductor volume and reduce the heat generation in uncooled portions of the tape. This structure provides such an enormous heat-transfer area that a helix of ½-in. calibre, 3-in. outside diameter and 2-in. average length is capable in principle of dissipating the entire available power of 1·7 MW. In practice, it proved impossible to maintain adequate flow, and the first two solenoids failed at somewhat less than half the power. On the basis of this experience, it was then decided to build a solenoid of twice the size (Fig. 1). Since impedance match to the same generators must be maintained, tape thickness remains invariant under the scaling. This increases the number of necessary slots and ribs to such an extent as to make manual fabrication impractical. The third solenoid was therefore constructed by the High Voltage Engineering Corporation on a semi-automatic jig built especially for the purpose. After two unsuccessful attempts and several modifications in materials, technique and design, a solenoid was completed and installed in its housing (Fig. 2). It generated 126 kilogauss at a power consumption of 1·88 MW. The test occurred in mid-summer, when operation is limited by the high temperature of the primary cooling water from the Charles River; in winter it will probably be possible to increase the power sufficiently to generate more than 130 kilogauss.

The second phase of the programme, construction of a new laboratory, has also been making satisfactory progress. The original proposal was favourably received, and by the time funds were provided through the Air Force Office of Scientific Research, initial studies had been completed and the major decisions had been made. It will comprise essentially a power plant of four motor-generator sets capable of supplying 8 MW of d.c. power continuously, 12 MW for a 15-min period, and up to 32 MW for a 2-sec period by the use of energy stored in flywheels. There will be ten cells or laboratories in which this power can be used, with space for expansion, and about 10^5 sq. ft. of area for supporting shops and laboratories. The generators will be separately controllable, so that four magnets can be operated simultaneously at 2 MW. each. The laboratory is expected to do for magnetic field research what laboratories like Brookhaven, for example, have done for nuclear physics. It will provide continuous fields up to about 250 kilogauss for visiting and resident scientists working in a number of different fields and also serve as a centre for advancing the art of magnet design. Construction of the laboratory is under the supervision of Prof. F. Bitter, and Dr. B. Lax will be its director. A broadly representative committee of prominent scientists is to guide all major policy decisions.

The third effort was first aimed at collecting and filing the sort of information on magnet design and performance data which are rarely if ever considered worth publishing. It began with a co-operative undertaking by a group of interested persons known as the "Magnet Society", and continues in the form of a punched card information file kept up by the staff of the new magnet laboratory. It is hoped that such information will ultimately be circulated in the form of periodical technical reports on magnet design and related subjects.

While working on the plans for the new laboratory, our group felt an increasing urge to discuss the project with a number of other scientists who might have occasion to use it. Since a new division of science, that of high magnetic-field research, was about to be born, it seemed logical to start things off with an international conference on the subject. The Air Force again generously agreed to provide sponsorship, and the date was set for November 1–4, 1961. Originally it was felt that this might coincide with the opening of the new laboratory, but as things turned out it will more nearly commemorate the ground-breaking. Papers to be presented at the conference will deal with the generation of high magnetic fields, as well as their use in plasma (ionized gas) physics, nuclear physics, solid-state physics and biology. Biological effects of magnetic fields have only recently attracted much attention, and the conference will provide the first opportunity for workers in this field to meet.

One cannot help but recall a similar conference in the field of low-temperature physics held in the Massachusetts Institute in 1949. It was originally intended to celebrate a great contribution to the field, namely, the invention of Prof. Collins's helium liquefier, but has since become a biennial event of great value. We

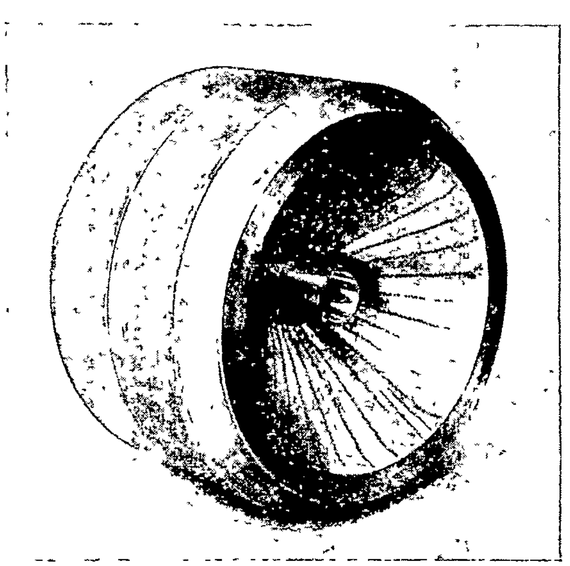

Fig. 2. Core of the new solenoid which generates 126 kilogauss at a power consumption of 1·88 MW. Calibre of the barrel is 1 in., outside diameter of the tape winding is 6 in. 300 gallons per min. of cooling water are pumped through the small channels which are aligned like the spokes of a wheel

sincerely hope that the high-magnetic field conference is destined to comparable success Like most divisions of science based on experimental difficulty, both cryogenics and magnetogenics are probably doomed to ultimate absorption into the more legitimate disciplines As in the case of cryogenics, however, high-magnetic field research will first experience a golden age of contributing a great many stimulating problems

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TRAINING OF INDUSTRIAL FOOD SCIENTISTS AND TECHNOLOGISTS

THE "Training of Industrial Food Scientists and Technologists in the United Kingdom" was discussed on September 5 by Section B (Chemistry) of the British Association for the Advancement of Science, meeting at Norwich

Britain's war-time food problems drew sharp attention to the practical importance of the application of scientific knowledge to the processing and distribution of food. Likewise difficulties in obtaining technologists skilled in this field of work became apparent, and to meet this need the then Ministry of Food set up a working party in 1948 to study means of training A direct result of these endeavours was the establishment of the National College of Food Technology in London and the introduction of courses in food science at the Royal College of Science and Technology in Glasgow Although these centres have flourished, it has recently become clear that their joint efforts are insufficient to meet the rising demand This discussion at the British Association was therefore particularly appropriate in timing and content

Sir Herbert Broadley (U N International Children's Emergency Fund) dealt with international aspects of the problem in his opening paper His theme, which was to be emphasized by subsequent speakers, was the opportunity open to the United Kingdom to assist the under-developed countries in a realistic way by placing our technological and scientific resources at their disposal In the field of food science and technology, great needs exist, and while much has already been done, the shortage of suitable experts remains as a limiting factor which cannot be resolved on a short-term basis Not only are experts needed in the field to tackle the most urgent problems, but training facilities are also required in the United Kingdom for overseas students Only very limited numbers of overseas nationals have been able to obtain such training partly because of lack of places and partly because of the limited research being undertaken in Britain on food problems of special importance to the under-developed countries One of the major malnutrition problems is associated with shortages of protein foodstuffs, and increasingly the international agencies are forced to seek help in this field from the United States There is therefore a great opportunity for the universities and especially for the new universities of Britain to make a contribution in this field

Dr J S Mounfield (principal of the National College of Food Technology) analysed the difference in function of the food scientist and the food technologist and emphasized the importance of integration with industry in the training of the technologist While it is true that the food technologist requires a rather less intensive knowledge than the food scientist of any single basic discipline such as chemistry, physics or biology, he must nevertheless be given some training in engineering and management to help him to solve the problems of large-scale processing

Dr Mounfield quoted a survey carried out about two years ago which suggested that the United Kingdom's needs for trained food scientists and technologists were between 150 and 250 per annum Compared with these numbers, the present output is very small In these circumstances, it is difficult to see how we can make more than a token contribution to the training of students from under-developed countries Furthermore, it is clear from applications received at the National College that inadequate basic scientific training in some countries denies students the opportunity to profit from facilities which can be offered here He proposed that special qualifying courses be organized in the United Kingdom to enable such students to reach the required entrance standards The admissions problem was complex, and to increase the effectiveness of existing facilities for study he recommended the formation of a committee to foster co-operative effort between the research associations, the food industry and the education authorities

Prof J Hawthorn (professor of food science, Royal College of Science and Technology, Glasgow) began by considering the educational principles governing the training of food scientists as opposed to food technologists and emphasized the importance of the basic disciplines of physics, chemistry, biochemistry and microbiology in the development of the student's thought He reported the first stage of a survey being carried out by his own department on opportunities for graduates in food science, and estimated the apparent annual demand of the United Kingdom at between 150 and 250 per annum, thus agreeing with Dr Mounfield's estimate, which had been obtained independently This apparent demand, however, is almost certainly less than the real need and even the apparent demand is far in excess of the numbers of graduates actually produced He then pointed out that the Universities of Nottingham and Reading have now followed the example of Glasgow and would introduce degree studies in this subject as from 1961 The University of Leeds would follow in 1962

Dr J W Corran (chief chemist of J and J Colman, Ltd) then opened the discussion by making a formal proposal to the meeting that it should recommend to the Council of the British Association that an *ad hoc* committee be formed to consider existing schemes for training food scientists and technologists, to suggest means of extending the available facilities, to consider whether the food research associations could contribute to such training schemes and to bring any recommendations to the notice of the appropriate overseas departments of the Government. He also suggested that the membership of such a committee should include both scientists associated with food science and also persons with experience of conditions overseas and in under-developed countries. After discussion, the proposal was adopted.

Perhaps the main impression left in the minds of those attending was the growing sense of urgency in this field of work, and the feeling that the United Kingdom could and should do more to assist the under-developed countries in their drive to raise the nutritional standards of their peoples. While it was comparatively easy to discuss training needs and facilities, real progress would depend on the work of the professional teachers who would be concerned with those essential but tiresome details of entrance qualifications, standards and course structures. The root of the difficulties lay not merely in the availability of money to be spent in developing such facilities but also in the differences in educational standards between the under-developed countries and the United Kingdom.

J. HAWTHORN

INTERACTION OF WEST FORD NEEDLES WITH EARTH'S MAGNETOSPHERE

By PROF. S. F. SINGER
Department of Physics, University of Maryland

W. E. MORROW[1], of the Lincoln Laboratory of the Massachusetts Institute of Technology, has suggested the use of a new method for intercontinental communication. It is based on the use of belts of orbiting dipoles which act as a scattering medium for microwaves. This technique could have significant advantages over other satellite communication techniques. However, it has been pointed out by others, particularly observational astronomers, that this operational project labelled West Ford would have some undesirable side-effects on optical astronomy[2] and on radio astronomy[3]. This article is not concerned with these effects nor with communications and related topics[4]. Instead I shall examine in some detail certain aspects of the interaction of the West Ford medium with the exosphere, in particular the interaction of the electric charge, which the needles are certain to carry, with the ionized portion of the exosphere. I wish to point out the possibility of making unique observations which can help establish the important parameters of this interaction. Therefore, so far as possible, this article will give numerical predictions of orbit changes. In addition, a useful modification of the experiment is suggested.

Forces acting on Small Particles in Near Space

The unique properties of the orbiting dipoles arise from the fact that they are so small. They have, in fact, close to the largest area-to-mass ratio (50 cm^2/gm.) of any artificial satellite, and approach an area-to-mass ratio of small ($\sim 100\mu$) interplanetary dust particles. Hence we can apply to the West Ford medium an analysis used for interplanetary dust in the vicinity of Earth[5]. [See also p. 321 of this issue.]

The motion of a particle is determined by the forces which act on it. Within the region of Earth's exosphere (taken to extend out to about 10 Earth radii) the gravitational force of Earth predominates and will be orders of magnitude greater than other forces, even for small particles. However, there are three other forces which need to be taken into account, particularly for orbiting particles: (1) radiation pressure; (2) the Lorentz force produced by Earth's magnetic field; (3) Coulomb force produced by the interaction with the ionized exospheric gas.

(1) The radiation pressure force is chiefly due to solar radiation and, to a smaller extent, to the radiation scattered from and emitted by Earth. The dynamic effects of the radiation pressure force have been investigated by Jones and Shapiro[6]. The effects predicted for the West Ford medium are given in a paper by Morrow and MacLellan[4] and would indicate that the medium disperses at a certain rate; also that the change in eccentricity produced by a resonant phenomenon would be particularly strong for polar orbits at altitudes of 3,700 km.

(2) The Lorentz force acts perpendicular to the velocity vector of the particle. It will be most pronounced in the polar regions where the magnetic field is strong and always perpendicular to the velocity vector of the particle. Only one general statement may be made about the effects of the Lorentz force: it cannot change the total energy of the particle. In other words, the semi-major axis of the orbit will remain constant, but the other parameters of the orbit may vary.

(3) The Coulomb (electrostatic) drag force has been shown to be one of the most important forces for particles moving in interplanetary space[7]. This electrostatic drag force acts generally between any charged body moving through a plasma and has therefore been also considered for the case of Earth satellites by various authors who, however, arrive at varying conclusions[8–13]. My view agrees most closely with that of Lehnert[8], who first showed that the Coulomb drag would not be particularly effective for Earth satellites of nominal dimensions.

The importance of the Coulomb force then hinges on two points: (a) the small size of the body; (b) the question of its charge. In general we may say that if the body were very much smaller than the Debye screening distance, then the question of the Coulomb drag should be investigated in detail. The dynamics of the charging situation was investigated by me, and I also discussed the competition between photoelectric emission and accretion of charge by ambipolar diffusion from the surrounding plasma. It was concluded that in interplanetary space dust particles would most likely be positively charged[7]. Whipple[14] has pointed out explicitly that the charge becomes less positive as one approaches Earth, and becomes negative in the ionosphere

Since the Coulomb drag produces a dissipation effect akin to friction, it will decrease the semi-major axis of the orbit continuously and eventually cause the particle to enter the dense atmosphere of Earth I am therefore particularly interested in investigating the effect of Coulomb drag on the particle's life-time

Analysis of the Coulomb Drag Force

For the purposes of discussion I shall adopt a treatment introduced by Chandrasekhar and Spitzer in which the orbiting particle will be referred to as the 'test particle' and the ions and electrons of the exosphere as the 'field particles' The drag deceleration is given by[15]

$$a_C = -A_D \left(\frac{m_1}{2kT_1}\right)\left(1 + \frac{m}{m_1}\right) G(x) \qquad (1)$$

where the subscript 1 always refers to the field particles,

$$x = (1\ 5)^{1\ 5}\, w/C_1$$

where $C_1 = (3kT_1/m_1)^{1/2}$, and is the root mean square velocity, and w is the test particle velocity ($\sim$ 5 km / sec)

$$G(x) = \frac{\varphi(x) - x\,\varphi'(x)}{2x^2}$$

$$\varphi(x) = 2\pi^{-1/2}\int_0^x \exp(-y^2)\,dy$$

$$A_D = 8\pi e^4 n_1 Z_1^2 Z^2 (\ln\Lambda)/m^2$$

$$\Lambda = 3(2Z\,Z_1\,e^3)^{-1}(k^3T_1{}^3/\pi\,n_1)^{1/2}$$

where n and Z denote particle concentration and charge, respectively, for the exosphere $Z_1 = 1$

With an exospheric temperature T_1 of 1,500° K, we obtain. $C_1 = 6\ 1$ km./sec for protons, $C_1 = 260$ km /sec for electrons Under these conditions $\ln\Lambda \sim 20$, $G(x) \sim 0\ 2$ for the field protons and less than 0·015 for field electrons Since the particle mass $m \gg m_1$, equation (1) reduces to

$$a_C = 1\ 23 \times 10^{-23}\, Z^2\, n_1/m \qquad (2)$$

In the derivation of equation (2) some approximations have been made, for example, the particle's velocity w was assumed constant and appropriate to a circular orbit at an altitude of 9,500 km No greater refinement appears necessary in view of the gross approximations which must be made in the calculation of the charge Z

Calculation of Electric Charge

The electric charge of the particle is given by its electric capacity multiplied by the potential of its surface For a conducting sphere of radius a cm ·

$$Z = 6\ 95 \times 10^6\, aV \qquad (3)$$

where V is measured in volts For a body of different shape the capacity must be calculated, in the absence of detailed data we will adopt a system of two spheres each with 'effective' radius of $1\ 5 \times 10^{-3}$ cm Thus for the West Ford needles, we take

$$Z \sim 2 \times 10^4\, V \qquad (4)$$

The calculation of the potential V also presents special problems since it depends on the position of the particle, and therefore in turn on the characteristics of the particle's orbit It also depends on the work function W and photoelectric yield γ of the particle material, its shape and its attitude towards the Sun As explained earlier, we calculate V in an analysis involving competition between the photoelectric effect and ambipolar diffusion[7] The ambient density above $\sim 1\ 5\ R_E$ (Earth radii) is given by Helliwell as

$$n_1 \sim 10^4\, r^{-3} \qquad (5)$$

where r is measured in terms of Earth radii

The photo-emission is determined by the ultraviolet spectrum of the Sun, that is, by N_ν (the photon flux per cm ²/sec with energy $h\nu \geqslant eV + W$). I assumed the relation $N_\nu = fV^{-\delta}$, where $f = 10^{19}$ and $\delta = 4$ On the basis of Hinteregger's measurements[16], a better set of values is

$$N_\nu = 2{\cdot}2 \times 10^{18}\, V^{-1.844}\ [\text{cm}^2\text{sec.}]^{-1} \qquad (6)$$

The equilibrium potential is given by ref 10

$$V_+ \sim +\,(\gamma f/n_e C_e)^{1/\delta} \qquad (7)$$

when the particle has a large positive potential and the ion current can be neglected In Earth's shadow, where photo-emission is negligible, the equilibrium potential is given by

$$V = -\,(kT_e/2e)\ln(m_i T_e/m_e T_i) \qquad (8)$$

In view of the uncertainties in γ, W, attitude and orbit orientation, I have decided to make some drastic approximations and represent the potential by·

$$V = 7{\cdot}5\,(r - 2) \qquad (9)$$

As can be seen, V varies from $-7\ 5$ V. in the F-layer to $+60$ V at 10 R_E Note that $V = 0$ at 2 R_E, the implication will be discussed later

It should perhaps be pointed out explicitly that another powerful charging process exists, namely, the high flux of fast electrons in the outer radiation belt, it is, however, quite localized and confined mainly to the vicinity of the lines of force which extend to 3–5 R_E in the plane of the magnetic equator. Its effect is to produce (or enhance) the negative charge (by accretion and secondary electron emission), this effect must be considered for orbits which intersect the outer radiation belt

By combining equations (2), (4), (5) and (9) we obtain for the Coulomb drag deceleration

$$a_C = \sim 3 \times 10^{-9}\,(r-2)^2\, r^{-3}/m$$

With the needle mass given[4] as 10^{-4} gm

$$a_C \sim 3 \times 10^{-5}\,(r-2)^2\, r^{-3}\ [\text{cm./sec}^2] \qquad (10)$$

This acceleration may be compared with the accelerations due to

(i) Radiation pressure

$$a_{RP} \sim \left(\frac{\text{solar constant}}{\text{speed of light}}\right)\left(\frac{50\ \text{cm}^2}{\text{gm.}}\right) =$$

$$2\ 5 \times 10^{-8}\ \text{cm /sec}^2 \qquad (11)$$

(ii) Gravity

$$a_G = 980\, r^{-2} \qquad (12)$$

(iii) Magnetic

With the circular velocity $w \sim 8 \times 10^5\, r^{-1/2}$ cm /sec, and with the magnetic field $B \sim 0\ 5\, r^{-3}$ gauss, we obtain.

$$\begin{aligned} a_M &= Ze\,w\,B/m \\ &= 10^{-5}\,(r-2)\, r^{-3.5}\ \text{cm./sec}^2 \qquad (13) \end{aligned}$$

Effects of Coulomb Drag

The changes in orbit and life-time of a satellite in the presence of a dissipative drag force F_D can be treated most simply by energy considerations[17]

The loss of energy per orbit in a circular orbit is $\Delta E = 2\pi r F_D$ and this will reflect itself in a lowering of the orbit by an amount Δr in a time T which is simply the orbital period

$$T = 2\pi\ (GM_E)^{-1/2}\, r^{3/2} \qquad (14)$$

Since the total (kinetic+potential) energy is given by $E = \frac{1}{2}\ GM_E m/r$, we obtain by differentiation

$$\Delta r = (-\Delta E)\ 2r^2/GMm \qquad (15)$$

We can therefore obtain an expression for the rate of shrinkage of a circular orbit as follows

$$\Delta r/\Delta t = 2\pi r F_D\ \frac{2r^2}{GMm}\,\frac{(GM)^{1/2}}{2\pi r^{3/2}} = \frac{2}{(GM)^{1/2}}\,\frac{F_D}{m}\ r^{3/2} = \frac{1}{\pi}\ T\ \frac{F_D}{m} \qquad (16)$$

which is $(1/\pi)$ times (the orbital period) times (drag deceleration)

We can also express $(\Delta r/\Delta t)$ as a function of r using equation (10) for Coulomb drag and using $T = 5{,}080\ r^{3/2}$ for orbital period

$$(\Delta r/\Delta t)_C = \sim 5 \times 10^{-2}\ (r-2)^2\, r^{-3/2}\ [\text{cm /sec }] \qquad (17)$$

This expression may now be compared with the neutral drag acceleration evaluated from

$$F_N/m = \tfrac{1}{2}\ C_D A \rho\ w^2/m = \tfrac{1}{2}\ C_D\ A(n_H m_H + n_0 m_0)\ w^2/m \qquad (18)$$

where C_D is the drag coefficient, A the projected area and ρ the density measured in gm /cm $^{-3}$.

Using $w^2 = (7\ 9 \times 10^5)^2/r$, $m = 10^{-4}$ gm , $A = 5 \times 10^{-3}$ cm ^{2}, $C_D \sim 2$; we obtain.

$$\begin{aligned} F_N/m &= 5 \times 10^{-3} \times 1\ 66 \times 10^{-24}\ (n_H + 16\ n_0)\ (6\ 25 \times 10^{11})\ 10^4/r \\ &= 5\ 2 \times 10^{-11}\ (n_H + 16\ n_0)/r\ [\text{cm /sec }^2] \qquad (18') \end{aligned}$$

In the case of a particle belt, it is more relevant to calculate $(\Delta r/\Delta t)$ rather than the change in orbital period

$$\begin{aligned} (\Delta r/\Delta t)_N &= 1/\pi\,(5{,}080\, r^{3/2})\, 5{\cdot}2 \times 10^{-11}\,(n_H + 16 n_0)/r \\ &= 8\ 4 \times 10^{-8}\,(n_H + 16\, n_0)\, r^{1/2}\, [\text{cm /sec }] \qquad (19) \end{aligned}$$

It is of considerable interest to establish the relative importance of the two drag forces In order to do this we must adopt a model for the structure of the magnetosphere

Observational information is limited to rather low altitudes The distribution of mass density below 800 km is known from analysis of satellite drag[18] The integrated thickness of the neutral hydrogen cloud around Earth is known from analysis of the profile of solar hydrogen Lyman-α (ref 19) Electron densities up to 1,500 km are known from rocket measurements[20] The electron density to several Earth radii is inferred (with some uncertainty) from whistler observations[21,22]

On the other hand, the *relative* distributions of the neutral constituents can be deduced from a theory of the exosphere[23] The neutral components describe ballistic orbits without collisions above the base of the exosphere (530 km) and the distribution of concentration with altitude is calculable in a fairly straightforward manner The ionized components (having much larger cross-section because of Coulomb interaction) are distributed according to the barometric formula[24] In each case the slope (or scale height) is determined by the temperature at the base of the exosphere Using the data referred to above for normalization, it is possible to construct a model of the exosphere[25] This model is shown in Table 1

Table 1 CONCENTRATION OF MAJOR CONSTITUENTS IN THE TERRESTRIAL EXOSPHERE (CM $^{-3}$)

r/R	O	O+	H	H+
1 100	$1\ 2 \times 10^7$	5×10^5	10×10^3	—
1 200	$2\ 7 \times 10^4$	$2\ 5 \times 10^4$	$6\ 6 \times 10^3$	9×10^3
1 300	$1\ 7 \times 10^2$	$1\ 7 \times 10^3$	$4\ 4 \times 10^3$	$6\ 6 \times 10^3$
1 400	1	$1\ 3 \times 10^2$	$3\ 1 \times 10^3$	5×10^3
1 500	—	11	$2\ 3 \times 10^3$	$3\ 7 \times 10^3$
1 75		< 0 1	$1\ 25 \times 10^3$	$2\ 1 \times 10^3$
2 00			8×10^2	$1\ 3 \times 10^3$
3 00			2×10^2	$3\ 5 \times 10^2$
4 00			82	$1\ 5 \times 10^2$
5 00			43	85
6 00			25	51
7 00			16	33
8 00			12	23
9 00			9 1	17
10			6 5	11

Both neutral and Coulomb drag decelerations are shown in Table 2, as well as the resulting values of $(\Delta r/\Delta t)$ It must be pointed out, however, that the Coulomb drag depends on many detailed factors (some of which were discussed earlier), involving the particular properties of the particle, of the exosphere, and of the Sun, as well as the particulars of the orbit

The zero value of Coulomb drag at $2R_E$ should not be taken literally, it merely indicates that somewhere in this altitude-range there may be a minimum As discussed elsewhere, small fluctuations of charge will produce a drag (since it depends on the square of Z and not on the sign), in particular, the day–night effect sets a lower limit to the Coulomb drag[5] This minimum may give rise to a high concentration of interplanetary smoke (particles $\gtrsim 1\mu$) near 2 Earth radii if they exist

Discussion

It would be of interest to calculate the life-times of the West Ford particles Since the details of the orbit have not been revealed, and, in particular. since the altitude and orbit inclination are not known, it does not seem worth while to attempt any refined calculations There are in any event uncertainties about the electric charge of the particle, about its attitude with respect to magnetic field and with respect to the Sun, and also about the actual values of air density

With initial orbit details available, the subsequent history of the West Ford particles can only be obtained by numerical methods It is clear, by referring to Table 2, where the neutral drag may be neglected It is clear also, by referring to equations (10), (11), and (13), that probably none of the other forces can be neglected, except for very special orbits (It is interesting to note the importance of the radiation pressure in relation to the Coulomb drag This is due to the geometric shape of the needles It should be noted that for a spherical interplanetary dust particle of the same mass, the radiation pressure acceleration is $\sim 5 \times 10^{-5}\ \pi a^2/m$ and is, relatively,

Table 2 DRAG DECELERATION AND ORBIT SHRINKAGE

r/R	a_N (cm /sec ^{2})	$(\Delta r/\Delta t)_N$ (cm /sec)	a_c (cm /sec ^{2})	$(\Delta r/\Delta t)_C$ (cm /sec)
1 1	9×10^{-3}	$1\ 7 \times 10$	$1\ 8 \times 10^{-5}$	$3\ 5 \times 10^{-2}$
1 2	$3\ 6 \times 10^{-5}$	$7\ 6 \times 10^{-2}$	$1\ 1 \times 10^{-5}$	$2\ 4 \times 10^{-2}$
1 3	$1\ 5 \times 10^{-6}$	$3\ 6 \times 10^{-3}$	$6\ 9 \times 10^{-6}$	$1\ 7 \times 10^{-2}$
1 4	$3\ 7 \times 10^{-7}$	1×10^{-3}	$4\ 0 \times 10^{-6}$	$1\ 1 \times 10^{-2}$
1 5	$2\ 1 \times 10^{-7}$	$6\ 1 \times 10^{-4}$	$2\ 2 \times 10^{-6}$	$6\ 8 \times 10^{-3}$
1 75	$9\ 8 \times 10^{-8}$	$3\ 7 \times 10^{-4}$	$3\ 6 \times 10^{-7}$	$1\ 4 \times 10^{-3}$
2 0	$5\ 5 \times 10^{-8}$	$2\ 5 \times 10^{-4}$	(0)	(0)
3 0	$9\ 5 \times 10^{-9}$	$7\ 9 \times 10^{-5}$	$1\ 1 \times 10^{-6}$	$9\ 6 \times 10^{-3}$
4 0	3×10^{-9}	$3\ 9 \times 10^{-5}$	$1\ 9 \times 10^{-6}$	$2\ 5 \times 10^{-2}$
5 0	$1\ 4 \times 10^{-9}$	$2\ 4 \times 10^{-5}$	$2\ 1 \times 10^{-6}$	$4\ 0 \times 10^{-2}$

100–1,000 times smaller when compared with the Coulomb drag[5,7].)

In a numerical computation, particular attention must be paid to the day–night effect, not only on radiation pressure, but also on the particle charge. In fact, because of fluctuations in radiation belt intensity, solar flares, and similar factors, the charge will fluctuate unpredictably. This effect will contribute to a spreading of the belt which is, in addition to the dispersion, calculated by the Lincoln Laboratory[4].

Nevertheless, the shrinkage of an initially circular orbit produced by loss of energy due to drag is a gross effect which should be observable, and which gives some information on air density as well as about the importance of Coulomb drag.

A Proposed Experiment

However, a much more refined experiment is possible which illuminates the interaction of small particles with the magnetosphere. It is based on a separation of the perturbing forces.

Consider, for example, an initially circular orbit at ~ 3,200 km altitude ($r = 1.5\ R_E$), nearly polar and perpendicular to the Earth–Sun line. In this special orbit the radiation pressure does not produce any appreciable perturbation (at least initially), and the Lorentz force perturbation can be determined quite simply, although it is again negligibly small. Since the velocity vector of the particle is more or less in the plane of the magnetic meridian, the chief perturbation will be orthogonal and cause a rotation of the orbital plane. The sense of the rotation establishes the sign of the electric charge on the particle. Using the analysis leading up to equation (13) I estimate the rate of rotation of the orbital plane as about 10^{-11} rad./sec., and rather unobservable.

Next consider a modification of the photoelectric properties of the copper needles. Specifically, one-third could be left with their present copper surface, one-third would be coated with a material of high work function W and low photoelectric yield γ (for example, platinum), and the remainder with a material of opposite characteristics (possibly a cæsium alloy). For our particular orbit, the copper particles would be negative, the platinum particles even more negative, while the cæsium particles would have a nearly zero potential, or perhaps be even positive.

Because of Coulomb drag the three belts will shrink at different rates. Using the analysis leading up to equation (17) I estimate a differential rate of shrinkage of orbit radius amounting to nearly 5 m. a day.

Thus the separation of this West Ford belt into three layers might be resolvable with available radar methods, provided the dispersion of the particles at injection can be held very small.

I am indebted to A. E. Lilley, W. E. Morrow, E. J. Öpik, I. I. Shapiro, and E. H. Walker for helpful discussions. The investigation was supported by the Air Force Office of Aero Space Research under grant *AFOSR*-61-57.

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NEWS and VIEWS

Royal Aircraft Establishment: Mr. R. J. Atkinson

MR. RONALD JOHN ATKINSON has been promoted to deputy chief scientific officer and has taken up the post of head of the Structures Department at the Royal Aircraft Establishment, Farnborough, in succession to Dr. Percy B. Walker, who has been appointed special consultant to the director of the Establishment. Mr. Atkinson, an Australian, came to Great Britain in 1936 with a Rhodes Scholarship from the University of Queensland. After studying at Oxford under Sir Richard Southwell, he went to the Imperial College of Science and Technology for a year, and later was with Fairey Aviation, Ltd., at Stockport. He joined the Royal Aircraft Establishment in May 1940, and was appointed head of the Fatigue Division of the Structures Department in 1955. He is a bachelor of engineering of the University of Queensland, holds the diploma of the Imperial College and is a Fellow of the Royal Aeronautical Society.

Research and Development in the Gas Industry

DR. J. A. GRAY, group leader of physical chemistry and chemical engineering at the Battelle Memorial Institute, Geneva, since 1958, has been appointed head of the new Basic Research Group which the Gas Council is establishing at its London Research Station at Fulham. Dr. Gray is a graduate of King's College, University of London, and prior to joining the staff of the Battelle Memorial Institute, was employed in the Alkali Division of Imperial Chemical Industries, Ltd. In forming the Basic Research Group, the Gas Council's intention is to provide the industry with the means of developing entirely new ideas for the production and utilization of gas without being bound by short-term considerations, or the need to produce results to a definite programme. Although, for administration purposes, it will form part of the Council's London Research Station, the Basic Research Group will operate independently, and the head of the Group will be a member of the

Council's Research Committee, and will exercise full control over the Group's scientific work.

Air Force Cambridge Research Laboratories

MAJOR changes were recently announced for the Air Force Cambridge Research Laboratories, Bedford, Massachusetts, by the commander, Brigadier-General B G Holzman The changes were aimed at strengthening the research programme and the management structure of the Laboratories, and preventing the tendency for overlap of work within the Directorates The separate staffs of the two Directorates of the Air Force Cambridge Research Laboratories, the Electronics Research Directorate and the Geophysics Research Directorate, plus a small headquarters staff, were combined to form a single headquarters staff that now serves both Directorates However, the various laboratories coming within these Directorates are not affected by the reorganization Heads of the four major staff elements are· Dr A M Gerlach, deputy for plans and programmes; Lt -Colonel Eugen Duff, deputy for administrative management, Mr V S. Dempsey, deputy for logistics; and Mr L E Woods, research information officer In addition, a new division was created to provide technical services to the Laboratories' scientists under the direction of Mr V. S Dempsey Dr L M Hollingsworth continues as director of electronics research Colonel Ernest A Pinson continues in the dual capacity of the Laboratories' deputy commander and director of geophysics research, positions he has held for the past year However, Colonel Edward M Douthett, at present assigned to Headquarters, U S Armed Forces, will assume duty as director of geophysics research in mid-November.

Revision of School Science Curricula

IN reply to a question from Viscount Samuel in the House of Lords on October 18 as to what action was being taken to implement the recommendation of the Advisory Council on Scientific Policy regarding the revision of school science curricula, the Minister for Science, Lord Hailsham, said that this has been carefully considered by himself and the Minister of Education In England and Wales the initiative for framing curricula and syllabuses rests with the teaching profession and, after a very thorough review, the Association of Women Science Teachers and the Science Masters' Association have recently proposed substantial revisions and modernization of existing syllabuses The Minister of Education is encouraging these and similar efforts to revise the syllabuses, and the proposals of the Science Masters' Association are being used by the General Certificate of Education examining bodies as the basis for the revision of their examination syllabuses which they are undertaking at the request of the Secondary School Examinations Council This Council advises the Minister on the general direction of the General Certificate of Education examination, and schools, universities, local education authorities and other bodies concerned are represented on it Corresponding action has been taken independently in Scotland through the Scottish Education Department

The CERN Laboratory, Geneva

IT was at the end of 1959 that the 25-GeV proton synchrotron came into operation in the CERN laboratory at Geneva (European Organization for Nuclear Research, CERN Annual Report, 1960 Pp 113 Geneva CERN, 1961) During 1960 the beam intensity was increased ten-fold to 10^{11} protons/sec and the experimental hall was equipped for beam transport In the past quarter 504 operating hours were devoted to nuclear physics experiments These experiments opened up a new energy region in the study of the elementary particles. On the synchro-cyclotron the experimental groups have continued to make distinguished contributions to meson physics Probably the most impressive of these was the measurement of g-2 for the muon to an accuracy of 2 per cent The completion of the new muon channel must mean major progress in muon physics within the next few years To cope with the increasing amount of experimental data, particularly from bubble chambers, an IBM 709 computer was installed during the year

The original programme defined in the convention of 1953 is now virtually complete This is a brilliant achievement but, as the report makes clear, CERN has no intention of resting on its laurels Work is in progress to improve the facilities on both the accelerators and a major contribution to nuclear physics is guaranteed for many years to come Even so, the director-general, in his introduction, refers to longer-term plans for a new accelerator in 1970 All this is an indication that the CERN laboratory is a lively and interesting place, an outstanding example of international collaboration Confirmation of this can be found by reading the report, which gives a vivid and particularly well-illustrated account of the laboratory's achievements and activities in 1960.

Political and Economic Planning

THE annual report of Political and Economic Planning for the year ended March 31, 1961, includes a list of publications, 1960–61, and of PEP staff for the year, but deals essentially with the research programme Continued progress is reported with the study of Britain and the European Common Market and the preparation of a report on Atlantic Tariff and Trade has proved to be a major undertaking This study is assisted with a generous three-year grant from the Ford Foundation. Two new major projects launched during the year were concerned with trade unions in a changing society and with present-day trends in the development of community mental health services, the former being supported by a substantial grant from the Leverhulme Trust Fund, and the latter by a grant from the Nuffield Provincial Hospitals Trust An inquiry is also being launched into the effects on wives and dependants when the chief male earner is imprisoned An examination of the work of professional and learned societies, with special reference to any difficulties imposed by modern industrial conditions, is also being carried out, and provisional help has been promised by the Department of Scientific and Industrial Research

Scientific Research in Australia

THE Research Review of the Commonwealth of Australia Scientific and Industrial Research Organization for the year ending June 30, 1960, is the complement of the shortened form in which the Organization's twelfth annual report was presented, with which it is intended to be read (Pp iv+418 Melbourne Commonwealth Scientific and Industrial Research Organization, 1961) It presents the scientific details of the research programme in much the same way as the earlier annual reports, each of its 48 chapters being devoted broadly to one division or section of the Organization. Lists of professional staff, published scientific papers and financial details are excluded and are given in the annual report.

Research Grants in Science and Medicine in Canada

The report on *University Support*, 1960–61 (Pp. 123. N.R.C. No. 6390. Ottawa: National Research Council and Medical Research Council, 1961. 50 cents), issued by the National Research Council and the Medical Research Council of Canada, records support totalling 8,169,564 dollars from the National Research Council and 2,304,467 dollars from the Medical Research Council. Of the former figure, 7,169,402 dollars represent direct support by way of scholarships in science and engineering (738,467), fellowships in science and engineering (396,758) and grants in aid of research through the Assisted Researches Committee (4,267,195), Prairie Regional Committee (232,458), Atomic Energy Control Board (650,000) or General Research grants (492,000), and 1,000,162 dollars indirect support, including 285,589 dollars for publication costs of research journals and 227,334 dollars for the administration of the programme. The latter includes 189,326 dollars for fellowships, 204,191 dollars for associateships and 1,899,809 dollars for grants in aid of research. The totals represent increases of 1.11 million dollars over 1959–60 and 7 million dollars over 1956–57. Besides listing the membership of the Councils and their officers and committees, the report lists the recipients of fellowships, bursaries and scholarships for the period 1960–61 and the subjects of research grants.

The Wildfowl Trust

The Wildfowl Trust has again prepared an annual report which is a model of presentation (*The Twelfth Annual Report of the Wildfowl Trust, 1959–1960*. By Hugh Boyd and Peter Scott. Pp. 185+24 plates. Slimbridge: The Wildfowl Trust, 1961. 17*s*. 6*d*. net). Practical administrative matters are described clearly and briefly, scientific topics are many and varied, and there are the usual high-quality photographs and line drawings which make the reading of the report an event to be anticipated rather than a duty to be overcome. Unfortunately, the twelfth year of the life of the Trust has seen a drop in the number of visitors to Slimbridge and Peakirk, and it is to be hoped that the decline is only temporary. Probably the most interesting and disturbing scientific article is one by J. M. and J. G. Harrison, who have collected evidence to show that radioactive contamination in birds is beginning to increase. While risks to wildfowl are but a tiny facet of the bigger problem of radioactive contamination, wildfowl may be particularly susceptible, especially those breeding in the far North, where the hazards from radioactive fall-out may be at their greatest. The added risks from the disposal of radioactive industrial waste products may again render wildfowl and seabirds more susceptible than other groups of birds.

London and Guildhall Museums

The Government and the Corporation of London and the London County Council have agreed in principle to establish one Museum dealing with the history of London. This will involve the amalgamation of the present London and Guildhall Museums and the full approval of each of the governing bodies is assured. The new Museum will be situated in the City of London, probably on a site close to the Roman Wall. Although details have not yet been agreed, it is probable that the governing body will consist of representatives appointed by the Prime Minister, the City Corporation and the London County Council. When the details have been agreed by the authorities, an Act of Parliament will be necessary to implement the scheme.

Rocket Propulsion Thirty Years Ago

The science and technology of rockets have advanced from being the hobby of a few apparent cranks to become major consumers of taxpayers' money, even in the universities the type of student who immediately after the War would have sought to accomplish research on the gas turbine, and later would have followed changing fashion and interested himself in nucleonics, now regards rockets as pointing the way to the stars. In spite of the great achievements of the age, however, it is interesting to look back at the pioneer work of another student at the Imperial College of Science and Technology, London. A paper, originally read by Mr. Paul Adorian in 1929, has been reprinted and the proceeds from the sale of these reprints is to be given to the Henry Tizard Memorial Fund. The paper contains little original matter, but it does give a picture of the approach to rocket propulsion more than thirty years ago, and a contemporary point of view of such developments as the Opel rocket cars, designs for jet aircraft curiously modern in style, and a German rocket gun which its open-minded inventor claimed could "shoot London from Berlin, or if you prefer it, shoot Berlin from London". Copies of the reprints are available from Mr. P. Adorian, 21 Denmark Street, London, W.C.2 (price 8*s*. or 1.25 dollars).

The Cambridge Rheology Club

A recent meeting of the Cambridge Rheology Club was held on September 11 in the form of an exhibition of rheological equipment. Mr. C. H. Rose (Cambridge Instrument Co.) demonstrated the dye dilution curve recorder and described its uses in the investigation of certain defects of the vascular system. Grant Instruments showed notably a low-temperature thermostatically controlled bath (Model *RB6P*). Miss M. Nicholas (Spillers, Ltd.) demonstrated the cattle cube hardness tester, which may also be used for strength tests on paper and similar materials. She dealt briefly with the statistical evaluation of the results. Dr. N. A. de Bruyne (Techne, Ltd.) discussed an extensive range of rheological instruments, including the gelation timer, capillary viscometer, various temperature control units and the automatic printing viscometer, still in the experimental stage. The next meeting of the Club will be held on November 27, at 7.45 p.m., when Dr. R. McKennell (Ferranti, Ltd.) will speak on "Rotation Viscometers and their Application to Non-Newtonian Measurements", followed by a demonstration of the Ferranti–Shirley viscometer. Further information can be obtained from Mr. H. G. Muller, Spillers, Ltd., Technological Research Station, Station Road, Cambridge.

Tables and Charts for Use in Gas Dynamic Studies

The Sandia Corporation has recently issued a very useful bibliography (*SCR*–299), by D. E. Randall, listing references which provide tabulated or graphical data regarding the atmosphere, gas flows, flow through shock waves, and information on the thermodynamic and transport properties of gases. Included in the list is a new Sandia monograph (*SCR*–288), by K. L.

Goin, entitled "Mach Tables for Real Gas Equilibrium Flow of Air in Hypervelocity Test Facilities with Total Temperatures to 10,000° K." This report tabulates the aerodynamic flow parameters for isentropic expansions and flow through normal shocks in the pressure range 100–4,000 atmospheres. Both the bibliography and the monograph are available, price 0 75 and 2 0 dollars, respectively, from the Office of Technical Services, Department of Commerce, Washington 25, D C

Gairdner Foundation Awards

GAIRDNER FOUNDATION awards have been made to the following in recognition of their contributions to the study of arthritis and heart diseases Dr Alan C Burton, professor of biophysics, University of Western Ontario, for his work on blood circulation, Sir Russell Brock, cardiac surgeon at Guy's Hospital and Brompton Hospital, London, for his work on heart surgery, Dr Jonas H Kellgren, professor of rheumatology and director of the Rheumatism Research Centre, University of Manchester, for his work on the incidence of rheumatoid arthritis, Dr Alexander B Gutman, director of the Department of Medicine, Mount Sinai Hospital, New York City, for his work on the treatment of gout, Dr. U S von Euler, professor of physiology, Karolinska Institutet, Stockholm, for his work on noradrenaline and its effects on body functions

The Royal Institution of Naval Architects: Awards

THE Council of the Royal Institution of Naval Architects has announced the following awards *Denny Scholarship in Marine Engineering* (1961), to Mr David K Brown (17), of Kelvinside Academy, Glasgow (£350 per annum for four years at the University of Glasgow), *Parsons Scholarship in Marine Engineering* (1961), to Mr Alan B Turner (21), of Messrs Alfred Holt and Co , Ltd , Liverpool (£400 per annum for three years at the University of Durham, King's College, Newcastle upon Tyne)

The Meldola Medal for 1961

THE Meldola Medal is the gift of the Society of Maccabæans and is normally awarded annually The next award will be made early in 1962 to the chemist who, being a British subject and under thirty years of age at December 31, 1961, shows the most promise as indicated by his or her published chemical work brought to the notice of the Council of the Royal Institute of Chemistry before December 31, 1961 No restrictions are placed upon the kind of chemical work or the place in which it is conducted The merits of the work may be brought to the notice of the Council, either by persons who desire to recommend the candidate or by the candidate himself, by letter addressed to the President, Royal Institute of Chemistry, 30 Russell Square, London, W C 1

The Association of Clinical Biochemists

THE following officers were elected at the annual general meeting of the Association of Clinical Biochemists *President*, Dr A L Latner, *Chairman*, Mr H Varley, *Honorary Treasurer*, Dr R Gaddie; *Honorary Secretary*, Dr J H Wilkinson, Westminster Medical School, Horseferry Road, London, S.W 1

Announcements

DR RALPH P HUDSON has been appointed chief of the Heat Division at the National Bureau of Standards, U S Department of Commerce He succeeds Dr Charles M Herzfeld, who was recently appointed associate director of the Bureau

THERE will be open days at the Humber Laboratory at Hull of the Department of Scientific and Industrial Research on November 9 and 10 Exhibits will include some of the work of the parent laboratory, the Torry Research Station at Aberdeen Both laboratories deal with the handling, processing and distribution of fish.

THE Welsh Soils Discussion Group will meet at the Department of Biochemistry and Soil Science, School of Agriculture, Bangor, on November 15. The subject for discussion is "Aspects of the Dynamic Organic Matter Cycle in Soils". Further information is available from Mr J A Taylor, University College, Alexandra Road, Aberystwyth

THE four hundred and tenth meeting of the Biochemical Society, for the presentation of original communications, will be held in the Department of Biochemistry, Manchester College of Science and Technology, on November 11 Further information can be obtained from Dr W J. Whelan, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S W 1.

THE office of the Biochemical Society and the editorial office of *The Biochemical Journal* have now moved to 20 Park Crescent, Regent's Park, London, W 1 All communications connected with *The Biochemical Journal* should be sent to that address

A SPECIALISTS' conference on culture collections will be held in Ottawa, Canada, during August 27–28, 1962 Topics for discussion will include fundamental and technical aspects of the preservation of culture of micro-organisms and other cells Special emphasis will be placed on the maintenance of morphological, physiological and genetical characteristics Attendance at the conference will be by invitation only. Persons interested in attending are invited to write to Dr S M Martin, Division of Applied Biology, National Research Council, Ottawa, Canada, before December 1, 1961

THE first Feldberg Foundation Lecture 1961 will be given by Prof. Herbert Hensel, director of the Physiological Institute of the University of Marburg, who will speak on "The Electro-physiology of Thermoreceptors" on November 2 in the Physiology Theatre, University College, London, Gower Street, W C 1 The Foundation was recently instituted (see *Nature*, 191, 1143, 1961) to encourage German–English scientific exchange for experimental medical research Further information can be obtained from Dr M Godfrey, Medical Research Council, 20 Park Crescent, Regent's Park, London, W 1

THE Czechoslovak Society of Arts and Sciences in America will hold its first national scientific congress in the Hotel Statler, Washington, D C , during April 20–22, 1962 There will be a two-day symposium on the contributions of the Czechoslovak science and arts to world culture The programme will include papers on science, medicine and technology, fine arts, literature and literary criticism, slavistics and linguistics, law and economics, history and political science, philosophy and sociology, and finally a special section will be devoted to Czechs and Slovaks abroad Further information can be obtained from Dr M Rechcigl, jun , secretary, Programme Committee, 1703 Mark Lane, Rockville, Md

DEVELOPMENTS IN ASTRONAUTICS

THOUGHTS on the seminar on "Astronautics", held in the University of Southampton during July 12–19, should be considered against the technology of the changing scene in air and space, an unsteady scene, its movements affected by engineering and scientific inventions The trend is to curtail weapon programmes, and in place of production quantities there is more limited manufacture of precision 'hardware', aircraft types are fewer and more highly specialized, benefiting from the development of rocket systems and instrumentation

The five groups of twenty-three interrelated lectures provided a clear picture of the scientific and technological aspects of the subjects, the result of painstaking planning by those to whom science was an intellectual adventure.

The seminar was organized by the N A T O Advisory Group for Aeronautical Research and Development and the University of Southampton Prof E J Richards, head of the first Department of Aeronautics and Astronautics in Great Britain (University of Southampton), introduced Mr E T Jones, deputy director of A G A R D , who welcomed the delegates, mentioning the fitness of the venue, the first of its kind, due to the enterprise of the professorial staff, particularly Dr B L Clarkson

Mr M. J Lighthill, director of the Royal Aircraft Establishment, gave the introductory lecture to about a hundred delegates from Europe and the United States Brilliantly he set the scene, introducing the subjects to be discussed, dealing with aims and organization of pure, and applied, space science and technology Space included the stars, planets, and everything known and predicted by the astronomers, to whom we are indebted for information, and with whom it is most desirable to co-operate

In pure science we are concerned with perturbations of orbits, macrometeorites in orbit, the fluctuating environment of plasma, solar activity, solar streams, and radiation effects, Van Allen belts, and plasma propulsion

In applied science we consider geodesy, navigation, transit satellites, observations, communications, geometric aspects of space, meteorology, information for synoptic meteorologists, the particular need for viewing clouds from above—thermal stability in satellites, solar cells, gyroscopes, and generally the application of discoveries useful for mankind For the future, *Blue Streak* and *Black Knight* would help in the further observation of the ionosphere and re-entry research as well as contributing to the communication satellites plan, now being handled by the Royal Aircraft Establishment and the General Post Office, the Commonwealth and the European Space Launcher Organization, this scheme is promising and will engender greater integration, economical development, technical adventure and co-operation over a wide field, and would attract the best men The co-operative scheme needs intensive planning for arranging the work, and a body to co-ordinate effort from design to the operating conditions This includes using existing institutions, avoiding extravagances, and avoiding the separation of aerodynamics, astronautics and space technology

Useful visits were made to the Royal Aircraft Establishment, the Radio Research Establishment, and the Royal Greenwich Observatory

"Orbits in the Solar System" was the subject of a lecture by Dr J G Porter, Royal Greenwich Observatory, and he was followed by Mr. D G King-Hele, Guided Weapons Department, Royal Aircraft Establishment, with a paper on "Earth-Satellite Orbits"

These papers dealt with orbits affected by perturbations As an astronomer, Dr. Porter was concerned with all the members of the solar group and their movements in continuously variable ellipses, and the method of defining the instantaneous orbit in which a body is travelling at a given epoch The figures in the published ephemerides of the Sun and planets are based on rectangular co-ordinates measured in astronomical units to seven decimal places, they are published so that observers may make a comparison with theory Tables of planetary co-ordinates for the next twenty years are available, and for Mars for every four days up to 2,000

Complementary to Dr J G Porter's talk, Mr D G King-Hele dealt in more detail with four main perturbations affecting the orbit of a satellite, caused by (1) the departure of the Earth's gravitational field from spherical symmetry, (2) the influence of atmospheric drag, (3) the luni-solar gravitational attractions, and (4) solar radiation pressure Near satellites, having a perigee height of less than 1,000 km , and apogee heights of less than 5,000 km , are affected particularly by 1 and 2 The third perturbation results from the asymmetry between the northern and southern hemispheres If the apogee height of a satellite is greater than 20,000 km (*Explorers* 5 and 10) the gravitational attractions of the Sun and Moon are important in addition to those already mentioned, and if the perigee height exceeds 1,000 km the force due to solar radiation pressure is greater than atmospheric drag

Prof T R F Nonweiler, Mechan professor of aeronautics and fluid mechanics, University of Glasgow, spoke on "Re-entry Orbits", discussing the two conditions in which re-entry will occur, either as the final result of the gradual contraction of the orbit by air resistance, or by deliberate perturbations (through the action of retro-rockets) The speed at the first stage of re-entry, say Mach 30, is changing to stage two at Mach 3 or 4, then to stage three at Mach 0 3–0 2, and finally the vertical drop While drag can be reduced, it is not possible to reduce the rate of heating, and this problem at hypersonic speeds has two satisfactory solutions, namely, ablation, and variable drag by flared skirt or parachute When retro-rockets are used to control the vehicle, the point of firing is important so that the vehicle hits the target Whatever the shape of the vehicle the maximum deceleration is the same, but it occurs at a greater height if the design is blunt and is of high drag, thus in the lower part of the atmosphere a blunt satellite has a lower speed and endures less heating A more streamline and faster-falling vehicle suffers intense heating in the denser part of the atmosphere where maximum deceleration occurs If the vehicle can be given some aerodynamic lift to modify the re-entry path, then the rate of heating and deceleration can be reduced

Captain P V H Weems, U S Naval Academy, rounded off the session on orbits by explaining his method of "Space Navigation" The basic concept is that the space observer's optical line of sight to the Earth's centre is in the observer's vertical Range is restricted to cislunar navigation and the space-vehicle is assumed to have sufficient power to enable it to be directed and navigated The Earth is used: (1) for fixing the position of the space observer's geographical position by observing visually the position of the Earth's centre relative to the stars, (2) for finding the distance from the Earth's surface stadimetrically by using the Earth's diameter as a base, (3) for guiding a space-vehicle by so changing its velocity as to cause the apparent path of the Earth's centre to follow a precomputed path The space navigator can fix the relative position of the satellite by using a two-way telescope, which would provide, simultaneously, fixes on the Earth's centre and the zenithal stars, and then tables will give the position The proposed *Space Almanac* has been started, by attempting to provide a space navigator for the *Echo* satellite with usable data, and it is intended for teachers and students one or two generations ahead of the astronaut

Prof E Vassy, of the University of Paris, in discussing "Physical Properties of the Upper Atmosphere", paid due tribute to the astronomers, and outlined the results from the *Veronique* research rocket, the programme being similar to that of the *Skylark*, there being close collaboration with the Royal Aircraft Establishment in the development of the mass spectrograph for ion measurement

Prof B Fraeijs de Veubeke, University of Liège, discussed the "Optimization of Space Vehicles and Launchers relative to their Missions", and showed that minimization of fuel consumption, initial weight, initial thrust for a given payload and given performance requirements lead to widely different missile configurations, and then there is the extensive analysis necessary to determine the minimization of cost of mission per unit payload

Prof L Broglio, Department of Aeronautics, University of Rome, explained "A Method for Solving Non-linear Thermal Problems in the Re-entry of Space Vehicles".

In "Some Recent Advances in the Theory of Control", Dr J K Lubbock, University of Cambridge, assessed the progress of control theory embracing non-linear systems, and dealt with the difficulties of analysis and optimization of feedback systems containing non-linear elements He reviewed the Fokker–Planck equation for the Markov processes, he believes that Pontryagins' general theory is the most significant theoretical advance in recent years

Dr W W Lines, Guided Weapons Department, Royal Aircraft Establishment, outlined the "Problems of Guidance in Astronautics" and dealt with methods for the injection of Earth satellites into orbit, and the problem of orbital transfer

Flight-Lieutenant J C Guignard, Institute of Aviation Medicine, discussed "Physiological Limitations of Man in Space" He said that from the physiological aspect, space begins at sea-level, and the basic problems extend to meet the conditions discussed in the seminar are being studied in simulation laboratories The absence of normal diurnal and seasonal cycles during flights of weeks or months demands new knowledge

Dr E V. D Glazier, Royal Radar Establishment, discussed the radio problems for interplanetary space probe missions where the range may be many millions of miles, but at present there are limitations on probe transmitter power and life, and on the extent of costly ground facilities Measurement of range is achieved by the secondary radar principle, and good tracking in range and angle can be obtained out to ten million miles with a slight extension of present techniques For reciprocal and rapid transmission during a Venus mission, more powerful transmitters and aerials of the Jodrell Bank type are essential

In a paper on "Hypersonic Aerodynamics", Mr K M C Bray, University of Southampton, said that perfect fluid hypersonic effects, real gas effects and high-altitude effects are three classes of problem that may occur simultaneously, but they must be studied separately in order to provide information for the design of the blunt-nosed ballistic vehicle or the slimmer boost glider type The designer needs to know the heat-transfer rate at the stagnation point, perhaps in the presence of ablation, then data about drag and dynamic stability Due to the Faraday effect, under hypersonic conditions radio signals to a vehicle suffer serious interference

Complementary to Mr K M C Bray's paper was "Laboratory Facilities and Techniques for Rarefied Gasdynamics Research", by Mr R L Chuan, director, Engineering Centre, University of Southern California, who stressed the need for more advanced designs of experimental apparatus to analyse the problems raised by Mr Bray Four types of facilities of various degrees of complexity and cost are needed the wind tunnel, shock tubes, molecular beam, and the rotating arm The study of hypersonic phenomena requires conditions in air of Mach number greater than 10, a Reynolds number less than 100, and a static temperature of about 300° K Pressure, temperature, density, speed, gas composition are basic quantities to be measured, the pressure being of the order of 10^{-6} atm , and this Mr Chuan had achieved by designing a low-density hypersonic tunnel around a cryopump cooled by a helium refrigerator achieving 20° K.

Mr D J Johns, College of Aeronautics, reviewed the design of the thin cylindrical structures typical of the liquid-fuelled launching vehicles, covering all the ground handling and flight conditions until impact on landing They included pressurization, axial and lateral loads, thermal, dynamic and space environment effects In general, the pressure-stabilized structure is lighter and simpler than the conventionally stiffened type The inflatable structure has minimum structural density, maximum volume/weight ratio, and unmatched packageability for the launch phase Prof A J Murphy, principal of the College of Aeronautics, spoke about the nature of the environment in outer space, and its significance for "Materials for Astronautic Vehicles", and said that the requirements discussed by Mr D. J Johns could be met by the use of metals of higher melting point, especially beryllium, molybdenum, columbium and tungsten, and also non-metals such as carbon, ceramics, or metal–ceramic mixtures Refractory metals withstand 1,370° C at stressed conditions but need protection against oxidation Heat-dissipating ablation surfaces of organic material are satisfactory.

Prof E J Richards (Southampton) discussed "Some Problems of Noises in Ballistic Missiles", briefly sketching the effect of noise-levels on structures and instruments between 100 and 180 decibels, at 140–150 db structures begin to crack, and at 170 db cracking is immediate To guard against trouble, there must be a detailed analysis of the environment of noise around a vehicle both during launching and at re-entry The source of most efflux noise of a rocket is about eighteen diameters downstream, the unsteady conditions with fluctuating pressures arising from turbulent mixing of the gas in the jet, from the rough flow in the boundary layer, and from the movement of the missile in turbulent air, fluctuating shock waves buffet the missile Noise may be real and radiated acoustically or as hydrodynamic pressure fluctuations exciting the structure and causing acoustical radiation inside it

Mr G K C Pardoe, chief weapons research engineer, De Havilland Co., spoke on "Space Flight Projects based on *Blue Streak*" His practical outlook brought together the theoretical and design philosophies discussed in other papers *Blue Streak* will have a fundamental part in the proposed research work on probes, satellite communication and meteorology, and this activity means that the European Space Launcher Organization should get off to a good start Mr Pardoe reviewed the *Eslo* three-stage launcher based on *Blue Streak*, with a second stage of French design Various third stages have been proposed and could produce high or low thrust, depending on the kind of mission, *Black Knight* with Bristol Siddeley *Gamma* engine would give a versatile performance. Mr C R Turner, of the De Havilland Co , spoke on "Communication Satellites", describing a world-wide coverage scheme based on *Blue Streak*

Prof P C Parkes, Control Engineering Department, University of Southampton, speaking in Russian, welcomed Prof A M Bobrov, chief scientist, Astronomical Society, Academy of Sciences, U S S R , who discussed the "Cosmological Neighbours of the Earth" from the astronomer's point of view, referring to the Sun, and the atmosphere of the Moon, Venus and Mars Astrobiological laboratories are growing plants under simulated Martian conditions A third belt of radiation has been located beyond the Van Allen belts

Dr R R. Jamison, assistant chief engineer (ramjets), Bristol Siddeley Engines, Ltd , in discussing "Advanced Air-breathing Engines", said that the use of these engines for astronautical work was as propulsion units for space-vehicles during the boost phase of space missions Studies based on development work show that the ramjet has a better fuel specific impulse than a typical rocket engine and so should be a better first-stage booster, faster and lighter It must operate up to speeds which are a significant proportion of orbital velocity, and research indicates that speeds much higher than the present operable Mach 6 can be achieved

Mr. S L Bragg, chief scientist, Rolls Royce, Ltd , discussed "Operation and Control of Liquid Propellant Rocket Engines", and explained the difficult handling problems of starting, keeping the engine running, and stopping. The *Blue Streak* engine is the *RZ*-2, using liquid oxygen and kerosene Accurate control of the moment at which the engine is shut down is essential to enable the accelerating vehicle to follow the flight plan After the stopping signal, there will be a brief time while the engine runs down, and on a multi-stage rocket it is desirable that the thrust should cease before the stages separate to avoid the possibility of subsequent collisions

Mr. P A E Stewart, rocket and satellite systems engineer, Hawker Siddeley Aviation, Ltd , in dealing with "Advanced Propulsion Systems for Space", reviewed the three main classes (*a*) high acceleration, covering free-radical rocket propulsion, open-cycle thermonuclear propulsion, and ionospheric recombination ramjets, (*b*) for medium acceleration there is solar energy, and (*c*) for low acceleration the closed-cycle nuclear system and variations of the electrodynamic types, electro-thermal or arc-jet, electrostatic or ion engine, the magnetohydrodynamic type, and the photon system

Group (*c*) attracted most interest, progressive laboratory work has been achieved in electrical propulsion and the arc-jet is well advanced Electric propulsion provides jet velocities 5–10 times those of nuclear engines, but as the thrusts are limited to pounds and fractions of a pound, they are promising only in space flight, but the problems are chemical and mechanical Then there is the question of space-flight test-beds. A CADMAN CLINTON

THE COLONIAL DEVELOPMENT CORPORATION

Annual Report, 1960

THE annual report and statement of accounts of the Colonial Development Corporation for the year ended December 31, 1960, the first to appear over Lord Howick's signature, who, as chairman, succeeded Sir Nutcombe Hume on December 1, 1960, follows the pattern of recent reports* Ninety-one continuing projects are reported as compared with 88 in the previous year, including 10 new projects totalling £11,675,000 Seven projects have been removed from the list and total commitments aggregated £109,448,000 compared with £96,145,000 in 1959 Of this aggregate, £14·6 million was in the Caribbean, £19 4 million (including £12 8 million for the Federation of Malaya) in the Far East, £22 7 million in East Africa, £22 0 million in Central Africa, £16 0 million in the High Commission Territories, and £14 7 million (including £205,000 in Ghana and £8 9 million in the Federation of Nigeria) Functionally 52 5 per cent of the committed capital was for basic development, 38 3 per cent for primary production and processing, and 9 2 per cent for commerce and industry

The report is at pains to emphasize the nature of the Corporation's task and some of its achievements in Africa It is believed that the Corporation can make an important contribution to the economic life of the emerging peoples for several reasons Its regional organizations have now been established for nearly ten years and its employees are able to relate business needs to the conditions of the region They have come to know well some leading people in the political and commercial life of their region, and are accustomed to working with them The Corporation is able either to take a shareholding or make a loan according to whether the principal need of a country is, at one time, for an equity type investment or, at another time, for a loan, and those in charge of the Corporation have become accustomed to working with many partners and have learned, for example, the importance of the pilot scheme stage in development projects

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* Colonial Development Corporation Annual Report and Statement of Accounts for the year ended 31st December, 1960 Pp vi+82 (London H M S O , 1961) 5*s* net

The achievements of the Corporation in Africa are illustrated by reference to the Mhlume Sugar Mill in Swaziland, built in partnership with Sir J L Hulett and Sons, on a Corporation irrigation project, which will have produced by the end of 1961 more than 40,000 tons of sugar, the new pulp mill at Usutu Forests in the same territory, with an annual output of 100,000 tons , the Lobatsi abattoir in the Bechuanaland Protectorate, the launching of the Kilombero Sugar Co , Ltd , in Tanganyika, with the International Finance Corporation, Dutch interests and the Standard Bank of South Africa, and the agreements concluded in Nyasaland to provide up to £1,300,000 for the Mudi River Water Board to relieve the water shortage in Blantyre and Limbe The value of the Corporation's activities has equally been clear in countries which have become independent since the Corporation was established

As regards future possibilities, the report stresses particularly that, while remaining fully commercial, the Corporation could and should give the help which smallholders need in loans in processing and marketing their produce and in establishing a few larger farms for demonstration and for experiments with new agricultural methods, because development of smallholder farming can contribute powerfully to the stability of a country In small or sparsely inhabited territory the Corporation can often do much and is frequently irreplaceable, as is shown by the hydroelectric scheme in Dominica and in St Vincent established by the Corporation, which provided the background for production of bananas, cocoa and copra Great importance is attached to the training of men in the territories in which the Corporation operates to undertake posts of responsibility both in local projects and the Corporation's organization as a whole The Corporation believes that the best training is that received on the job provided a real effort is made to explain and demonstrate by those appointed to supervise, but in view of the absence of minimum educational background in many territories the Corporation decided last year to offer, on a very limited basis, scholarships to promising students as a contribution towards solving the educational difficulty

When the Corporation's report was discussed in the House of Lords on June 27, the Earl of Perth emphasized that in the main this was a matter which should be handled through the new Technical Aid Department, and that the Corporation should limit itself to giving training and occasional scholarships to particular people who were to work on a particular project Generally, warm approval of the work of the Corporation was expressed in the debate criticism centring rather on the Government's financial policy to the Corporation and the delay in giving effect to the recommendations of the Sinclair Report The Earl of Perth, however, mentioned that the Government had followed closely the essential recommendations regarding stock representing loans advanced for 'finance house' business and for stock representing abandoned schemes and fructification interest, but laid down that repayment for this stock should depend on the Corporation, after its various obligations, having a profit of more than £250,000 The only variation was in respect of the recommendation for '*B*' Stock, which involved a fluctuating subsidy and thus no Parliamentary control, and here the Government insisted that the agreed rate must be paid, but as these investments would be ready for equity investment the Government would continue with the fructification period for seven years The suggestion for a reserve or allowance for exploration was met by allowing the Corporation to use the first £250,000 of its true profits for whatever purpose it saw fit Lord Perth also said that it was not expected that the Corporation, which was not at the moment in need of funds, would require increasing funds indefinitely it might be expected from time to time to sell off successful ventures He thought the Corporation would still be able to play a limited part in the newly independent countries but that it was right that the Corporation should concentrate on the remaining Colonial territories

Research Schemes and Loans, 1960

The schemes made under the Colonial Development and Welfare Acts in the period April 1, 1960–March 31, 1961, which are detailed in the latest returns*, bring the total commitments from April 1, 1946, under the Acts to £275,318,622, of which £20,798,424 is for research schemes The totals for the year in question are £35,291,739 for development and welfare and £2,186,869 for research schemes Of the latter, £1,085,365 is for agriculture, £400.624 for medicine, £223,784 for tsetse research and trypanosomiasis, £150,786 for social science, £108,360 for fisheries, £56,401 for pesticides, £25,727 for locust control, and £6,803 for economic research

Of the research schemes, £446,149 is towards the cost of research organizations controlled by the East African Agriculture and Fisheries Research Council, but there are other considerable contributions to the cost of work sponsored by the Medical Research Council, the Agricultural Research Council or regional organizations, for example, £30,000 for the Medical Research Council's laboratories at Fajara, Gambia, and £11,750 for entomological research there, £133,516 for continuation of services, other than the Leprosy Research Centre controlled by the East African Council for Medical Research, 1960–63, £27,432 to the West African Council for Medical Research and its associated units, £27,103 to the West African Building Research Institute, £22,412 to the West African Institute for Trypanosomiasis Research, 1960–61, £43,320 for the Regional Virus Research Laboratory, Trinidad, £148,000 for the Regional Research Centre, Faculty of Agriculture, University College of the West Indies, and £8,000 to the Agricultural Research Council Unit of Experimental Agronomy and Weed Research Organization, 1961–62

Other major grants which may be noted are £42,827 for immunological work on trypanosomiasis and preparation of precipitin sera at the Lister

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* Colonial Development and Welfare Act Return of Schemes made and of Loans approved under the Colonial Development and Welfare Act in the period from 1st April, 1960, to 31st March, 1961 (House of Commons Paper 240) Pp 58 (London H M S O , 1961) 3*s* 6*d* net

Institute of Preventive Medicine, £39,898 for tsetse fly breeding at the University of Bristol, £34,303 for continuation of the preparation of the Flora of Tropical East Africa, £62,933 for continuation and expansion of agricultural research and land-use planning in Tanganyika, £62,645 for the Agricultural Research and Experimental Station, Nyasaland, £56,550 for establishment of a Citrus Research Unit in the British Caribbean, £79,549 for continuation of the Institute of Social and Economic Research in the West Indies; £48,812 for the Tropical Metabolism Research Unit in Jamaica, and £20,934 for soil surveys in British Guiana

Development and welfare schemes of scientific interest include various contributions to Royal College, Nairobi, and the University College of East Africa, Makerere, as well as to the University College of the West Indies, and £450,000 for the construction of a College of Arts, Science and Technology, Malta, which received grants totalling more than £5 million during the year, £421,907 for intensification of African agriculture under the Swynnerton plan in Kenya, and a further £102,804 for the Veterinary Department, £518,300 for forestry development in Tanganyika, and £148,445 for continuation and expansion of agricultural research and land-use planning, £246,000 for development of broadcasting in Uganda, and £429,132 for soil conservation and land-use planning in Basutoland, 1960–64, £18,877 was contributed for the activities of the Commission for Technical Co-operation in Africa South of the Sahara and the Scientific Council for Africa South of the Sahara, and there were numerous contributions for education at secondary and primary levels

THE NORFOLK BROADS

On August 1, the Minister of Housing and Local Government announced in the House of Commons that he had accepted the advice of the National Parks Commission that the unique character and complex problems of the Norfolk Broads make them unsuitable for designation as a National Park It was therefore appropriate and timely that the British Association should discuss some of these problems during its Norwich meeting Sections E (Geography) and K (Botany) held a joint symposium on September 1 at which the main contributions were made by Dr Joyce Lambert of the Botany Department, University of Southampton, Mr C T Smith of the Geography Department of the University of Cambridge and Mr Charles Green, an archæological consultant to the Ministry of Works

The factors which render the Broads unsuitable as a National Park stem largely from the rapid changes which are taking place at the present time and which are all recent in effect, if not in origin The silting up of the Broads, with the consequent overgrowth by vegetation had scarcely begun to reduce the area of open water when the Tithe Commission mapped the area in the mid-nineteenth century The degeneration of mowing marsh into scrub and carr follows changes in agricultural practice in the past half-century These physiographical and ecological developments clearly have a profound significance in terms of the ever-increasing use of the Broads as a holiday area, a use which introduces its own problems, both ecological and economic While these changes have only recently forced themselves on the attention of students of the broadland scene, they are bound up with the basic problems concerning the age and origin of the Broads, which formed major themes in the Norwich discussion and which involve rather longer periods of time

Most nineteenth-century writers from Robbards and Taylor in the 1820's to Gregory in 1892, sought to interpret the Broads as residual natural lakes resulting from the silting up of a 'great estuary', the three arms of which occupied the valleys of the Rivers Waveney, Yare and Bure Many of these writers assumed that the 'great estuary' persisted well into historical times A few workers sought to invoke glacial agencies In 1952, J N Jennings published a monograph on *The Origin of the Broads* (No 2 in the Royal Geographical Society's Research Series) This was the first weighty piece of original research to appear for almost half a century and represented the results of several years labours, mainly in the valleys of the Bure and its tributary, the Ant Jennings suggested that the Broads can be explained in terms of a geologically very recent marine transgression during which a thick wedge of estuarine clay was laid down over the early valley peats of the Norfolk river valleys He suggested that the Broads themselves were initiated as discontinuous lakes, lying in natural hollows in the peat, filled with water ponded back between the flanges of the clay and the upland valley margins (by-passed broads) or in tributary valleys the mouths of which were blocked by the clay (side-valley broads) Jennings examined the possibility, mooted on several occasions to explain particular broads, that all the Broads were man-made For a variety of reasons he came to the conclusion that "a general theory based on peat cutting appears to be wholly unacceptable"

Almost before his monograph was in print, Jennings had modified his views in the light of new work. Dr Lambert, with whom he had collaborated closely in work on the valleys of the Bure and the Ant, had obtained convincing evidence that the Broads of the Yare valley are artificial She employed the same boring methods and the same pollen analysis technique, to determine the stratigraphy of the clays, peats and muds, as Jennings had used A reconsideration of the evidence from the Bure and the Ant valleys suggested that deep extraction of peat in historical times was responsible for all the by-passed broads, though, for lack of evidence, the side-valley broads were not as yet included in the general theory This recognition of the fact that most, if not all, of the Broads are man-made did not, of course, detract from Jennings's interpretation of the natural evolution of the river valleys in which the Broads are located

In 1960, Jennings and Lambert published a re-appraisal of the whole problem of the evolution of the Broads They found it "difficult, in view of the stratigraphical evidence to conceive a substantial alternative" to the theory that all the Broads are deep peat diggings which have been afterwards flooded Breydon Water is excluded from this explanation since it is clearly not a broad in the accepted sense and is merely the surviving part of a formerly more extensive Bure–Yare–Waveney estuary

Evidence from the field of historical geography assembled by Mr C T Smith and the results of archæological work, interpreted by Mr Charles Green and Mr J N Hutchinson, supported the new hypothesis The combined evidence was published under the title *The Making of the Broads* (No 3 in the Royal Geographical Society's Research Series)

At the Norwich symposium, Dr Lambert reviewed the physiographical and botanical evidence on which their findings are based Boring has revealed that the basins in which the Broads lie have uniformly steep sides and virtually level floors with resulting rectangular profiles Islands and peninsulas with similar vertical sides have exactly the same stratigraphy as the surrounding fenland They are interpreted as balks left in the extraction of peat to a depth of 10–12 ft In old surveys, including tithe and enclosure maps and in modern air photographs these islands and peninsulas can be correlated with boundaries between holdings

Two of the more important difficulties in accepting human agencies to account for the existence of the Broads relate to the enormous amount of peat involved and the need to postulate changes in water-level, which would permit the excavation of peat to depths well below those of present-day workings, and eventually lead to the flooding of the pits Mr C T Smith described the search for evidence which would resolve these difficulties Nothing in the published records of broadland suggested an 'artificial' origin The large-scale changes caused by the extraction of an estimated nine hundred million cubic feet of peat and the flooding of the workings appear to have made no impact on local tradition and folk-lore However, a careful and detailed study of documents and maps supplied strong circumstantial evidence in support of the deductions based on the stratigraphy References to turbary and the sale and consumption of turves show that large-scale peat digging was well under way by the twelfth century Much of the consumption must have gone unrecorded but examination of terriers, deeds, account rolls and registers, such as those of Norwich Cathedral Priory and St. Benet's Abbey, give an indication of the scale of operations. The purchase of 400,000 turves by Norwich Cathedral Priory in 1316 is typical These same records also show that by the beginning of the fourteenth century the flooding of the turf pits had begun While extraction continued well into the fifteenth century it was increasingly difficult and expensive, and involved first the dredging of peat with simple scoops and then the making of turves Soon other fuels replaced turves in the records and references to turbaries were replaced by references to open water and fisheries.

The archæological evidence put forward by Mr Charles Green showed that emergence from the Romano–British submergence reached a peak in the thirteenth century. The excavations on the site of the power-station at South Denes, Great Yarmouth, indicate a relative fall in sea-level of the order of 13 ft This means that deep extraction of peat from the Norfolk valley floors was possible in 'dry' pits Similar evidence revealed a reversal of the downward movement of sea-level, initiated toward the end of the thirteenth century, which clearly accounts for the slow but inexorable flooding of the peat-diggings and the formation of what we now call the Norfolk Broads

As a piece of scientific detection, the story unfolded to the Norwich symposium was a masterpiece It is, however, not only of historical or academic interest The state of dynamic change, which Dr Lambert showed to underlie all the present-day problems of the Broads, both scientific and practical, stems directly from these surprising beginnings The vegetation of broadland does not, as was formerly thought, result from the overgrowing of large continuous sheets of open water There is a fundamental distinction between the vegetation which has grown up on the solid fen, much modified by a variety of human interference, and that which is encroaching on the basin of the Broads. The filling of the man-made lakes with freshwater mud, which has made this encroachment possible, only reached a critical stage about the beginning of the nineteenth century It has, however, already resulted in a rapid reduction of the open water available to the holiday maker Dr Lambert is continuing her ecological work in the Broads, but the number of problems which still remain to be tackled, some of which she touched on, is very large The need for more research was the theme of the discussion which followed the reading of papers Any new work which may be instituted will owe much to Dr Lambert and Mr Jennings and their colleagues

C KIDSON

THE SOIL AS AN ENVIRONMENT FOR ANIMAL LIFE

A SYMPOSIUM on "The Soil as an Environment for Animal Life" was held on September 1 by Section D (Zoology) at the Norwich meeting of the British Association for the Advancement of Science

Sir John Russell pointed out that soil contains four groups of components a mineral framework, organic matter, mineral matter from the bodies of organisms and soil solution. The relative proportions of organic and inorganic substances largely determine the suitability of the soil as a habitat arable soils, with the lowest organic content, contain fewest organisms, while grasslands are usually richer

The organic matter is derived from plants, their remains being reduced by organisms in the soil to their original simple origins residues remaining form the humus. This is black, has colloidal properties, and in the climate of Great Britain contains about ten times as much carbon as nitrogen If non-nitrogenous substances are added to the soil, atmospheric nitrogen is fixed, while if nitrogenous substances are added the humus splits off nitrogen as ammonia or nitrogen gas

The mineral framework of the soil contains particles ranging from 2 mm to 0 02 mm in equivalent diameter these are the sand and silt Clay particles less than 2μ in equivalent diameter differ from the larger particles, they carry a negative electric charge, and can attract positively charged ions such as calcium and hold them against leaching Clay is colloidal, with a great power of absorbing water it expands on wetting and shrinks on drying, producing large cracks in the soil

Calcium compounds, especially calcium carbonate, are as important as clay and organic matter in their biotic effects While calcium carbonate is present the soil remains neutral, but as it dissolves in rain-water containing carbon dioxide and is leached out the soil becomes acid The flora and fauna then become more restricted, though some organisms, such as *Plasmodiophora* and the amœbæ, do well At a certain stage earthworms disappear plant residues then remain on the surface and decompose there, forming 'mor'

Organic and mineral soil components mingle to form crumbs, the optimum crumb size for plant growth ranging from 5 to 1 mm Good crumbs are stable even under rainfall, while bad ones break down in wet conditions, forming a slurry Mineral particles and crumbs have pore spaces between them, the total pore space being increased by crumb formation

For organisms to survive in the soil, five conditions are necessary living space, air, water, suitable temperature and freedom from injurious factors The pore spaces provide the living room, and are also the water reservoirs The chief cause of water loss is suction by plant roots evaporation causes less than might be supposed The surface layer dries quickly and protects the soil beneath, and there is no evidence of capillary movement of water from wetter to drier parts except just above the water table

Diffusion of air into the pore spaces is very rapid, and the composition of air 6 in down on arable land differs little from that of the atmosphere, except that the amount of carbon dioxide is greater, reaching 0 1 per cent on bare soil and 0 2 per cent under crops In grassland soil the carbon dioxide content is greater 3 per cent has been recorded This high concentration does not appear to affect the soil population seriously

Soil temperatures reflect those of the air, but fluctuations are heavily damped Variations are greater on arable land than on grassland

Harmful factors are numerous and may be drastic These include acids, products of anaerobic decomposition such as hydrogen sulphide, the presence of parasites and predators, etc Soil bacteria act as scavengers, removing harmful substances very effectively

The question of food for the soil fauna has been studied most fully by the foresters Leaves and twigs form litter on the soil surface in it a mixed population develops and makes the primary attack on the food material it contains Then follows the secondary decomposition, in which organisms nurtured in the first stage are eaten by predators that either capture their prey or poison it and feed on its decomposing body, also the excretions of primary decomposers are eaten by coprophagous animals The final residue is humus, this persists in the soil, but is slowly decomposed, mainly by bacteria, the final end-products being carbon dioxide, ammonia, water and a few mineral salts These will be starting material for a new generation of plants

Dr F Raw discussed "The Interdependence of the Soil and its Fauna" The soil can be considered an environment created by animal activity from weathered mineral substrate and plant remains The material from which the soil developed, the climate and the vegetation influence the animal communities developing in the soil, and these in turn influence the soil profile Agricultural practices that affect the fauna may produce great changes in the biological and physical properties of the soil

The greatest diversity and abundance of soil animals are found in virgin habitats such as permanent grassland or woodland The arthropods in a permanent grass field in Cambridgeshire included 17 orders or classes, including uncommon groups such as the Pauropoda and Protura Smaller arthropods were especially numerous About 70 per cent of the Arthropoda occurred in the top 6 in, though some groups were more numerous at lower levels In general, the smaller forms live deeper in the soil

In arable soil the arthropod population is greatly reduced In the Barnfield mangold plots at Rothamsted, the plots receiving mineral fertilizers yielded about 15,000 individuals per square yard, while in the dunged plots the number of individuals was almost twice as great, and the population was more diverse The same was true of the earthworms, though the dominant *Lumbricus terrestris* was less affected by dunging than other species

Earthworms are the principal agents that bury plant remains, mix them with the soil and so accelerate decomposition and the release of plant nutrients In undisturbed habitats their activity can have a profound effect on the soil profile, and their presence or absence can result in the development of two fundamentally different soil types, 'mull' and 'mor' This was illustrated by measuring the amount of leaf litter buried by worms in several apple orchards near Wisbech, by placing a known number of weighed leaves on the ground beneath wire cages at leaf-fall and recording the number and weight of leaves remaining in the spring In grass orchards the number and weight of leaves buried was closely correlated with the estimated fresh weight of *Lumbricus terrestris* Some orchards had about 20 cwt of *L terrestris* per acre, which buried 8–9 cwt dry weight of leaves—enough to bury all the leaf-fall Cultivation did not appear to affect the abundance of *L terrestris* greatly nor did regular applications of farmyard manure or compost

In a grass orchard with a long history of heavy spraying with copper fungicides *L terrestris* was not found, although it was abundant in surrounding unsprayed soil In this orchard no leaves were buried, they decomposed slowly on the surface, and a mat of partially decomposed leaves, about 2 in thick, had accumulated, so that the soil profile had typical 'mor' characteristics

"The Soil Fauna and its Contribution to Fertility" was the subject of Dr A Macfadyen's paper The soil contains enormous numbers of individual animals, and their action appears to be three-fold The larger species tunnel through the soil and carry litter downwards and mineral matter upwards at the same time they aerate and drain the soil and, by crumb formation and in other ways, render it a good medium for the growth of higher plants and micro-organisms

The second function performed by the soil animals is a direct chemical one due to their feeding activities They assist in the breakdown of litter in the soil, though here they make a poor showing compared with the micro-organisms

The third property of the soil fauna has only recently come under investigation It appears that quite apart from their own direct metabolic effects on litter breakdown, soil animals act as catalysts of the metabolism of the bacteria, fungi and other micro-organisms This catalytic action appears to operate through the cropping of senescent colonies, the re-distribution of slow-moving food materials, the local removal of antibiotic substances, the re-concentration of nutrients and the spreading of spores to places where they can flourish. The biological properties of the microbes are such that high-energy food materials become inaccessible and energy becomes dammed up. The animals are the 'dam-busters' that disperse the accumulations of energy and restore the flow—and with it the nutrients needed by the higher plants.

It is not known yet how important these catalytic actions are, nor which of them are the most significant. Clearly, over the whole range of soil types, from forest and pasture to moorland and degraded arable land, the details of the picture are going to vary enormously, but the few experiments reported indicate that the animals' 'catalytic' role greatly exceeds their metabolic role as decomposers.

Current experiments are aimed at building up a good knowledge of the metabolic rates of isolated organisms under conditions as close to those in Nature as possible. At the same time systems are being developed containing micro-organisms and essential soil constituents of varying degrees of complexity but with predictable metabolic behaviour. It is hoped to go on from there to introduce different kinds of animals into the cultures and observe their effects on total metabolism, and to analyse the biochemical basis of these effects. In this way it is planned to bridge the gap between field conditions and laboratory experiments.

Dr. C. L. Duddington discussed "Predacious Fungi and Soil Eelworms". Predacious fungi are micro-fungi that attack small animals, including protozoa, rotifiers and eelworms. They are abundant in most fertile soils. We can distinguish two kinds of predacious activity among the fungi that attack eelworms. Some capture eelworms alive, while others are internally parasitic, attacking the host through the agency of sticky spores or by other means. At least two species of *Nematoctonus* combine both methods. Most of the work on predacious fungi attacking eelworms has been concentrated on the trap-forming species.

In the common *Arthrobotrys oligospora* and others the eelworm traps consist of systems of sticky networks, formed from curled, anastomosing branches of the mycelium. Eelworms that happen to touch the networks stick to them like flies on a fly-paper. Other species, like *Dactylella cionopaga*, have short, sticky lateral branches, while in *D. ellipsospora* and others small sticky knobs are attached to the mycelium by short stalks.

Some predacious fungi have mechanical traps. In *Dactylaria candida* rings, composed of three curved cells, are attached to the mycelium by short stalks, eelworms accidentally pushing their heads into the rings become wedged. In some species, such as *Dactylella doedycoides*, the cells of the rings are sensitive to touch, if an eelworm passes its body into the ring the three cells swell suddenly inwards, holding the eelworm by constriction.

Whatever trapping mechanism is used, the end result is that the eelworm is killed, and its body is invaded by hyphæ that consume its contents.

A survey of 49 British arable soils produced 82 records of predacious fungi, belonging to 20 different species. *Arthrobotrys oligospora* was the commonest; both eelworm-trapping and internally parasitic species were represented. There is direct evidence that predacious fungi prey on eelworms in the soil under natural conditions, for mycelium with traps and captives has been found on slides immersed in soil both in field plots and in the laboratory. There is also convincing evidence that their predacious activity is enhanced by the presence of decomposable organic matter in the soil. There is reason to believe that the organic matter affects the activity of the fungi directly and not, as has formerly been supposed, through its stimulating effect on the population of free-living eelworms in the soil.

The importance of predacious fungi in limiting the soil nematode population under natural conditions has yet to be fully investigated, but experiments on the use of predacious fungi for the biological control of plant pathogenic eelworms suggest that their effect is not negligible. Soil treatment designed to enhance the activity of predacious fungi was successful in controlling pineapple root-knot eelworm in Hawaii, and in Britain the number of larvæ of cereal root eelworm invading the roots of oat seedlings has been significantly reduced by soil inoculation with *Dactylella thaumasia* combined with the addition of organic matter to stimulate the fungus.

C. L. DUDDINGTON

DOMESTIC FOOD CONSUMPTION AND EXPENDITURE, 1959

THE survey for 1959 of the National Food Survey Committee* completes twenty years of continuous recording of domestic food consumption in Britain. Two reports cover the years 1940–49 and since 1950 a report has been published each year. These twenty years have seen a complete change from severe restriction in food supplies, followed by a period of gradual increase, to the point recently reached at which the housewife with money to spare looks for greater variety and shows a preference for what may be called 'convenience foods', processed and packed to save time and trouble.

A gap necessarily intervenes between the year of survey and the publication of these annual reports. As a result, they often clarify and extend facts already known in a general way. For example, the present report throws light on the increase in expenditure on food known to be taking place during the period under review. The average weekly expenditure a head increased from 28*s.* 5*d.* in 1958 to 29*s.* 3*d.* in 1959. This was partly due to an increase in the price of foods such as butter, meat, cheese and fruit, but there was also a shift in demand from staple foods such as bread, flour, potatoes and fresh fish to fresh fruit, fresh green vegetables and a wide variety of processed foods. Expenditure on processed foods prepared ready for the table, which include canned and frozen foods, cakes, pastries and biscuits, has risen from 4*s.* 3*d.* a head a week in 1955 to 5*s.* 5*d.* in 1959. Doubtless, as the range of such foods increases and advertising becomes more clamorous, the expenditure on them will continue to rise, though a certain lack of variety in the taste of foods prepared by mass-cooking methods may eventually drive some housewives back to the kitchen stove In the section dealing with the effect of the housewife's age on the household diet, it is shown that young housewives devote up to 19–24 per cent of their total food expenditure to these 'convenience' foods, while older housewives devote considerably less The fact that 'single-earner' families with two children spend more on them than childless couples shows how useful they can be to the busy mother Their cost in terms of food is increased by packaging and advertising, but this is offset by saving in time and less wastage The expenditure on cooked and canned meats rose 5 per cent from the previous year, and on prepared fish the rise was 14 per cent The most striking change in food habits in Britain, apart from the growing preference for processed foods, is the increase in consumption of poultry Total supplies of poultry and rabbits are 31 per cent higher than pre-war, although there has been a considerable decrease in supplies of rabbit

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* Ministry of Agriculture, Fisheries and Food. Domestic Food Consumption and Expenditure 1959. Annual Report of the National Food Survey Committee. Pp. vi+142. (London: H.M.S.O., 1961.) 8*s.* 6*d.* net.

Comparison of the nutritive value of the diets of the different social classes with the recommendations of the British Medical Association showed that intakes of all nutrients, except protein, considerably exceeded the figures of the British Medical Association Total protein intakes were 'borderline' in the lower income groups and the national average 99 per cent of the recommendation of the Association As income rises, the calorie value of diets and their content of all the nutrients also rises The diet of the most prosperous classes is 'richer' in these respects than that of all other classes This perhaps suggests that wealthy people eat too much, but on the other hand it might be taken to imply that the recommended allowances are set too low Such questions could be clarified only by correlating national food consumption data with data on the health of different sections of the population—an extremely difficult task

Study of the relation between food consumption and family composition and size gave a less favourable picture In households containing four or more children the diets were inadequate in certain respects Intake of calories was 94 per cent of the recommended level, and that of protein, calcium and riboflavin 82, 81 and 95 per cent respectively These deficiencies may not seem great, but it must be remembered that these families contain a high proportion of growing children whose health and development depend largely on proper feeding At the other end of the life-scale an improvement is recorded In earlier reports it was shown that the diets of old age pensioners were unsatisfactory , it is good to note that their diets included in this survey were adequate for all nutrients

Interesting facts are given about geographical differences in food consumption These give rise to many fascinating speculations on feeding and cooking habits in different parts of Britain, but it would be foolhardy to hazard guesses on the merits and efficiency of housewives according to locality

JOYCE DOUGHTY

NITROGEN IN SHEEP SALIVA

IN a series of articles, M Somers, of the Institute of Agriculture, University of Western Australia, describes the factors influencing the secretion of nitrogen in sheep saliva (*Austral J Exp Biol and Med Sci* , 39, Part 2, April 1961)

Measurements were first made on the distribution of nitrogen in the mixed and parotid salivary secretion of sheep and on the amount of nitrogen secreted daily in the parotid saliva The distribution of nitrogen in the mixed saliva followed the same pattern as that in the parotid secretions obtained when the glands were in a quiescent state The concentration of nitrogen in both fluids depended on the rate of secretion Although the concentration of total nitrogen in the mixed and parotid saliva increased during short-term stimulation, the pattern of nitrogen distribution was not affected During prolonged stimulation of the parotid glands, the nitrogen composition of the saliva changed

Quantitatively, urea was the most important nitrogen compound in both the mixed and parotid saliva, accounting for 60–70 per cent of total nitrogen The average volume of saliva secreted daily by one parotid gland was 2,380 ± 43 8 ml and the average total nitrogen content was 295 5 ± 5 77 mgm when the nitrogen intake was 14 8 gm /day

Somers later examined the influence of nitrogen intake on blood urea nitrogen and on the total nitrogen and urea nitrogen in the parotid saliva of sheep Urea was added to the rumen of three merino sheep at the rates of 5, 10 and 20 gm daily to increase the overall nitrogen intakes from 10 7 gm in the basal ration to 13 0, 15 4 and 20 0 gm a day A marked increase in the total amount of nitrogen secreted in the parotid saliva was found to occur as the nitrogen intake of the sheep increased A similar high correlation was found between the level of nitrogen intake and the amount of urea nitrogen secreted in the saliva daily

Between the highest and the lowest levels of nitrogen intake the magnitude of the increases were 0 26 gm of total nitrogen and 0 18 gm. of urea nitrogen/gland/day The concentration of urea nitrogen in the blood increased with the level of nitrogen intake of the sheep, but fluctuations in the level of blood urea could not be accounted for by nitrogen intake alone A significant correlation was found between the concentrations of urea nitrogen in the blood and in the saliva This offered a partial explanation of the higher concentrations of salivary urea resulting from the increased nitrogen intakes

Further investigations were made of the factors affecting the nitrogen fractions in the parotid saliva of sheep with special reference to the influence of production of ammonia in the rumen and fluctuations in level of blood urea Quantitative measurements were made on the volume of saliva secreted hourly by the parotid glands of Merino sheep over a period of 24 hr on three separate days At the same time fluctuations in the concentration of urea and total nitrogen in the saliva were studied in relation to the production of ammonia in the rumen and changes in the level of blood urea which occurred throughout the day

Levels of both rumen ammonia and blood urea followed a relatively simple cyclical pattern over each 24-hr period However, two distinct phases were apparent in the salivary nitrogen cycle The first, a short phase, followed the changes in stimulation associated with eating The second phase was prolonged and more complex It was influenced by changes in the level of blood urea, but additional factors also operated in the second phase

Finally, Somers examined the influence of injected urea on the quantitative recovery of urea in the parotid saliva and the urinary excretions of sheep Differential responses occurred in the amount of urea nitrogen and total nitrogen secreted in the parotid saliva, according to the nitrogen status of the sheep, when 1 4 gm of urea nitrogen was injected into the blood daily Of this amount of nitrogen, 0 1 gm could be recovered in the secretion from one parotid gland daily when the sheep were in positive nitrogen balance, and 0 3 gm when the sheep were in negative nitrogen balance The urinary excretion accounted for 1 0 gm of the 1 4 gm of urea nitrogen injected daily when the sheep were in positive nitrogen balance, but only 0 3 gm /day at the lower nitrogen status The implications of these findings have been considered in relation to a urea conservation mechanism in sheep

CEREBRAL INFARCTION

IN 1959, in England and Wales, cerebrovascular disease was the certified cause of 75,150 deaths (14 per cent of the total mortality), and, according to the Registrar General's analysis, about half these were a direct result of infarction of the brain The morbidity associated with this disease is also formidable, death may be preceded by months or even years of mental deterioration or paralysis

For more than a century autopsies on cases of cerebral infarction have revealed frequently an unexpected absence of significant lesions in the local cerebral arteries, and attention has periodically been directed to the possible role of the carotid arteries in this condition In a study by Dr Peter Yates and Dr E F Hutchinson on behalf of the Medical Research Council, 100 cases of suspected cerebral ischæmia were investigated in detail *post mortem*, and in 35 of these cerebral infarction was found[*] The whole course of the cerebrovascular tree from the aorta to the brain was examined, the vertebral as well as the carotid arteries being dissected, and the pathological findings were correlated with clinical records, Dr Yates was mainly concerned with the pathological side of the work, and Dr Hutchinson with the clinical aspects

Of the cases of cerebral infarction, little more than half showed occlusion or significant narrowing of the local arteries, but serious obstructions of the extracranial cerebral arteries was found in 90 per cent Such a high frequency of extracranial arterial obstruction in cases of cerebral infarction suggests that surgery may have a wider application in the early stages of cerebrovascular disease than has been generally recognized

The authors emphasize, however, that cerebral infarction rarely has a single cause, and that it is usually the result both of obstruction in extracranial or intracranial cerebral arteries and of systemic disease The decisive part played by general systemic factors, especially those that result in a lowering of the blood pressure, is amply shown in this report Even when cerebral arteries, whether extracranial or intracranial, are grossly narrowed, the blood circulation in the brain may be maintained for long periods, provided that there is an adequate collateral supply, but if hypertension or anæmia occurs in addition, the precarious balance may be upset and cerebral infarction precipitated

[*] Privy Council Medical Research Council Special Report Series, No 300 *Cerebral Infarction—The Role of Stenosis of the Extracranial Cerebral Arteries* By Peter O Yates and Edward C Hutchinson Pp viii+95+20 plates (London H M Stationery Office, 1961) 14s net

STANFORD–PACIFIC NAVAL LABORATORY CONJUGATE POINT EXPERIMENT

By J. E LOKKEN, J. A SHAND and Sir C. S WRIGHT, K C B , O.B.E.
Pacific Naval Laboratory, Esquimalt, British Columbia
AND
L. H. MARTIN, N. M. BRICE and R. A. HELLIWELL
Radioscience Laboratory, Stanford, California

A CLOSE correlation has been discovered in the times of occurrence of very low frequency and extra low frequency electromagnetic phenomena observed at high-latitude conjugate locations The observations were carried out jointly by the Pacific Naval Laboratory of the Defence Research Board of Canada and the Radioscience Laboratory of Stanford University during January 1961. For this experiment the Pacific Naval Laboratory extended the range of its 0 003–3 c p s investigations of geomagnetic micropulsations to include the natural electromagnetic background up to about 30 c p s Similarly, Stanford very low frequency investigations were broadened to cover from 15,000 c p s down to about 5 c p s Combined observations were made at Byrd Station, Antarctica (80° S , 120° W), and at two near conjugate locations in the vicinity of Hudson Bay in the Canadian Arctic The nearest, 200 miles south of the conjugate point on the Vestine model, was at Great Whale River, 55° 17′ N , 77° 46′ W , and the other at Fort Churchill, 58° 42′ N , 94° 03′ W , some 500 miles west of the conjugate point The geomagnetic co-ordin-

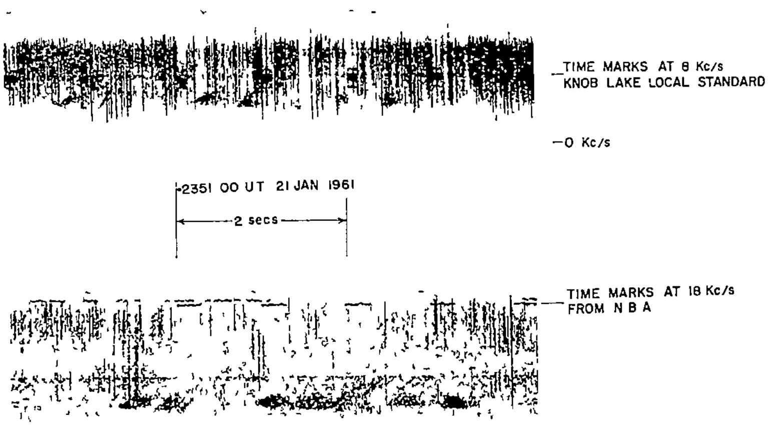

Fig 1 Six bursts of noise in the 5–6 kc p s frequency region appearing at Knob Lake (above), and at Byrd Station about 0 8 sec later (below)

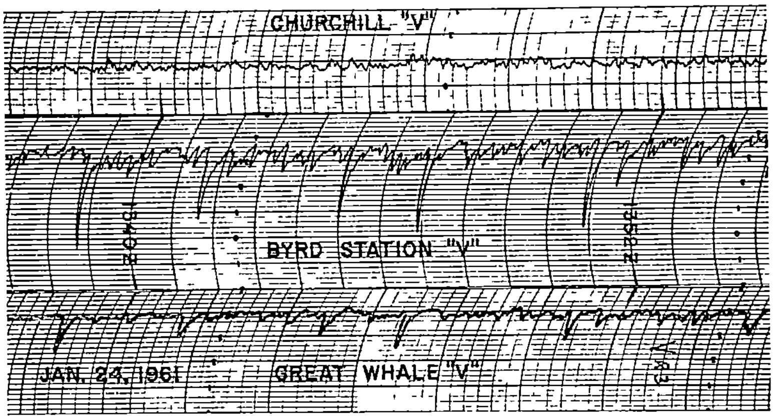

Fig 2 Envelopes of the activity received on the 2–30 c p s (V) system at three auroral stations The record for Churchill is in inverted relation to the other two

ates of these stations are 70 4° S , 337 5° E , 67 8° N , 350° E , and 68 7° N , 322 8° E , respectively

Stanford very low frequency equipment consisted essentially of a vertical single-turn loop antenna with an effective area of 80 square metres, coupled to a broad-band audio-frequency amplifier[1] The signals were recorded on magnetic tape Observations were made at synoptic intervals of 2 min each hour so that later comparison could be made with data from the various stations of the Whistlers-East and Whistlers-West networks Of particular interest in this regard were the observations made at Knob Lake, Canada, 67 5° N , 1° E geomagnetic, some 400 miles south-east of the Vestine point conjugate to Byrd Station These records were kindly loaned by Dr M G Morgan Observations taken at points along the magnetic meridian passing through Byrd Station (very low frequency traverse) were also available

Pacific Naval Laboratory equipment[2] at the conjugate stations consisted of multi-turn mumetal strip-cored detectors oriented to measure the geomagnetic fluctuations between 0 003 and 3 c p s in the conventional *X*, *Y* and *Z* geographical axes At pre-arranged times the *X*, *Y* and *Z* detectors at these stations were sharply tuned to one of four frequencies (0 8, 1 1, 1 6 or 2 2 c p s) Single components were monitored at Ottawa and Victoria In addition, signals in the 2–30 c p s bands were received on horizontal two-turn loops of 3,000 ft circumference The *X*, *Y* and *Z* outputs and the envelope of the 2–30 c p s band were continuously recorded on Esterline-Angus charts, and magnetic tape records were made at pre-arranged intervals

Analysis of synoptic very low frequency records taken at Byrd Station and Knob Lake has shown that various strong bands of very low frequency emission activity in the 200–1,200 c p s range are often present at the same times Table 1 shows the correlation coefficients versus frequency calculated from simultaneous intensity measurements It was found that the emission bands showing a distinct lower frequency cut-off at Byrd Station appeared more frequently at Knob Lake than those bands showing no distinct lower border Discrete bands of very low frequency emissions were also often closely related Fig 1 shows a sample of the close correlation in time and character of noise bursts at about 5 kc p s as observed at Byrd Station and Knob Lake The emissions shown arrive at Knob Lake 0 8 ± 0 1 sec prior to their appearance at Byrd Station Timing at both Byrd and Knob Lake was measured independently, utilizing the time pips from Station *NBA* so that the only significant limitation on the accuracy of the measurements is the lack of definition of the emissions themselves (Typical whistler time delays at 5 kc p s for one traverse of the path at these latitudes lie between 1 5 and 2 5 sec)

Signals recorded in the 2–30 c p s range also show close conjugate correlation Fig 2 shows a comparison of the envelopes taken at Byrd, Great Whale and Churchill It can be seen that the first two have a higher degree of correlation, not only of the bursts in amplitude, but also in much of the detailed structure between the bursts

Although only a few magnetic tapes of these extra low frequency signals have been analysed to determine the spectral composition, it seems that four distinct and independent bands of noise are often present These bands are usually centred about 8, 14 and 20 c p s , and one, more variable than the others, centred between 25 and 30 c p s The bands centred about 8 c p s and 14 c p s predominate (Fig 3) and are found at all three auroral stations The amplitude bursts in Fig 2 are associated with enhancements in the lower frequency bands, usually 8 c p s It seems probable that each band is made

Table 1 SPEARMAN RANK CORRELATION COEFFICIENT VERSUS FREQUENCY FOR 48 CONSECUTIVE HOURLY SAMPLES OF VERY LOW FREQUENCY NOISE AT BYRD AND KNOB LAKE

Frequency in c p s	200	300	400	500	700	1,000	2,000	3,000	4,500	7,000	10,000
Correlation coefficient	+0 46	+0 48	+0 51	+0 59	+0 48	+0 52	+0 14	+0 04	+0 26	+0 30	+0 17

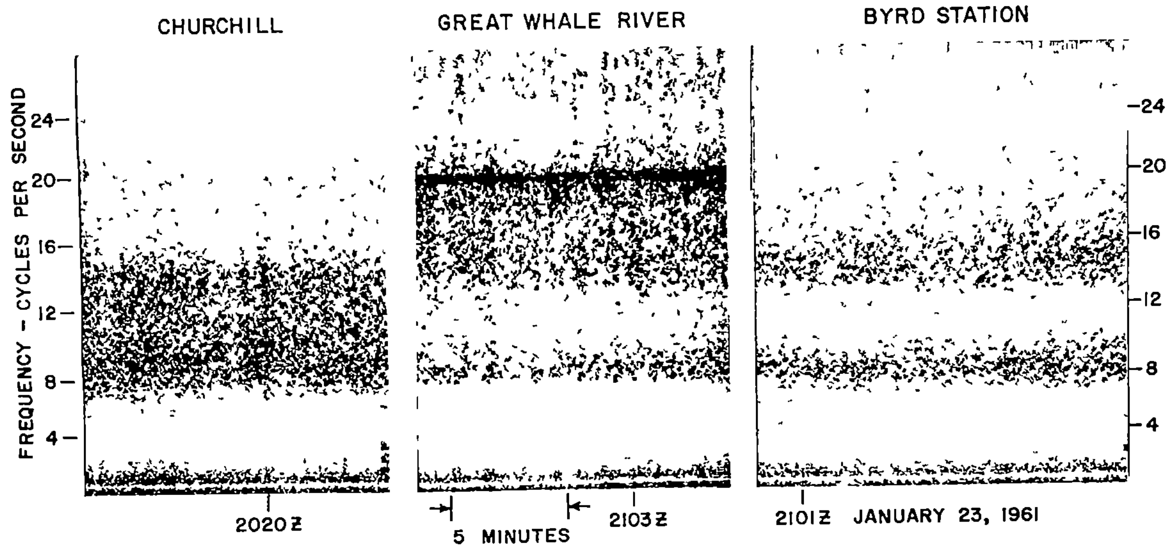

Fig 3 The spectral distribution of signals received by the 2–30 c p s system at three auroral stations at nearly similar times The narrow band near 20 c p s at Great Whale River is of undetermined origin

up of a multitude of discrete signals, similar to 'chorus' in the very low frequency regions In the frequency-range below 30 c p s world-wide lightning is a chief source of noise received on a vertical loop However, lightning does not appear to contribute substantially to signals received on the horizontal loop

In the micropulsation-range (0 02–3 c p s), although activity at the auroral stations was significantly more complex, during undisturbed times all stations often showed a marked similarity Enhancements of activity accompanied by visible auroræ usually showed a closer relationship in time and character between Great Whale River and its near conjugate at Byrd Station than between any other pair Auroræ were, of course, not visible in the antarctic summer, but from the character of the micropulsation records they were probably present Enhancements due to geomagnetic activity associated with sudden increase on ordinary magnetograms were received by the 2–30 c p s equipment on several occasions These were generally due to frequencies well below 2 c p s which were of large amplitude and usually coincident within 2 min at Byrd Station and Great Whale, while their occurrence and timing were often more independent at Churchill

It seems that the similarity of records taken at Great Whale and Byrd results from their close conjugate relationship This view is supported by the generally lower correlation of Churchill with Great Whale and Byrd Very low frequency observations taken in the Antarctic show little correlation of activity below 3 kc p s at stations 1,200 km apart (Byrd Station and South Pole Station), and results from the very low frequency traverse indicate that at these frequencies significant differences between simultaneous recordings appear at distances of 200 km Although this suggests that much of the very low frequency emission activity observed in the auroral regions may be initiated in the lower ionosphere, it does not exclude the possibility that these emissions may be generated at a much greater height The small time difference between the occurrence of identical events at opposite ends of a field line suggests that these events may result from particles impinging on the Earth's magnetic field not far from the equatorial plane

This work was supported in part by National Science Foundation grant 13532 and partly by the Defence Research Board of Canada under Project *D*45-95-11-39

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INTERPLANETARY DUST NEAR THE EARTH*

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THE problem of gravitational accretion of interplanetary dust[1–3] by a planet has recently been the subject of renewed attention In particular, the possibility of observing dust impacts experimentally at various distances from the centre of the Earth by means of rockets, satellites and space probes has stimulated an inquiry into the concentration of dust around the Earth[4,5] Beard[4] has predicted that the dust concentration should increase as the inverse 3/2 power of distance, that is, as $r^{-3/2}$, and by a factor of 10^3 over the value in interplanetary space Whipple[6] has recently published a compilation of rocket and satellite data which exhibits a rapid increase of counting-rate as the Earth is approached, by about

* Substance of a paper at the Spring Meeting of the American Geophysical Union, Washington, D C, April 1961

10^5 He identifies this phenomenon with the existence of a permanent 'dust belt' around the Earth

The purpose of the present article is to point out that a more complete theory does not lead to a continuous increase in dust concentration as Earth is approached, but rather to a maximum at about 2,000–3,000 km above sea-level, giving rise to a 'dust shell' The increase amounts to a few times the value of the concentration of dust in interplanetary space, rather than a factor of 1,000 as given by Beard[4,7] However, since detectors do not measure concentration, but instead count particles having a certain momentum above a minimum value, the expected increase in counting-rate as one approaches the Earth is in fact larger, but still not in accord with Whipple's compilation We are therefore left with the conclusion (1) that other factors besides gravitational enhancement take a role in a proper theory, for example, particles in captured orbits[5,6,8], or (2) that the variations with time in the intensity of the dust particles are very strong so that this effect must be taken into account in assembling a compilation

First I will neglect all forces except gravitational forces, hence, the results given here apply to large dust particles, certainly for dust particles of the order of 100 microns (For smaller dust particles radiation pressure forces must be considered, as well as force arising from the electric charge of the particle, notably the Lorentz force and Coulomb drag[8]) It is assumed that the geocentric velocities of these dust particles are isotropically distributed This means that at large distances from the Earth, where the gravitational potential due to it is negligible, the particles have randomly oriented velocities with respect to the Earth Furthermore, these velocities are small, of the order of a few km per sec This conclusion is arrived at by considering the action of the Poynting–Robertson effect on the orbits of small dust particles[2,9]

The following notation is used distances r are measured in terms of R, the radius of the planet, denoting $y = R/r$, velocities are measured in terms of $v_\infty = (2GM/R)^{1/2}$, the escape velocity from the surface of the planet The energy ratio (GMm/R) to $(\frac{1}{2} mu^2)$, where u is the (geocentric) velocity at infinity of the particle, will be denoted by E Thus

$$E = 2GM/Ru^2 = v_\infty{}^2/u^2 \qquad (1)$$

From conservation of energy it follows that

$$\tfrac{1}{2} mv^2 = \tfrac{1}{2} mu^2 + GMm/r \qquad (2)$$

Hence

$$v^2 = u^2 + v_\infty{}^2 y \qquad (3)$$

At a level determined by r, the speed of the particle is defined by equation (3) The allowed directions are defined as follows at any point r (i) particles incident with zenith angle θ between 0 and a certain value θ_c become accreted by the planet and therefore contribute to the density only when moving towards the planet, (ii) particles incident with θ between θ_c and $\pi/2$ return again to infinity and therefore contribute twice

The angle θ_c corresponds to the trajectory which grazes the surface of the planet Hence, by conservation of angular momentum

$$rv \sin \theta_c = Rv_R \qquad (4)$$

where

$$v_R{}^2 = u^2 + v_\infty{}^2$$

Hence

$$\sin^2 \theta_c = y^2 \frac{u^2 + v_\infty{}^2}{u^2 + v_\infty{}^2 y} \qquad (5)$$

I now make the reasonable assumption that the geocentric velocities u of the particles are isotropically distributed, that is, they are at large distances from the Earth I also take a unique value of u, although my approach can be generalized in a straightforward manner to deal with a distribution of velocity values

Since by Liouville's theorem the phase space density D remains constant, the density ρ at r will be given by

$$\rho(r) = c[\int_0^{\theta_c} v \sin \theta \, d\theta + 2\int_{\theta_c}^{\pi/2} v \sin\theta \, d\theta]$$
$$= C(u^2 + v_\infty{}^2 y)^{1/2} (1 + \cos \theta_c) \qquad (6)$$

With the help of equation (5)

$$\rho(r) = C(u^2 + v_\infty{}^2 y)^{1/2} \left[1 + \left(1 - y^2 \frac{u^2 + v_\infty{}^2}{u^2 + v_\infty{}^2 y}\right)^{1/2}\right] \qquad (7)$$

The normalization constant is obtained by setting $y = 0$

$$\rho_\infty = 2Cu$$

If all velocities are now expressed in terms of v_∞, then

$$\frac{\rho(y)}{\rho_\infty} = \left(\frac{u^2 + y}{4u^2}\right)^{1/2}\left[1 + \left(1 - y^2 \frac{u^2 + 1}{u^2 + y}\right)^{1/2}\right] \qquad (8)$$

The function is plotted in Fig 1 and shows some extremely interesting features. In the first place, it is seen that the density in cislunar space, in particular close to the Earth, is always enhanced over the density in interplanetary space The enhancement is particularly pronounced for small values of u The remarkable feature, however, is the fact that a pronounced peak exists always above the atmosphere

When u exceeds $3^{-1/2}$ the density ρ_R very close to the Earth drops below the free-space density This can be seen more clearly by setting $y = 1$ in equation (8) Hence

$$\rho_R/\rho_\infty = [(u^2 + 1)/4u^2]^{1/2} \qquad (9)$$

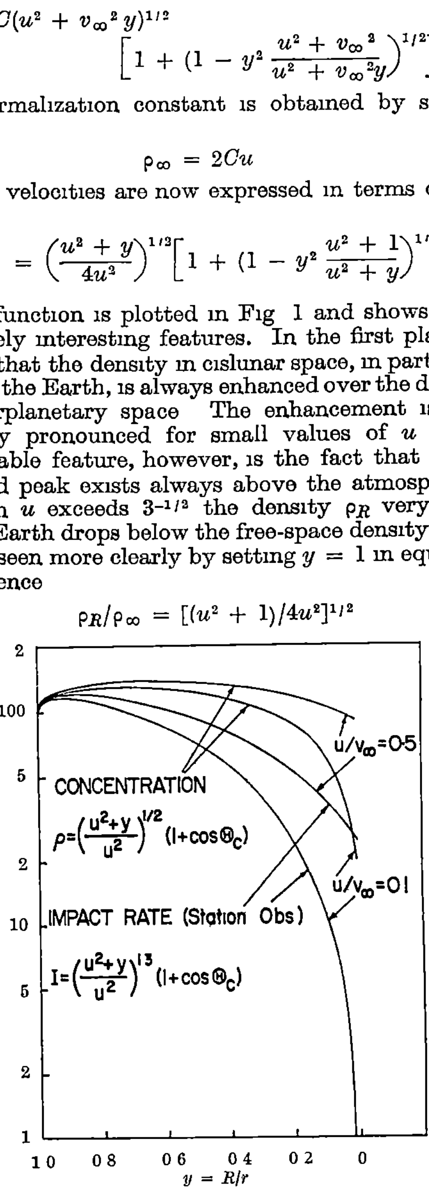

Fig 1 Concentration of zodiacal dust as a function of distance from the centre of the Earth Also plotted is the impact-rate which would be observed by an instrument moving at very low speed with respect to the Earth (case 1) The graphs convey the spread to be expected with geocentric velocities of the zodiacal dust in interplanetary space of about 1–5 km per sec

which for small values of u reduces to $(2u)^{-1}$ and for large values of u to 1/2.

For small values of u also equation (8) can be replaced by

$$\rho(y)/\rho_\infty = (2u)^{-1}\, y^{1/2}\,[1 + (1 - y)^{1/2}] = \rho_R\, y^{1/2}\,[1 + (1 - y)^{1/2}] \qquad (10)$$

We can investigate the remainder of the expression (10). It reduces to 1 at $y = 1$. If we replace y by $1 - \varepsilon$, then the expression reduces to $1 + \varepsilon^{1/2}$. Therefore, we see that there is always a peak below $y = 1$, and therefore at some altitude above sea-level. This peak is produced by a 'shadow effect' of the planet.

The rate at which dust particles are accreted by the Earth can also be investigated; this rate is given by

$$\rho_\infty\, (\pi R^2)\, u\, (1 + v_\infty{}^2/u^2) \qquad (11)$$

The expression is seen to have a minimum value when the geocentric velocity u equals the sea-level escape velocity v_∞ (= 11·2 km./sec. for the Earth). From the equation of continuity we can also deduce the mean radial velocity near sea-level. It is equal to equation (11) divided by ρ_R. Using equation (9) we obtain

$$v_{\text{rad}} = 1/2\,(u^2 + v_\infty{}^2)^{1/2} \qquad (12)$$

Comparison with Observations

We can use the results of the preceding section to calculate two quantities which can be checked by observations: (1) the absolute value of the rate of accretion by the Earth; (2) the relative counting-rate of a meteor impact detector as a function of distance from the Earth (the absolute rate depends on the sensitivity of a particular detector).

(1) *Accretion.* A normalization must be made to the density of interplanetary dust in the vicinity of the Earth's orbit. Photometric investigations based on measurements of the zodiacal light give values for ρ_∞ which vary in the range of 2×10^{-23} to 5×10^{-21} gm./cm.³ (refs. 10 and 11). Using this range of values in eq. (11), Table 1 is obtained.

Table 1. ACCRETION OF INTERPLANETARY DUST TO THE EARTH (in tons per day)

ρ_∞ (gm./cm.³)	u/v_∞ = 0·1	0·2	0·5	1·0
2×10^{-23}	2,450	1,260	605	485
5×10^{-21}	$6·1 \times 10^5$	$3·1 \times 10^5$	$1·5 \times 10^5$	$1·2 \times 10^5$

An estimate for the minimum value of u/v_∞ can be obtained by assuming the heliocentric orbits of the dust particles to be completely circular and inclined to the ecliptic at an angle of about 2°, a mean value for the nearer planets. The Earth's orbital velocity is 29·8 km./sec., giving a geocentric velocity of the dust particles of more than 1 km./sec. Again, since the eccentricity of the Earth's orbit is 0·0176, the perihelion to aphelion velocity ratio turns out to be $1 + \varepsilon/(1 - \varepsilon)$, or about 3 per cent of the orbital velocity. We can therefore take $u = 1$ km./sec. as a lower limit for the geocentric velocity of interplanetary dust, with most of the dust component responsible for the zodiacal light in the range 1–5 km./sec. ($u/v_\infty \sim$ 0·1–0·5).

It can then be seen from Table 1 that the lower limit of ρ_∞ of about 2×10^{-23} gm./cm.³ gives an accretion-rate in accord with the generally quoted value of about 1,000 tons per day.

(2) *Rate of impact.* Before any comparison is made with observations, it must be realized that it is never the concentration, given by equation (8), which is measured. Generally, an acoustic detector counts the number of particles which impart a momentum greater than some minimum value p_m.

But assuming (1) that detectors have similar values of p_m, and (2) that the momentum of impact is proportional to particle momentum (at least over the range of velocities encountered here), we can calculate the expected counting-rate vs. distance. (Actually, the results are fairly insensitive to assumption (1), see ref. 12.) However, it is quite important to bring in another factor, namely, the velocity of the vehicle on which the detector is mounted.

I have therefore considered three cases[12]: Case 1, stationary observer, that is, a rocket near peak altitude; Case 2, a space probe moving vertically outward with local escape velocity; Case 3, a satellite in circular orbit.

The results for Case 1 are shown in Fig. 1, using a dust particle cloud the particle-radii of which follow the law $f(a)da = a^{-2·8}\,da$. It can be seen that a peak still exists for the rate of impact but at a lower altitude than for concentration. However, the total variation of impact-rate is much greater between infinity and the vicinity of the planet: it increases between a factor of 4 (for u/v_∞ = 0·5) up to a factor 200 (for u/v_∞ = 0·1).

Conclusions

My results predict the existence of a modest dust shell around the Earth, instead of a variation with a factor of 10³–10⁵ increasing uniformly without a maximum[4,6].

Experimental evidence concerning a concentration of interplanetary dust in the vicinity of the Earth is as yet very scanty. The data come from different detectors, different types of vehicles, namely, rockets, satellites, and space probes (corresponding to the three cases mentioned), and were obtained at different times[13]. Discrepancies among individual measurements amount to orders of magnitude at times[14], indicating the possibility of real variations with time, due perhaps to a non-zodiacal dust component.

It appears to be premature, therefore, to draw any firm conclusions from the experimental data at present available about the existence of a dust shell of the type discussed here. Nevertheless, a confirmation of its presence with a maximum as shown, for example by Fig. 1, would be of great importance in establishing the validity of the assumptions underlying the theory of the present work.

I thank M. Dubin, E. J. Öpik and C. W. McCracken for illuminating discussions. This work was supported by a grant of the National Aeronautics and Space Administration, *NsG*-58-60.

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NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF PROTEINS: THE HYDROGEN BONDING OF WATER IN BOVINE SERUM ALBUMIN SOLUTIONS

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THE nature of the 'water of hydration' in aqueous protein solutions has been the subject of much investigation and discussion[1-4], but there is little direct experimental evidence as to whether it exists in a 'frozen' form, comparable with ordinary ice[3], or in some less highly organized state It appeared that measurement of the nuclear magnetic resonance peak positions of water protons in concentrated protein solutions might furnish some information on this point In this communication results are presented for bovine serum albumin solutions at 25° and *p*H 6 8

Arnold and Packard[5] have observed that the chemical shift of the hydroxyl proton signal of ethanol is dependent on temperature, moving upfield by about 1 5 p p m (with respect to the methylene signal) over the temperature range $-117°$ to 78° This was believed to arise from the breaking of hydrogen bonds[6] Similar shifts have been observed on dilution of alcohols[7-9] and phenols[10,11] with non-polar solvents and have been satisfactorily interpreted as due to the dissociation of hydrogen-bonded polymers

Cohen and Reid[7] observed that at 29° the peak position for water dissolved in carbon tetrachloride is at a field 3 68 p p m higher (after bulk magnetic susceptibility correction) than for liquid water Schneider, Bernstein, and Pople[12] found that for water vapour the peak position is 4 58 p p m higher than for liquid water at 0°, susceptibility correction being made Very recently, Tiers and Kowalsky[13] have found that the peak position for liquid water, measured with respect to the methyl group resonance of dissolved sodium 2,2-dimethyl-2-silapentane-5-sulphonate as internal reference (assigned a scale value $\tau' = 10\ 000$), moves to higher field with increasing temperature At 0°, $\tau' = 4\ 983$, at 25°, $\tau' = 5\ 219$ The peak position reported by Cohen and Reid[7] for water in carbon tetrachloride corresponds to 8 93 on the τ' scale The vapour value of Schneider *et al* [12] is approximately 9 56 on the τ' scale, but to be useful this value requires a rather large negative correction for 'solvent shift'[14], which appears to take place for all substances on passing from the vapour to the liquid state, whether hydrogen-bonded or not Since the magnitude of this correction for water is unknown, the value 8 93 τ', based on water in carbon tetrachloride, has been chosen as representing the best approximation to the resonance position of water protons when all hydrogen bonds are broken Assuming, with Pauling[15], that in ice every proton is involved in a hydrogen bond and that in liquid water at 0°, 15 per cent of these bonds are broken, I calculate that for completely hydrogen-bonded liquid water, if it were capable of existence, we would observe the proton resonance at 4 23 τ' We thus have a total difference of chemical shift of 4 70 p p m between hydrogen-bonded and non-hydrogen-bonded protons in water, and since peak positions are reproducible within ± 0 005 p p m in the present work (see below), a change in hydrogen-bonding involving 1 per cent of the water protons should be measurable, while changes involving only a few tenths of 1 per cent should be easily detectable

A 'Varian *V*-4300-2' 40 00-Mc per sec spectrograph was used, together with a 'Varian' variable-temperature probe, sample spinner, audio-oscillator, Hewlett–Packard '522-*B*' frequency counter and 'Varian' recorder Approximately 0 5 ml samples of protein solutions were used, using 5 mm (outside diameter) 'Pyrex' tubes Samples were maintained at 25 0 ± 0 2°, maintenance of a constant temperature is particularly important when external referencing is used, as described later All tubes were evacuated and freed of air by successive freezing and thawing, they were then sealed under 0 5 atmosphere of pre-purified nitrogen

The bovine serum albumin used was purchased from Pentex, Inc (Kankakee, Ill) and was designated 'Lot 9*G*–10', it was found to contain 3 5 per cent moisture Solutions were prepared in deionized water The *p*H of all solutions was found to be 6 80 ± 0 05, no buffers or salts were added

For measurement of water peak positions, internal referencing was first attempted It was found that, measured with respect to sodium 2,2-dimethyl-2-silapentane-5-sulphonate (2 per cent)[13], the water peak of an 11 9 per cent bovine serum albumin solution shifted $-0\ 102$ p p m compared with pure water It was also noted that the reference peak, normally narrow and prominent, became increasingly broad at higher protein concentrations, being barely observable in the 11 9 per cent solution and unobservable in the 23 9 per cent solution This appeared to indicate that the sulphonic acid anion was fairly strongly adsorbed by the protein and was experiencing the latter's much longer correlation time[16] It therefore could not be regarded as sampling all regions of the solution indifferently, as a reliable internal reference should It was found that 1 per cent of 2,2-dimethyl-2-silapropanol-1 (ref 13) showed much less but still perceptible broadening of the methyl peak and gave also a smaller apparent shift, $-\ 0\ 072$, of the water peak in the 11 9 per cent solution *Tert*-butanol (2 per cent), which is much more soluble than the silapropanol, showed no broadening and an apparent shift of water peak of only $-\ 0\ 019$ in 11 9 per cent protein solution To avoid any suspicion of spurious shifts, however, it seemed best to rely chiefly on an external reference For this purpose, hexamethyldisiloxane (obtained from the Dow-Corning Corp) was sealed into 1 mm (internal diameter) capillaries, which were then inserted into the 5 mm nuclear magnetic resonance sample tubes Peak positions were measured by the 'frequency changing' method already described by Tiers[17] At least six measurements were made on each system, values obtained on increasing and decreasing sweeps were averaged to eliminate systematic error from this source. Measurements were found to be reproducible within ± 0.005 p.p.m. average deviation at most, usually the limits were narrower. Only differences in peak positions between water and protein solutions were of interest, and therefore no correction for difference of bulk diamagnetic susceptibility between solvent and reference was necessary.

Solutions of methylamine hydrochloride, potassium acetate, and potassium butyrate were prepared from reagent or C.P. materials and their *p*H values were adjusted to within ± 0.01 unit using a 'Radiometer' *p*H meter. The nuclear magnetic resonance tubes were sealed under purified nitrogen in the same manner as the protein solutions.

Water proton peak positions were measured at 25.0° with respect to hexamethyldisiloxane as external reference for pure water and for solutions containing 0.030, 0.060, 0.119 and 0.239 gm. of bovine serum albumin per c.c. of solution (W_2). There was an appreciable broadening of the signal at higher protein concentrations (up to approximately 5 c.p.s. half-height width at $W_2 = 0.239$), but this did not impair the measurements. The observed differences in peak position are shown in the third column of Table 1, in which $\Delta\delta(\delta_{\text{water, protein sol.}} - \delta_{\text{water}})$ is recorded. Because the diamagnetic susceptibility of the protein solution differs somewhat from that of water, a correction must be made when employing an external reference. The volume susceptibility χ_s of the protein solution can be calculated from

$$\chi_s = W_1\chi_1 + W_2\chi_2$$

where χ_1 and χ_2 are the gram susceptibilities of water and (dissolved) protein, respectively. We have taken χ_1 as -0.721×10^{-6} (ref. 18*a*) and have calculated χ_2 for bovine serum albumin from the Pascal constants and constitutive corrections, as listed by Pople, Bernstein and Schneider[18b]. In column four of Table 1 the calculated values of χ_s are listed. From these the value of the corrected shift of the water peak, $\Delta\delta_{\text{corr}}$ in column 5 is calculated, using the relationship[19]

$$\Delta\delta_{\text{corr}} = \Delta\delta_{\text{obs}} + \frac{2\pi}{3}(\chi_1 - \chi_s)$$

It can be seen that, within experimental error, the differing magnetic susceptibility is entirely responsible for the apparent shift in the water peak of the protein solution.

In the most concentrated bovine serum albumin solution ($W_2 = 0.239$), it can be calculated that the carboxylate group concentration is approximately 0.38 *M*, the ammonium and guanidinium group concentration (not considered separately) approximately 0.32 *M*, and the peptide group concentration approximately 2.2 *M*. Direct measurement at *p*H 6.8 of effect of methylammonium ion (0.5 *M*), acetate and butyrate ion (1 *M*), and *N*-methylacetamide (2.5 *M*), as models for these groups, showed that in the protein solutions they would not affect the position of the water peak within the experimental error of measurement.

It appears that even in very concentrated bovine serum albumin solutions there is no change in the hydrogen-bonding of the water molecules which involves more than 0.1–0.2 per cent of the water protons. By 'change' is meant a disruption of the hydrogen bonds normally existing in water, or the formation of new additional hydrogen bonds (see below) whether with other water molecules or with the protein.

Table 1. OBSERVED AND CORRECTED RELATIVE PROTON PEAK POSITIONS FOR WATER IN SOLUTIONS OF BOVINE SERUM ALBUMIN (BSA), 25.0°

BSA conc., gm./c.c. solution (W_2)	Water conc., gm./c.c. solution (W_1)	$\Delta\delta_{\text{obs}}$	$-\chi_s \times 10^6$	$\Delta\delta_{\text{corr}}$
0	1.000	0	0.721 ($=\chi_1$)	—
0.030	0.978	−0.006	0.724	+0.002
0.060	0.955	−0.008	0.727	+0.006
0.119	0.912	−0.031	0.733	−0.004
0.239	0.821	−0.060	0.746	−0.006

Let us first suppose (somewhat naively) that protein molecules, on being introduced into water, have no effect on the structure of the water other than to interrupt it at the protein–water interface. Taking the bovine serum albumin molecule as a cylinder 145 Å in length and about 30 Å in mean diameter[20], it can be estimated that about each there will be a monolayer containing at least 1,000 water molecules, corresponding to about 28 per cent 'hydration'. Assume that each interfacial water molecule suffers the breakage of one hydrogen bond. In a solution for which $W_2 = 0.24$ and $W_1 = 0.82$ (Table 1), it is readily calculated, assuming a molecular weight of 65,000 for the protein, that about 5 per cent of the total hydrogen bonds of the water would be broken. This should produce a $\Delta\delta$ of $+0.235$, exceeding the experimental error by fifty-fold. It seems, then, that the interruption of the water structure must be compensated by the formation of other hydrogen bonds, either to the peptide linkages or other polar groups of the protein, or in some other way. We have seen that the interaction of water molecules with carboxylate, amino, and peptide linkages in the concentration present in such a protein solution has an essentially negligible effect on the water resonance position, so that the replacement of water–water hydrogen bonds by bonds to these groups is not directly seen as such.

In addition to forming hydrogen bonds with the protein, the interfacial water molecules may become re-arranged in such a way as to form bonds among themselves to the same degree as in water alone, despite the interrupting interfaces. It has been recognized for some time[21] that when hydrocarbons are dissolved in water the heat effect is often slightly negative but that there is a large decrease in entropy, indicating a clustering of water molecules about the intruding hydrocarbon but no breakage of hydrogen bonds. These observations have been recently reviewed and their significance for protein structure pointed out by Kauzmann[4]. It is not unreasonable to suppose that a similar clustering of water molecules occurs about the hydrophobic portions of dissolved proteins (at least 40 per cent of the amino-acid residues of bovine serum albumin can be regarded as hydrophobic), the present results appear to be consistent with this supposition.

Kauzmann has pointed out[4] that the 'icebergs' presumed to form about hydrophobic areas of the protein should not be identified with the 'frozen water' which has been thought by several authors to exist around proteins, and to which a very important part in protein structure and function has been assigned by Klotz[3]. The hydrophobic icebergs have in fact a relatively low thermodynamic stability and would tend to lead to aggregation and insolubility. The nature of this 'ordered' or 'frozen' water, which presumably constitutes the major share of the water of hydration, is still in doubt. The evidence presented here indicates that this ordered water cannot be regarded as having a structure like that of ice (a conclusion also indicated by other lines of evidence) If we suppose that the water of hydration (taken as 0 28 gm per gm protein) has an ice-like structure and that its protons exchange rapidly with the surrounding water, I expect that in the bovine serum albumin solution in which $W_2 = 0\ 24$, a $\Delta\delta$ of approximately $-$ 0 067 would be observed, compared with pure water, which again is well outside my experimental precision It is possible, of course, that there is a disruption of water hydrogen bonds near hydrophobic areas of the protein, and that this is just compensated by more extensive bonding in the 'ordered' or 'frozen' portions of the hydrate water But it appears more logical at present to regard the present results as supporting Kauzmann's conclusions concerning the nature of the water in the hydrophobic areas, and to regard the remainder of the hydrate water as having a degree of hydrogen bonding equal to that of pure water

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PURIFICATION OF URINARY CONJUGATED ŒSTROGENS BY GEL FILTRATION

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A GEL filtration method for de-salting and group-separation of water-soluble substances has recently been worked out by Porath and Flodin[1] This method is based on a column procedure similar to chromatography in which the stationary phase is comprised of a new type of gel ('Sephadex', manufactured by Pharmacia, Ltd, Sweden) The present report suggests that this technique might be of use for the purification and group-separation of conjugated œstrogens in pregnancy urine It might also form the basis of a simple assay method for determination of œstrogens in pregnancy urine

In the early experiments a solution of 5 μgm of sodium œstriol glucusiduronate (Sigma, Ltd, U S A) and/or sodium œstrone sulphate (Schering, Ltd, Germany) in 1 ml of 0 15 *M* acetate buffer (*p*H 7 0) was applied directly to a 1 × 50 cm. 'Sephadex' (*G*25) column As soon as this solution was percolated into the gel, elution was performed with distilled water One-ml. fractions of the effluent were collected and, after evaporation of the tubes. the amount of œstrogen present in each fraction was measured by a modified Kober reaction[2] In these experiments the two conjugated œstrogens were eluted in one distinct peak, which was invariably found in fractions 40–43 Other conjugated œstrogens, such as the sodium salts of 17β-œstradiol-17-glucusiduronate, 17β-œstradiol-3-sulphate, 17β-œstradiol-17-sulphate, œstriol-3-sulphate, 17β-œstradiol-3-phosphate and 17β-œstradiol-17-phosphate, were also eluted in fractions 40–43

In the next series of experiments the conjugated œstrogens listed above were added to male urine which was then percolated through the 'Sephadex' column Usually a concentration of 2–5 μgm. of conjugated œstrogen per ml of urine was used 1–20 ml of these urine samples were applied to the columns without previous purification As soon as the urine was completely percolated into the bed material, elution was performed with distilled water and 1-ml fractions of the effluent were collected Since the main part of urinary pigments was eluted in fractions 22–36, it was not possible to apply a direct Kober reaction to these tubes Therefore the individual fractions were incubated with an extract of the digestive tract of the snail (*Helix*

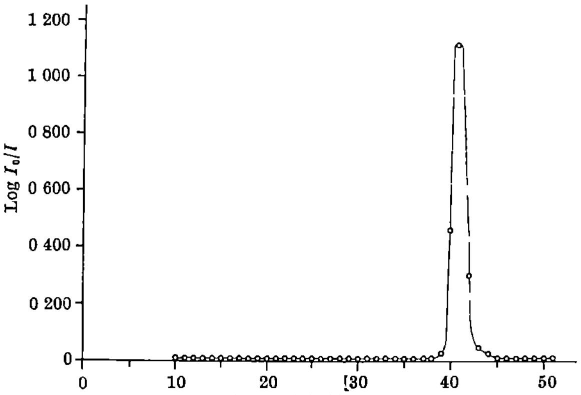

Fig 1 Elution curve obtained by gel filtration of 2 ml of male urine containing 10 μgm of sodium œstriol glucusiduronate 'Sephadex' (*G*25) column 1×50 cm, eluant distilled water, running time 4 hr

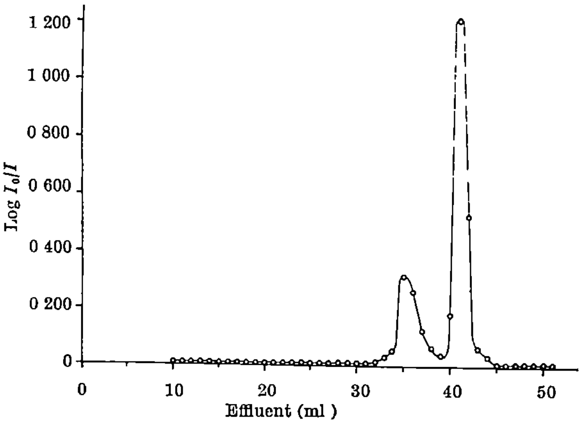

Fig. 2. Elution curve obtained by gel filtration of 1 ml. of pregnancy urine; experimental conditions as in Fig. 1

pomatia, purchased from L'Industrie Biologique Française) and, following enzyme hydrolysis, each fraction was extracted with ether. The ether was evaporated and the amount of œstrogen liberated was measured by the Kober reaction. Under these experimental conditions all the conjugated œstrogens already mentioned added to male urine were eluted in one single zone (fractions 40–43) as shown in Fig. 1.

Approximately 90 per cent of the conjugated œstrogens added to male urine could be recovered within this zone. Also in those experiments where as much as 20 ml. of the urine samples were applied to the columns, all the Kober-positive material was found to be concentrated to fractions 40–43.

On the other hand, when late-pregnancy urine was processed under the same conditions, two distinct peaks were found, one in fractions 33–36 and the other in fractions 40–43 (Fig. 2).

As judged from the elution curve, the second peak of pregnancy urine corresponds to the single peak (Fig. 1) obtained from male urine containing one or more of the reference compounds already named of conjugated œstrogens. It was also found that the addition of small amounts of œstriol glucusiduronate and œstriol sulphate to late pregnancy urine (5–10 µgm. per ml.) resulted in a definite enlargement of the second peak of the elution curve. About 90 per cent of the Kober-positive material present in the two peaks obtained from pregnancy urine was characterized by the Brown method as œstriol. The predominant conjugate of the second peak was found to be identical with œstriol-16 (17 ?)-glucusiduronate. In preliminary work on the conjugated œstrogen present in the first peak, one main compound was found. This appears to be a form hitherto unknown of conjugated œstriol. In paper-partition chromatography or in countercurrent distribution work this compound was found to be more polar than œstriol glucusiduronate.

The gel-filtration technique seems to be most useful for group-separation and purification of conjugated œstrogens in pregnancy urine. Since the recovery from the columns is almost quantitative, the gel filtration seems to be suitable for the isolation of unknown conjugated œstrogens.

As judged from the high yield obtained after enzyme hydrolysis of the purified fractions, the enzyme inhibitors usually present in urine specimens seem to be removed to a considerable extent by the column procedure. This fact made it possible to develop a quantitative method for the estimation of œstrogen in pregnancy urine. The method is based upon gel filtration and enzyme hydrolysis of the purified fractions containing the Kober-positive material. An additional advantage of the proposed method is that after the elution with distilled water the gel is completely regenerated. Furthermore, the columns can be used daily for several weeks without repacking. Since one technician can easily carry out up to 100 determinations a week, this method seems to provide a suitable means for serial estimations of urinary œstrogen excretion in normal as well as in pathological forms of pregnancy.

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CHROMIUM-51 TAGGING OF THE α-CHAIN OF HUMAN HÆMOGLOBIN

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THE separation of the α- and β-chains of hæmoglobin by elution from 'Amberlite *CG*-50', utilizing a urea gradient at *p*H 1.9, has become an almost routine procedure in the study of the tryptic peptide patterns of hæmoglobin[1]. Work by Pearson[2] and by Ebaugh[3] indicated that $Na_2{}^{51}CrO_4$ preferentially labels the β-chain of human hæmoglobin, and these observations suggested to me a method for determining the degree of cross contamination of one chain by the other when separated on 'Amberlite' columns. Under the conditions of my experiments, however, tagging of the hæmoglobins by chromium-51 was almost exclusively limited to the α chain of human hæmoglobin *A*.

Hæmoglobin solutions were prepared by standard techniques, following washing of the red cells with normal saline, by lysis with water and toluene. One sample of hæmoglobin was further purified by crystallization from ammonium sulphate solutions following Drabkin's method[4]. Globin preparation was carried out by the technique of Anson and Mirsky[5]. 'Radiochromate' (Abbott) of high specific activity (0.547 mc. chromium-51/ml., 0.00118 mgm. chromium/ml.) was utilized to tag the various hæmoglobin or globin preparations by any one of several methods. Globin (1 gm./100 ml.) was dissolved in 11.7 per cent formic acid, *p*H 1.4, and approximately 80 µc. of chromium-51 added. Following incubation for 90 min. at 32°, the solution was dialysed against water at 4° until no further chromium-51 was lost. The preparation was found to have bound 13 µc. chromium-51/gm. of globin or 0.71 × 10^{-4} mole chromium/mole globin. To

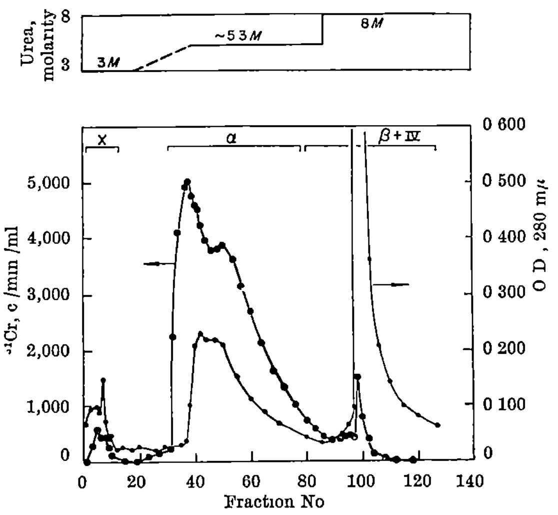

Fig 1 Separation of α- and β-chains of chromium-51 globin-*A* on '*IRC*-50' Interrupted urea gradient, *p*H 1 9

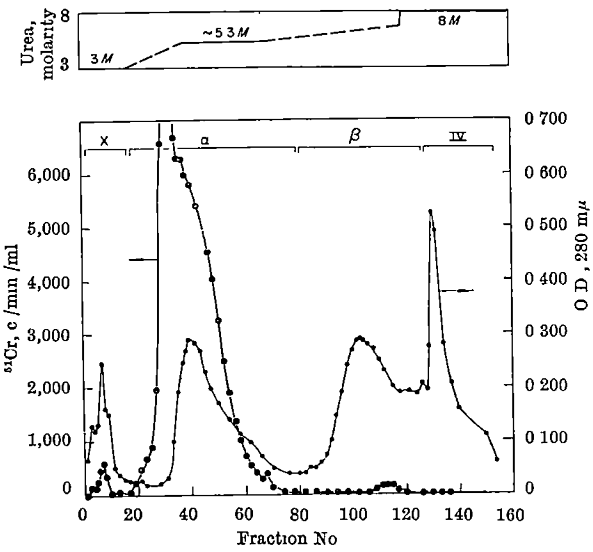

Fig 2 Separation of α- and β chains of chromium-51 on '*IRC*-50' Interrupted urea gradient, *p*H 1 9

hæmoglobin solutions (approximately 1 gm /10 ml) were added the required amounts of dry reagents to constitute a standard acid–citrate–dextrose solution, while purified hæmoglobin powders were dissolved directly into acid–citrate–dextrose solution (approximately 1 gm /10 ml) In each instance, approximately 75 μc of chromium-51 were incubated with the hæmoglobin solution for 90 min at 32°, following which the preparations were dialysed until free of unbound chromium-51 The uptake of chromium-51 was approximately 22 μc chromium-51 per gram of hæmoglobin or 1 1 × 10^{-4} mole chromium/mole hæmoglobin Following chromation of the hæmoglobin, globin was prepared from the tagged material by acid–acetone cleavage

Separation of the α- and β-chains was carried out by my modification of the technique of Wilson and Smith[1] utilizing an interrupted gradient of urea to achieve wider separation of the two chains This technique, described in detail elsewhere[6], uses an initial wash of the column with 3 *M* urea, until the non-protein peak at 280 mμ has been removed (fraction *X*, Figs 1 and 2) The progress of elution is automatically monitored at 280 mμ by a direct recording instrument An increase in the urea gradient is instituted and maintained until the peak of the α-chain has been eluted (approximately 5 2–5 3 *M* urea) at which time the urea concentration is held steady until no further protein can be washed from the column (α fraction, Figs 1 and 2) The gradient may then be resumed and continued until the second major peak (β fraction, Fig 2) is eluted before switching to 8 molar urea (to remove the final pigmented material (fraction IV, Fig 2)) or elution with 8 molar urea may be instituted immediately (Fig 1) Sample counting was done in a well-type scintillation counter on 3-ml aliquots of suitably diluted material, and the results recorded as c p m / ml of solution

The results of two typical experiments are recorded in Figs 1 and 2 The former experiment involved globin prepared from a hæmoglobin solution, the latter, globin prepared from material purified by ammonium sulphate fractionation Except for some non-protein bound chromium-51 which was eluted from the column in the first 300 ml , virtually all the chromium-51 appeared in the α peak Dialysis of this fraction resulted in no loss of chromium-51 activity Tryptic peptide patterns of the material in the β + IV peak of Fig 1 showed very slight contamination of the β-fraction with α-chain peptides, while the α-chain digests were essentially free of any peptides specific for the β-chain No such contamination of the β-chain is suggested in the experiment described in Fig 2 Total recovery of chromium-51 in this experiment amounted to 96 5 per cent of the material applied to the column Calculations of the activities recovered in the various fractions from this run showed 1 75 per cent of the chromium-51 in fraction *X*, 95 8 per cent in the α-fraction, 1 95 per cent in the β-fraction and 0 5 per cent in fraction IV (Table 1) Protein recovery and c p m /mgm of protein are also listed in Table 1

Table 1

Fraction	Protein recovered* (mgm)	Protein recovered (per cent)	Chromium-51 recovered (per cent)	Activity/mgm c p m /mgm
X	0	0	1 75	—
α	290	48 4	95 8	4,290
β	213	35 5	1 95	118
IV	107	17 8	0 5	60
Totals	610	101 7	100 00	

* Protein determinations done by technique of Lowry *et al* (ref 7) Starting material, 600 mgm labelled globin *A*

Although there seems to be little doubt that, under the conditions utilized in these experiments, chromium-51 is bound to the α-chain, the work of Pearson[2] and of Ebaugh[3] suggests preferential binding to the β-chain of human hæmoglobin *A* Pearson showed twice the specific activity in hæmoglobin *H* (β_4) compared with hæmoglobin *A* ($\alpha_2\beta_2$) when the hæmoglobins were tagged with chromium-51 and separated at *p*H 7 0 on starch-block electrophoresis Also, recombination experiments at *p*H 4 7 between chromium-51 tagged hæmoglobin *A* and untagged hæmoglobin *C* yielded no label in the recombined hæmoglobin *C* fraction which, once again, suggested that the chromium-51 was in the β-chain Ebaugh reports experiments utilizing techniques similar to mine for separating the α- and β-chains of hæmoglobin *A* at *p*H 1 9 in which the label was also found in the β-chain, although no peptide analyses were done to prove this association Except for differences in the method of labelling chromium-51, and minor differences in chain separation, the procedures as carried out in our respective laboratories seem comparable Whether minor differences in *p*H, in mole ratios of chromium to hæmoglobin or in incubation time are of primary importance in determining the site of chromium-51 binding remains to be seen It is also possible that conditions exist which permit preferential binding of chromium to either chain of hæmoglobin Work is now in progress to test some of these hypotheses as well as to determine whether chromium-51 binds to a specific site, peptide or amino-acid in the α-chain under the conditions of the experiments reported here

This investigation was supported by U S Public Health Service grant No *A*2956 from the National Institutes of Health, Bethesda, Maryland

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EFFECT OF PROGESTERONE ON PREGNANCY

Method of Administration and Effect of Progesterone

THE conclusion that progesterone is a key hormone in the maintenance of pregnancy in both women and laboratory animals has been challenged It was stressed that the concentration of progesterone in the systemic blood in several species is at its highest value at the onset of labour when myometrial activity and pharmacological reactivity greatly increase, and also that systemic progesterone therapy does not suppress uterine activity in late pregnant women These observations aided the formulation of the theory of local effect of placental progesterone[1–3]

It was proposed by this theory that placental progesterone affects the myometrium directly, that is, before being carried round by the systemic circulation Once in the blood-stream the biological potency of progesterone was assumed to be weakened by its 'binding' to some plasma constituents, which in turn affected its 'transport' to target organs While the local effect of placental progesterone has been demonstrated in a variety of experiments by the functional asymmetry of the uterus, the mechanism by which this effect is enforced remained uncertain

The experiments to be reported here bring the regulation of progesterone effect from the realm of speculation into that of experimentation, by showing the relative ineffectiveness of intravenous progesterone administration, specifically when the hormone is given in a fat emulsion containing lipids

New Zealand white rabbits in our colony with a litter size of more than five usually deliver spontaneously on the thirty-second day of pregnancy However, when induced on the morning of the thirty-first day, by 1 I U oxytocin ('Pitocin') intramuscularly, they promptly deliver their uterine contents[4,5] If induction is carried out on the morning of the thirtieth day, labour is delayed and prolonged in spite of repeated oxytocin treatment, and many placentæ are retained[6] Thus oxytocin induction is only predictable in the 31-day rabbit, and induced labour is so characteristic that it has been used as a measure of oxytocic potency[5,7].

If 12–24 hr before induction on the thirty-first day the animal is treated with 5 mgm progesterone in oil intramuscularly, she does not deliver and remains uninducible so long as progesterone treatment (5 mgm /day in oil, intramuscularly) continues[4,5] If progesterone treatment is given less than 12 hr before induction oxytocin is effective on the thirty-first day[4,5] This and other observations[8] suggest that the progesterone effect has a latency period of 12–24 hr It is of considerable significance to explain this long period of latency and shorten it if possible

We have been supplied with a progesterone preparation which has been dissolved in 'Lipomul', a mixture of fat, lecithin, a polymer, and water This mixture (without progesterone) has been used extensively and in large quantities in human therapy for its high calorie content

We investigated in rabbits the possibility that intravenous 'Lipomul'-progesterone can shorten the latency period of the progesterone effect when used in massive doses Thus 40 mgm progesterone in 10 ml. 'Lipomul' was injected intravenously to pregnant rabbits on the thirty-first day of pregnancy 3 and 6 hr. before induction with oxytocin The animals promptly responded to oxytocin and delivered as characteristic of the normal 31-day pregnant rabbit. Thus these pilot experiments showed that the latency period is not reduced by massive doses of progesterone (~10 times the effective dose of progesterone, in oil. intramuscularly) injected directly into the systemic circulation

These negative results prompted the question whether this type of progesterone treatment is effective 12–24 hr later, for 5 mgm progesterone dissolved in oil and given intramuscularly is effective A group of animals received 40 mgm progesterone in 10 ml 'Lipomul' intravenously on the thirtieth day and induced 24 hr. later on the thirty-first day All animals but one delivered as normal untreated rabbits do The one which failed to deliver on the thirty-first day was successfully induced on the morning of the thirty-second day in spite of the fact that the dose of progesterone was 40 mgm This failure in progesterone therapy was unexpected in a laboratory where pregnancy in rabbits is regularly maintained by 5 mgm (or less) progesterone in oil intramuscularly, not only in this type of experiment, but also in the ovariectomized and placental dislocated animal, or after term in the intact animal We could only account for this failure in progesterone therapy by the route of administration or by the solvent used ('Lipomul')

The difference between the effect of intramuscular and intravenous progesterone was determined by

Table 1

Rabbit No	Litter size	Total weight (gm.)	Induced delivery on day 31	31	32	32	33	Progesterone administration and solvent
*A*46	5	257	5					40 mgm in 'Lipomul' (i v)
*R*211	8	298	8					
*M*9	9	365	9					
*A*42	9	418			9			
**R*243	10	420	10					
**R*215	7	294	7					
*K*11	8	430	8					10 mgm in oil (i v)
*A*16	10	420		10				
*A*7	9	371	9					10 mgm in propylene glycol (i v)
*C*12	5	276	5					
*I*14	12	534	12					4 mgm in 'Lipomul' (i m)
*R*240	10	386	10					
*A*47	6	371		6				25 mgm in propylene glycol (i v)
*R*1	9	400				9		
*R*2	4	196				4		
*WH*2	4	157				1	3	
*R*13	7	357		5	2			
*WH*20	5	332			5			
*G*2	8	386					8	100 mgm in 'Lipomul' (i v)
*K*10	9	307					9	
*WH*6	9	470			2	7		5 mgm in propylene glycol (i m)
*R*251	6	278			6			8 mgm in 'Lipomul' (i m)
*F*15	5	293			5			10 mgm in 'Lipomul' (i m)
*K*8	10	419					10	40 mgm in 'Lipomul' (i m)

All animals received progesterone treatment on day 30 (except *) and were induced twice a day, starting on the morning of the thirty-first day

**R*243 and *R*215 received progesterone 3 and 6 hr , respectively, before induction on the thirty-first day

intravenous treatment of a group of 30-day pregnant rabbits with 10 mgm progesterone dissolved in oil or in propylene glycol The effect of intramuscular treatment was already known[4,5] The injections were made through a 26-gauge needle which broke up the oil particles into small droplets All these animals delivered a normal litter 24 hr later when induced by oxytocin on the thirty-first day Thus 10 mgm progesterone when administered intravenously is ineffective, whereas 5 mgm intramuscularly is effective

Using another group of 30-day pregnant rabbits the intravenous dose of progesterone was increased to 25 mgm (25 mgm progesterone, dissolved in 0 5 ml oil, was suspended in 1 5 ml propylene glycol) These animals did not deliver on the morning or the night of the thirty-first day, but usually on the thirty-second day Thus 25 mgm progesterone given intravenously is effective although for only a short period Thus about five times more progesterone is needed for the same effect when the treatment is intravenous than when it is intramuscular

If 25 mgm progesterone is effective when administered intravenously and 40 mgm 'Lipomul'-progesterone is not, then 'Lipomul' must oppose the progesterone effect If the dose of progesterone is increased to 100 mgm the intravenous 'Lipomul'-progesterone becomes effective, in spite of the proportional increase of 'Lipomul' from 10 to 25 ml Thus the effect of 'Lipomul', inhibiting the exogenous progesterone action, can be overcome by increasing the dose of progesterone

When only 8–10 mgm 'Lipomul'-progesterone is given intramuscularly it is effective in defending pregnancy in the 31-day rabbit against oxytocin induction

What is of interest in these preliminary experiments is the fact that the progesterone effect is greatly dependent on the route of administration and the solvent used It is known that the *in vitro* effect of progesterone is greatly diminished when the hormone is administered in plasma[9] It is also demonstrated that the local application of progesterone into the uterine horn is very effective and results in a functionally asymmetrical uterus As a result of this asymmetry the animal is capable of delivering the contents of the untreated horn while maintaining pregnancy in the treated horn[10] Recent experiments showed that intramyometrial progesterone treatment is effective in pregnant women (Coutinho *et al*, and Bengtsson, L P , personal communications)

Thus problems of progesterone therapy may be partly overcome by discovering methods of administration which permit the ready transport of systemic progesterone to target organs Such methods may be suggested by experiments on progesterone transport Studies on the superimposed regulation of steroid effects are important, for we may easily overlook the biological significance of a steroid simply because its concentration in the systemic blood does not fit in with our expectations

This work was supported by the National Institutes of Health (*RG*–7942), the Population Council, and the Muscular Dystrophy Associations of America, Inc

We are grateful to the Upjohn Co for a generous supply of 'Lipomul' and to Dr Harold Upjohn and Dr Tapani Luukkainen for many stimulating discussions

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Effect of a Phospholipid Factor on the Activity of Progesterone

It has been demonstrated that the effect of intravenously administered progesterone is dependent on the solvent used. Pregnancy was prolonged in rabbits by 25 mgm. progesterone in oil–propylene glycol suspension, whereas 40 mgm. progesterone in 'Lipomul' was ineffective (previous communication).

The possibility having been considered that certain compounds in 'Lipomul' counteract the effect of exogenous progesterone in prolonging rabbit pregnancy, it was of interest to determine if 'Lipomul' itself would alter the physiological effect of endogenous progesterone.

New Zealand white rabbits, 21–26 days pregnant, were given 150–200 ml. 'Lipomul' intravenously during a period of 8–12 hr. This treatment resulted in premature labour. Rabbits pre-treated by 5 mgm. of progesterone, intramuscularly, could not be induced by higher doses of 'Lipomul'.

Increasing the amount of 'Lipomul' or the speed of infusion resulted in delivery with dead litter, while further increase led to maternal death in convulsions. Further experiments have shown that the active part of 'Lipomul' is the lecithin fraction. The other components of this emulsion[1] were not effective in producing premature labour.

The lecithin in 'Lipomul' is derived from soya bean phosphatides. It is still obscure whether or not pregnant mammals have phospholipids which could control the effect of progesterone. Work is in progress to purify further the soya bean phosphatides and to isolate from the blood and placenta of pregnant women a fraction with similar characteristics.

This work was supported by grants from the National Institutes of Health (*RG*–7942), the Population Council, and the Sigfrid Jusélius Foundation, to which we wish to express our thanks.

Our thanks are due to Dr. Harold L. Upjohn of the Upjohn Co. for the generous supply of 'Lipomul' (an intravenous fat emulsion) and its components.

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A PERCHLORIC ACID–NAPHTHOQUINONE METHOD FOR THE HISTOCHEMICAL LOCALIZATION OF CHOLESTEROL

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The histochemical demonstration of cholesterol by adaptations[1] of the Liebermann–Burchard reaction has never been satisfactory owing to diffusion of the coloured product and to destruction of the tissue by the concentrated sulphuric acid used in the method. The bismuth trichloride method[2] for cholesterol appears to be somewhat capricious when used in histochemistry[3]. The method to be described, a perchloric acid–naphthoquinone reaction, is more sensitive and gives better localization of cholesterol than either of the older methods.

Frozen sections from tissues fixed in calcium–formol are mounted on glass slides and dried in air. The reagent is 0·1 per cent 1,2-naphthoquinone-4-sulphonic acid in a mixture of ethanol–perchloric acid (60 per cent)–formaldehyde (40 per cent)–water (2 : 1 : 0·1 : 0·9 v/v). The sections are painted with reagent and heated on the bottom of an oven at 60–65° for 5–10 min. until the red colour which first appears turns dark blue. (Sections left floating in calcium–formol for some days react more briskly and intensely than those freshly cut.) A drop or two of 60 per cent perchloric acid is placed on the section and a coverslip lowered into position. The dark blue colour of cholesterol is stable in perchloric acid for a few hours and then gradually turns greyish black. The colour is not stable in either water or glycerine-jelly.

To test the specificity of the method, 5 mgm. samples of the following compounds were spot-tested on glass slides with the perchloric acid–naphthoquinone reagent.

Lipids: Red turning dark blue: cholesterol (3-hydroxy, Δ^5), cholesterol oleate, cholesterol palmitate, cholesterol stearate, cholesterol chloroformate, 7-dehydrocholesterol (3-hydroxy, $\Delta^{5,7}$). Red turning pale blue: cholesterol acetate. Red: lanosterol (3-hydroxy, Δ^8). Black: α- and β-carotene mixture (15 : 85), crystalline vitamin A. Brown: oleic acid, linoleic acid, stearic acid, triolein, tristearin, ovolecithin, brain cephalin, brain cerebroside. No colour: cholestane, cholestanol (3-hydroxy), cholestanone (3-oxy), cholestenone (3-oxy, Δ^4), sphingomyelin.

Amino-acids: Pale pink turning yellow: tryptophan. Pink turning brown: tryptophan-formaldehyde carboline. No colour: tyrosine, phenylalanine, histidine, proline, hydroxyproline, arginine, cystine, cysteine, methionine, glycine.

Purines and Pyrimidines: No colour: adenine, guanine, xanthine, uracil, cytosine, thymine.

Carbohydrates: Pink turning brown: glucose, galactose, mannose, lactose, sucrose, inulin. No colour: inositol, glucosamine, galactosamine.

Other organic compounds: Dark blue: resorcinol. Yellow: phenol, catechol. Orange: indole. Brown to black tars: pyrrole, furfural. No colour: benzene, hydroquinone, pyridine, pyridoxine.

From these spot tests, it is concluded that 3-hydroxy-Δ^5 (or $\Delta^{5,7}$)-steroids and their esters are the only physiological substances which develop a dark blue colour with the perchloric acid–naphthoquinone reagent. Steroids without either the 3-hydroxyl or Δ^5 groups are unreactive, but a second double bond in ring *B* (for example, Δ^7) does not inhibit the

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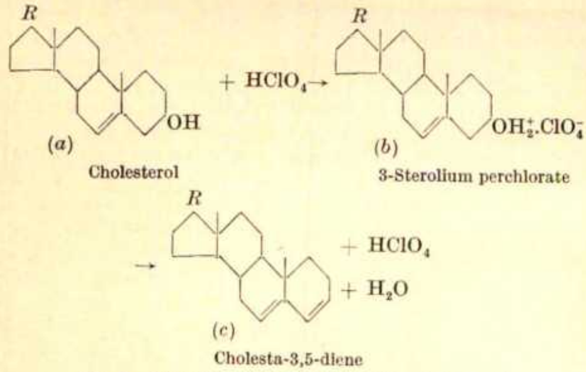
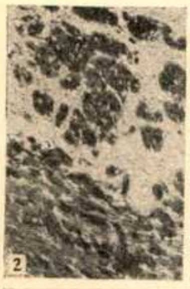
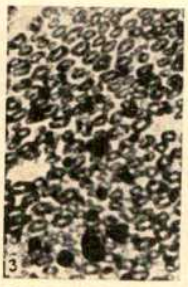
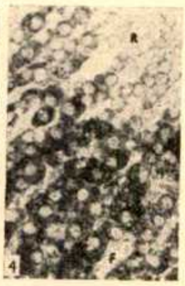
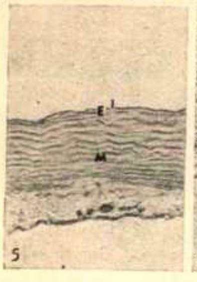


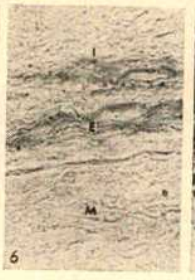
Fig. 1. The action of perchloric acid on cholesterol

reaction. The functional groups of the steroid nucleus demonstrated by this method are therefore essentially the same as those detected by the Liebermann–Burchard reaction[4].

The chemical mechanism of the perchloric acid–naphthoquinone reaction is unknown but previous investigations[5] have shown that perchloric acid has the specific effect, shared only with hexafluorophosphoric acid, of reacting with 3-hydroxy-Δ^5 steroids (Fig. 1*a*) to form insoluble 3-sterolium salts (Fig. 1*b*). The insolubility of these salts may explain the improved localization obtained with this method when compared with the Liebermann–Burchard reaction. By eliminating a molecule of water, excess perchloric acid produces cholesta-3,5-diene (Fig. 1*c*) from the sterolium salt, as does sulphuric acid from cholesterol[6]. Addition of acid to the 3,5-diene may be responsible for the transient red colour which is first formed, but the development of the dark blue colour appears to depend on a reaction between the diene and naphthoquinone. The diene structure is essential, since no colour develops in tissue sections brominated between the perchloric acid and naphthoquinone stages. Although naphthoquinones are dienophiles, Diels–Alder adduction of the diene to the quinone is not a plausible explanation for the reaction, for cholesta-3,5-diene does not undergo diene synthesis at the relatively low temperature used in the test[7]. A related method, using phenol and perchloric and molybdic acids, has been used to identify cholesterol on chromatograms[8]. A diene–quinone reaction may be the basis of both methods, for phenol is probably oxidized by these acids to a benzoquinone.





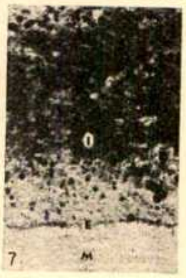


Fig. 2. Rat brain. Myelinated tracts in corona radiata. (Perchloric acid–naphthoquinone (PAN, × *c.* 95)

Fig. 3. Rat sciatic nerve. Staining of myelin sheath but no reaction in axon. (PAN, × *c.* 465)

Fig. 4. Rat adrenal cortex. Intracellular cholesterol in zona fasciculata (*F*) but little staining of reticularis (*R*). (PAN, × *c.* 385)

Fig. 5. Rat aorta. No staining of elastic lamellæ but slight reaction in interlamellar spaces. *I*, intima; *E*, internal elastic lamella; *M*, media. (PAN, × *c.* 136)

Fig. 6. Aorta of 36-year-old man. Faint staining of the reduplicated internal elastic lamella (*E*) and elastic fibres in the adjacent intima (*I*). (PAN, × *c.* 285)

Fig. 7. Aorta of 58-year-old man. Accumulation of cholesterol in intima (*I*) and around internal elastic lamella (*E*). (PAN, × *c.* 95)

The specificity of the perchloric acid–naphthoquinone method for cholesterol was confirmed histochemically by the substantial reduction in colour after the tissue sections had been extracted in acetone at 4° for 18 hr. The success of extraction by acetone, in which cholesterol is highly soluble, indicates that the reaction in tissues is due to lipid. The slight residual staining after extraction may be due to the difficulty of removing all lipid from tissue sections[9] or to the binding of lipid to protein.

In rat tissues, a dark blue colour reaction is seen in the myelin sheaths of the central (Fig. 2) and peripheral (Fig. 3) nervous systems, in the zona fasciculata (Fig. 4) and glomerulosa of the adrenal cortex, in the interstitial cells of the testis and in the lutein cells of the corpus luteum. Slight staining is seen in neuroglial fibres but axons are unstained, as are the unmyelinated axons of the leg nerve of the lobster.

Elastic lamellæ in the normal human and rat aorta are unstained, even though these lamellæ contain lipid[10], including phospholipid[11]. Only traces of cholesterol are present in the interlamellar spaces of the media of the human and rat aorta (Fig. 5), but a strong reaction is given by the lipid-laden macrophages which are frequently found in the more superficial parts of the human arterial intima. The junctional zone between media and intima, the point where phospholipid first accumulates in the early lipoidal lesions of human atherosclerosis[12], contains relatively little cholesterol but the internal elastic lamella itself is sometimes faintly stained at this stage (Fig. 6). The lipid masses of the mature atherosclerotic plaque contain abundant cholesterol (Fig. 7).

A pale pink reaction is seen in the zymogen granules of the rat pancreas and submandibular gland and a weaker background colour of the same hue is seen in many tissues. It is concluded from the spot-tests that the pink colour in tissues is due to tryptophan and possibly carbohydrate, whereas the well-localized dark blue colour obtained with the perchloric acid–naphthoquinone method is a highly specific histochemical test for cholesterol and related steroids.

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PROTEOLYSIS OF THYROGLOBULIN BY THYROID ENZYMES

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SINCE the thyroid hormone is held by peptide bonds within the molecule of its storage form, thyroglobulin, it is generally accepted that hydrolytic enzymes are concerned with its controlled release into the blood stream. The proteolytic activity of thyroid tissue has been shown to vary with the physiological status of the animal as, for example, that following the administration of thyrotrophin or of anti-thyroid drugs[1]. Thus, the purification and the study of the properties of the enzyme (or enzymes) concerned could afford more exact information as to the release mechanism and perhaps help to clarify some types of thyroid disease.

It has been demonstrated that iodinated tyrosines and thyronines can be liberated by incubation of thyroid homogenates[2], saline extracts of the gland[3] or by the action of a partially purified thyroid proteinase on thyroglobulin[4]. The enzyme preparation used by the last-named workers contained peptidase activity. Further purification studies[5] led to the separation of a peptidase fraction ('APATase'), which readily hydrolyses preferentially the pepsin substrate, acetyl-L-phenylalanyl-L-tyrosine[6] (APAT), and a proteinase of high specific activity. The proteinase still retained, however, some activity towards the peptide, L-cysteinyl-L-tyrosine, also a substrate for pepsin[7]. This property has been shown to be due to the presence of another peptidase ('cysteinyltyrosinase') which can be separated from the proteinase[8]. The actions of proteinase and APATase are not influenced by the usual enzyme activators, whereas cysteinyltyrosinase is metal-dependent[8].

The *p*H optimum of the proteinase is 3·5 (hæmoglobin substrate), while the two peptidases, APATase and cysteinyltyrosinase, show peaks of activity at *p*H 4·0 and 5·3 respectively. Like pituitary[9] and adrenal[10] acid proteinases, the thyroid enzyme, when freed from the two peptidases already mentioned, appears to have no action against simple peptide substrates.

We have carried out a series of experiments in which thyroid proteinase and APATase have been incubated, under varying conditions, with preparations of rat thyroglobulin labelled *in vivo* with iodine-131.

Thyroglobulin substrates were prepared as follows: eight female rats, average weight about 200 gm., were each injected intraperitoneally with 90–100 μc. carrier-free iodine-131 as iodide. 48 hr. later the animals were killed, the thyroid glands dissected out, pooled, blended by hand in a glass homogenizer with ice-cold isotonic saline (0·6 ml.), insoluble material removed by centrifugation and the supernatant brought to 42 per cent saturation with ammonium sulphate at 25° C. The precipitate, after standing for 2 hr., was separated, washed with 42 per cent ammonium sulphate, dissolved in water (0·6 ml.) and dialysed overnight. A precipitate which separated was discarded and the supernatant adjusted to 0·015 *M* *tris* buffer (*p*H 7·2) and 0·015 *M* ethylenediamine tetraacetic acid (EDTA), dialysed against the same concentrations of buffer and chelating agent (200 ml.) for 30 hr. and then against distilled water for 17 hr. The faintly opalescent salt-free solution was then heated in a sealed tube at 80° C. for 4 min. to reduce the content of endogenous proteinase, as it has been found that thyroglobulin tends to retain this enzyme during salt fractionation[11]. Protein was then precipitated by adding four volumes of acetone below − 5° C., followed by a small drop of saturated sodium chloride. The precipitate was dissolved in water (about 0·7 ml.), dialysed overnight and held in the frozen state until required.

The enzyme samples were prepared from pig thyroid glands, substantially as described[5], but were further processed by two treatments with buffered EDTA (as for thyroglobulin substrate), to reduce the activities of traces of metal-dependent peptidases, followed by prolonged dialysis against distilled water. The proteinase had an activity of 1,200 units/mgm. protein when incubated for 30 min. at 37° C. and *p*H 3·5 with bovine hæmoglobin substrate, followed by spectrophotometric determinations at 280 mμ (compare Anson[12]). This corresponds to a purification factor of about 400, a twenty-fold improvement on the preparation used in our previous work[4]. The peptidase (APATase) contained 8 units/mgm. protein, one unit being defined as that quantity of enzyme which liberates 1·0 μM tyrosine from APAT (5 μM per ml.) in 60 min. at 37° C. and *p*H 4·0. When incubated at a concentration of 1 mgm./ml. with APAT, the proteinase released the merest trace of tyrosine, as determined chromatographically, whereas APATase, under identical conditions but at a concentration of 0·1 mgm./ml., hydrolysed about 25 per cent of the substrate. The sample of APATase was slightly contaminated by proteinase (10 units/mgm. protein).

Action of purified proteinase on thyroglobulin. In our earlier investigations[4] with labelled thyroglobulin and partially purified proteinase, both prepared from pig thyroid, appreciable hydrolysis was observed at *p*H 3·5. However, in the present work, it became clear from the results of preliminary experiments, using rat thyroglobulin, that substantially greater quantities of labelled components were liberated at higher *p*H, even though the proteinase gave a smooth *p*H-activity curve with bovine hæmoglobin substrate, being only one-fifth as active at *p*H 5·2 as at *p*H 3·5.

In the following experiment the nature of the labelled substances released at the higher *p*H was examined. A half of a thyroglobulin solution prepared as above was mixed with an equal volume of 0·1 *M* ammonium acetate buffer *p*H 5·2. Duplicate tubes containing 0·1 ml. thyroglobulin solution and 0·05 ml. proteinase solution (equivalent to 60 units) were incubated for 2 and 16 hr., while water replaced the enzyme in duplicate control tubes for each period

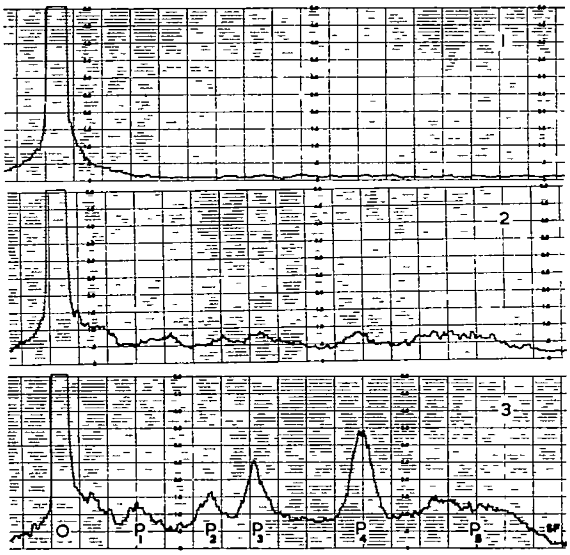

Fig 1 Distribution of iodine-131 on paper chromatograms of thyroglobulin after incubation with thyroid proteinase at 37° C and *p*H 5 2 Chromatograms developed with *n*-pentanol/propionic acid/water (20 3 15) (1) 16-hr control, (2) after 2 hr , and (3) after 16 hr with thyroid proteinase Scannings made with instrument set at full-scale deflexion, 500 c p m , slit width, ⅛ in , scan speed, 3 in /hr , time constant, 40 sec
O = origin, *SF* = solvent front, P_1–P_5 = main areas of radioactivity

A similar set, but buffered at *p*H 3 45 with 0 1 *M* ammonium acetate, was included The tubes were tightly stoppered and shaken during incubation at 37° C Following incubation, 0 025 ml samples from each tube were spotted on to washed Whatman No 3 paper and descending chromatograms developed with *n*-pentanol/propionic acid/water (PPW, 20 3 · 15 by vol) Marker spots contained mono- and di-iodotyrosine and thyroxine (25, 25 and 50 μgm respectively) Duplicate paper sheets (series *B*) were prepared with further 0 025 ml samples from all tubes after these had been standing for 48 hr in the frozen state After cutting the sheets into strips the latter were scanned foi radioactivity using a Nuclear Chicago 'Actigraph II' automatically recording instrument Rapid scans indicated marked increases in the radioactive components released at *p*H 5 2 compared with *p*H 3 45 Accordingly, further examination was restricted to the incubations at the higher *p*H

Fig 1 illustrates the results of slow scanning of the strips corresponding to 2-hr and 16-hr tests and that of a 16-hr control Duplicates corresponded closely. Apart from the origin peak—presumably residual protein—five main areas of activity are discernible (P_1–P_5), each area increasing and becoming more clearly defined with the longer incubation period The control scans showed only background activity except at the origin

Approximate values for percentage hydrolysis were derived from estimates of the areas of migrated radioactivity and those at the origins The results of the calculations are given in Table 1, which lists the duplicate values for the relevant strips from each of the two series of chromatograms (*A* and *B*) run in PPW with 0 025 ml samples On averaging each of the sets of four values, it appears that about three times as much radioactivity had been released after 16 hr as after 2 hr (approximately 27 and 10 per cent respectively) In view of the variations within the calculations, the exact agreement of three of the duplicate percentages must be regarded as fortuitous

In order to identify the peaks of radioactivity, the residual solutions (0 1 ml) from the incubation tubes were precipitated by addition, with warming, of ethanol to 70 per cent concentration, in the presence of carrier mono- and di-iodotyrosine, thyroxine and a sample of purified pig thyroglobulin (0 5 mgm) prepared by the method of Derrien, Michel and Roche[13] After centrifuging, the residues were washed with 0 2 ml 70 per cent ethanol and the combined supernatants evaporated in micro beakers in a desiccator (phosphorus pentoxide) The residues were then spotted on to paper with ethanol/water/conc ammonium hydroxide (7 2 1), the beakers being monitored for completeness of application, and chromatograms developed with PPW. The radioactivity scans were similar to those of the 0 025 ml samples directly applied The peaks corresponding to P_3 and P_4 (Fig 1), which had the same mean R_F values (0 37; 0 59) as marker spots of mono- and di-iodotyrosine respectively, were further characterized by eluting the areas with the alkaline ethanol solvent and then developing chromatograms of the eluate residues in *n*-butanol / 2*N* acetic acid (1 1) Well-defined areas with peaks at R_F 0 43 and 0 54 accounted for nearly all the radioactivity on the individual strips This was checked by radioautography and by subsequent spraying of the strips with ninhydrin to visualize the carrier iodo amino-acids, which demonstrated exact coincidence between radioactive and coloured zones Although eluted and re-run in butanol/acetic acid the peaks P_1 and P_2 (as in Fig 1) could not be characterized although P_2 did have a component at the same R_F as mono-iodotyrosine It is possible that they represented, in part, small peptides The elongated peak (P_5) of R_F 0 70–0 90 suggested a double character on radioautography, the higher part corresponding to marker thyroxine On eluting and re-running in *n*-butanol/*n*-pentanol/2 *N* ammonium hydroxide (BPA, 1 1 2), radioactive peaks were found at R_F values 0 00, 0 08, 0 14 and 0 20, with a broad spread of radioactivity through R_F 0 32 corresponding to thyroxine Thus, P_5 appeared to consist of a mixture, but the evidence strongly suggested thyroxine as one of the components An unequivocal demonstration of the direct release of thyroxine by the proteinase preparation was obtained in the following experiment

An apparent synergistic action of proteinase and APATase on thyroglobulin In the preparation of the

Table 1. Hydrolysis of Thyroglobulin by Thyroid Proteinase at *p*H 5 2

	Incubation period—2 hr				Incubation period—16 hr			
	A		*B*		*A*		*B*	
Tests								
Migrated (*M*)	7,500*	7,060	10,800	11,000	26,000	27,800	24,000	29,200
Origin (*O*)	89,900	84,500	84,400	85,800	69,800	74,100	68,700	72,900
O + *M*	97,400	91,560	95,200	96,800	95,800	101,900	92,700	102,100
M as per cent *O* + *M*	7 7	7 7	11 4	11 4	27 2	27 3	25 9	28 6
Controls (origin)	92,400	93,900	99,100	98,200	101,700	106,400	97,500	99,500

* Figures are given as counts per min of iodine-131 (corrected for decay back to day of injection)

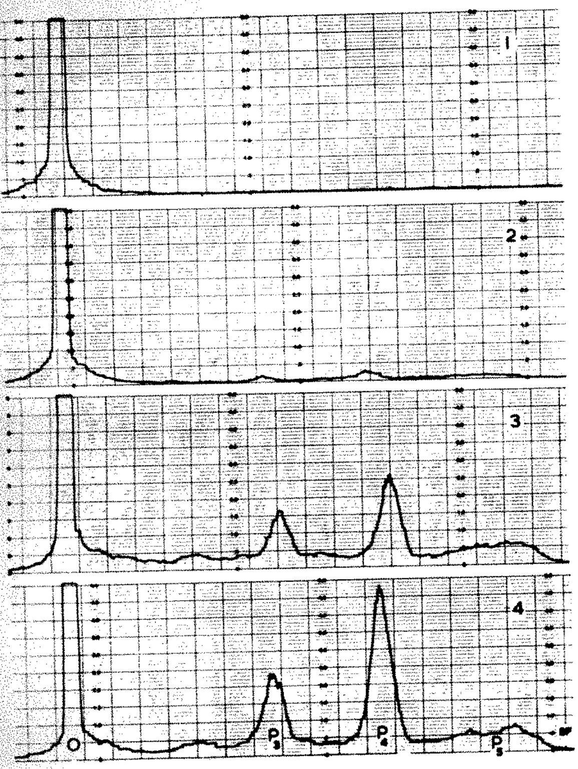

Fig. 2. Distribution of iodine-131 on paper chromatograms of thyroglobulin after incubation with thyroid proteinase and/or APATase at 37° C. and *p*H 5·25. Chromatograms developed with *n*-pentanol/propionic acid/water (20 : 3 : 15). (1) Control; (2) after 16 hr. with APATase; (3) after 16 hr. with proteinase; (4) after 16 hr. with proteinase plus APATase. Scannings made with instrument set at: full-scale deflexion, 1,500 c.p.m.; slit width, ⅛ in.; scan speed, 6 in./hr.; time constant, 20 sec. O = origin; SF = solvent front; P_3–P_5 = main areas of radioactivity

labelled thyroglobulin sample for this experiment the step involving dialysis against buffered EDTA was omitted as the chelating agent was present during incubation. The thyroglobulin solution was adjusted to 0·015 *M* ammonium acetate, *p*H 5·25, and 0·015 *M* EDTA, and 0·1 ml. added to each of eight incubation tubes which were tightly stoppered and pre-incubated for 1 hr. at 37° C. Duplicate tubes then received 0·05 ml. of solutions of (*a*) proteinase (60 units), (*b*) APATase (0·4 unit) and (*c*) proteinase (60 units) plus APATase (0·4 unit), the two control tubes having water only added. After 16 hr. incubation 0·025 ml. samples were applied to paper, run in PPW and the strips scanned as in the previous experiment. The tracings are shown in Fig. 2. Duplicates agreed closely. In the case of the proteinase alone there are again well-marked peaks (P_3, P_4) corresponding to mono- and di-iodotyrosine. Noteworthy is the virtual absence of peaks P_1 and P_2 (compare Fig. 1) and the greater relative concentration of radioactivity in the higher R_F region of P_5 coincident with thyroxine marker. Most striking, however, is the considerable augmentation of radioactivity in the areas corresponding to mono-iodotyrosine and, particularly, di-iodotyrosine, in the tracings of the samples where the two enzymes were associated. APATase alone released very little radioactivity and this could have been due to a trace amount of proteinase in the APATase sample (equivalent to 0·5 proteinase unit in the 0·15 ml. of incubated solution). The contributions of the three main areas, P_3, P_4 and P_5, to the migrated radioactivity, in the case of the proteinase alone and proteinase plus peptidase, as derived from the 0·025 ml. scans, are shown in Table 2.

Table 2. CONTRIBUTIONS OF RADIOACTIVE AREAS, P_3, P_4 AND P_5 (FIG. 2), TO TOTAL MIGRATED RADIOACTIVITY FOLLOWING TREATMENT OF THYROGLOBULIN WITH (*a*) PROTEINASE ALONE AND (*b*) PROTEINASE PLUS APATASE AT *p*H 5·25

Radioactive area	Proteinase (duplicates)		Proteinase + APATase (duplicates)	
P_3	4,900*	5,200	7,400	8,300
P_4	7,900	7,900	15,100	15,700
P_5	6,700	6,600	7,900	8,400
Total migrated	28,100	28,700	39,300	40,900
Total migrated less P_3, P_4, P_5	8,600	9,000	8,900	8,500

* Figures are given as counts per min. of iodine-131 (corrected for decay back to day of injection).

P_3 = mono-iodotyrosine; P_4 = di-iodotyrosine; P_5 = thyroxine-containing area.

The high R_F radioactive areas (P_5) for both proteinase and combined enzymes were unequivocally demonstrated to contain thyroxine as one of the components by further chromatographic examination of the residual solutions (0·1 ml.) from the relevant incubation tubes. Following development in PPW and elution of the areas coincident with marker thyroxine, the duplicate eluates were combined, carrier thyroxine added and the eluate residues then rechromatographed with the solvent system BPA. The 'thyroxine' areas were eluted and the residues re-run in *n*-butanol / 2*N* acetic acid (1 : 1). The chromatograms were checked by radioautography at each stage. Fig. 3 depicts the final tracings, which show in each case a well-defined thyroxine area at R_F 0·72 which coincided exactly with the carrier thyroxine—visualized by ninhydrin—with a minor peak of higher R_F which was not characterized. The total radioactivity in the thyroxine areas was higher when the enzymes were associated but, in view of the involved chromatographic and elution procedure, this difference may not be significant.

It may be concluded from the above results that thyroid acid proteinase is able to degrade thyroglobulin with the release of mono- and di-iodotyrosine and thyroxine. However, it is also possible that even the trace amount of APATase in the purified proteinase sample was the catalyst responsible for the release of the iodo amino-acids from peptides

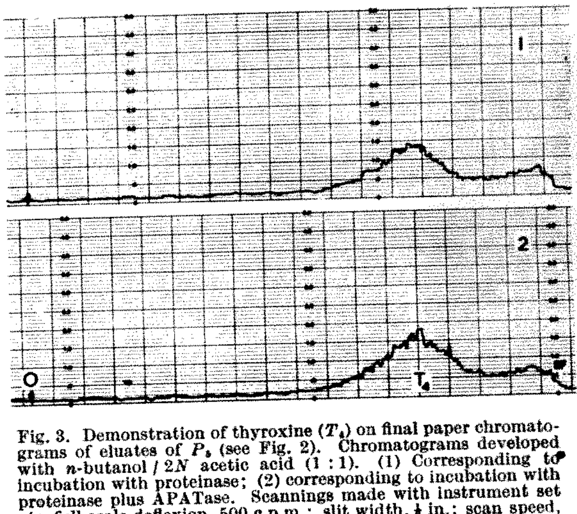

Fig. 3. Demonstration of thyroxine (T_4) on final paper chromatograms of eluates of P_5 (see Fig. 2). Chromatograms developed with *n*-butanol / 2*N* acetic acid (1 : 1). (1) Corresponding to incubation with proteinase; (2) corresponding to incubation with proteinase plus APATase. Scannings made with instrument set at: full-scale deflexion, 500 c.p.m.; slit width, ⅛ in.; scan speed, 3 in./hr.; time constant, 40 sec. O = origin; SF = solvent front; T_4 = thyroxine

produced by the proteinase as intermediates. In any event, as the peptidase had almost negligible action on intact thyroglobulin, it seems clear from the results that the two enzymes can act synergistically in the hydrolysis of the protein. It is not possible at this stage to make a concise evaluation of the respective contributions of the two enzymes, particularly as specific activities were not determined. In this connexion our earlier observation[4] may be recalled that the specific activities of enzymically released mono- and di-iodotyrosine were higher than those of the amino-acids isolated from the same sample of labelled thyroglobulin after complete chemical hydrolysis. This implied, possibly, a 'terminal turnover' in the biosynthesis and degradation of the iodo amino-acids.

Our present investigations are designed to clarify further the nature of the primary actions of thyroid acid proteinase and peptidases.

Mr. R. E. Loughlin was associated in preliminary experiments of this investigation. One of us (J. D. M.) has held the Thomas and Elizabeth Ross Scholarship and we are also indebted to the National Health and Medical Research Council for a grant in aid of this work.

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DEMONSTRATION OF INTERACTION PRODUCTS OF ADENINE AND ADENOSINE PHOSPHATES WITH FORMALDEHYDE

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RECENTLY, Alderson[1] ascribed a special role to adenylic acid as the mediator of the mutagenic action of formaldehyde on *Drosophila* larvæ, the main postulated agent being an 'adenylate dimer' produced by the interaction of formaldehyde with the amino-group of this purine derivative in the food mixture. The evidence for such interaction products rests mainly on slight alterations in the ultra-violet absorption spectra[2] after incubation of purine bases, nucleotides, etc., with formaldehyde. In view of the scheme suggested by Alderson[1] for the utilization of the postulated metabolite in deoxyribonucleic acid synthesis and its role in mutagenic activity, further demonstration and, if possible, isolation of such reaction products seemed desirable: experiments have been made therefore to see whether they could be resolved from the reaction mixture by chromatography or paper electrophoresis.

In preliminary tests adenine, 2 mgm./ml., was incubated for 18 hr. at 38° C. in presence of 0·1 per cent formaldehyde. After confirming the effect on the maximum of the absorption curve, 20 μgm. samples (10 μl.) were chromatographed by the ascending method using various solvent systems. The positions of the areas containing purine were made visible by the usual method of photographing with ultra-violet light. Using isopropanol / $2N$ hydrochloric acid (65 : 35) evidence of several compounds was obtained, some with greater R_F values and some with less R_F values than adenine (Fig. 1). This is in accordance with the possibility of the formation of monomethylol and dimethylol derivatives, and the 'dimer' (A—CH_2—A) containing the methylene bridge by conjugation of two adenine residues. Formaldehyde may also react with the imino group at the 9 position of the free purine ring. Adenosine, after reaction with formaldehyde, gave evidence of a component moving faster than the adenosine comparison spot in the isopropanol–hydrochloric acid solvent and in methanol / 90 per cent formic acid / water (160 : 30 : 10).

When adenosine-3′-phosphate was examined after formaldehyde treatment, no separation of components was obtained. In all cases clean separation of the products, some of which are relatively unstable, seemed to be disturbed by the long exposure to the solvents, as shown by trailing.

Experiments were made therefore to see whether useful separation could be obtained by treatment with various ion-exchange materials including types of synthetic resins. When solutions of adenosine and formaldehyde-treated adenosine containing 41 μgm./ml. were exposed for 2 hr. to 'Amberlite *IRC* 50 (H)' resin, 0·5 gm.–8 ml., a much higher proportion of the unchanged adenosine was abstracted as shown by the absorption curves in Fig. 2, and by Table 1. Complete separation of the interaction products from the mixture was not achieved, however.

By paper electrophoresis a clear demonstration of reaction products was obtained with formaldehyde-

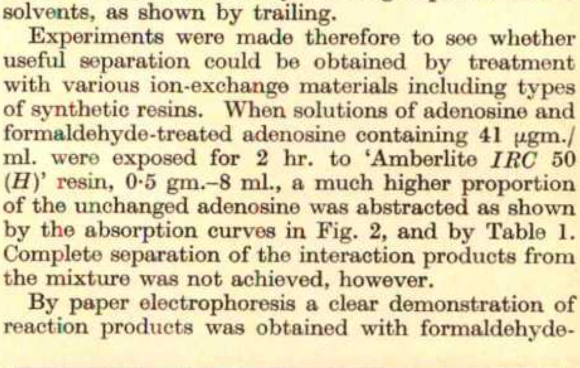


Fig. 1. Chromatography of adenine: (*a*), before; (*b*), after treatment with formaldehyde

Table 1. EFFECT OF ION-EXCHANGE RESIN ON ADENOSINE AND FORMALDEHYDE-TREATED ADENOSINE

mμ	Optical density before resin treatment*		Optical density after resin treatment	
	Adenosine	Adenosine treated with formaldehyde	Adenosine	Adenosine treated with formaldehyde
250	0·85	0·78	0·398	0·695
252	—	—	0·445	0·80
254	1·008	0·95	0·482	0·90
256	—	—	0·51	0·98
258	1·125	1·18	0·53	1·075
260	1·156	1·24	0·545	1·15
262	1·15	1·28	0·545	1·22
264	1·131	1·31	0·538	1·26
266	1·084	1·33	0·517	1·27
268	1·019	1·31	0·483	1·27
270	0·92	1·23	0·438	1·22

* Diluted 1–200 for analysis.

treated adenine (20 μgm.) or adenosine-3′-phosphate (60 μgm.); 2 m.amp. per 5 cm. strip for 5 hr. in 0·2 *M* acetate–acetic acid buffer, *p*H 4. Adenine–formaldehyde compounds with less mobility than the untreated marker were detected (Fig. 3) while adenosine-3′-phosphate showed a well-separated spot with greater mobility than the unchanged compound. Formaldehyde-treated adenosine solutions did not give good separation. It would appear that the structures of the riboside and any reaction products are too similar to be influenced differentially by the 'transporting charge' but that the phosphate group confers properties allowing the relatively small effect of the formaldehyde group to be utilized in the separation. Resolution was further improved when 0·1 *M* buffer (4 m.amp. per strip) was used for 3–4 hr. (Fig. 4). Similar separation of products from adenosine-5′-phosphate after exposure to formaldehyde is illustrated in Fig. 5, which shows the unchanged ribotide, and one (possibly two) compounds with greater mobilities than the 5′-phosphate.

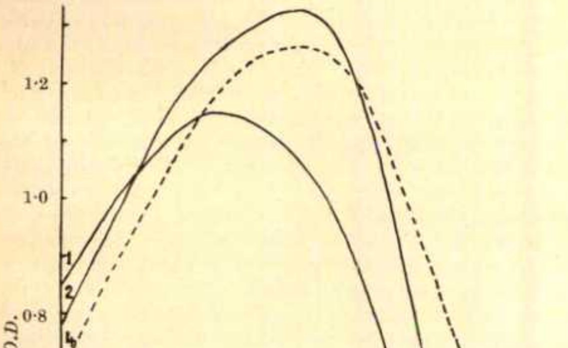
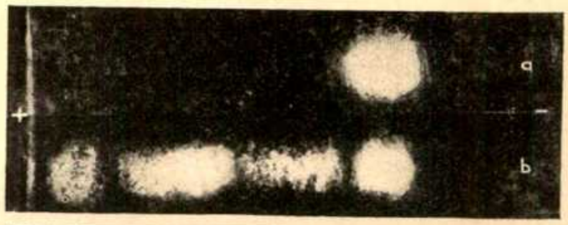
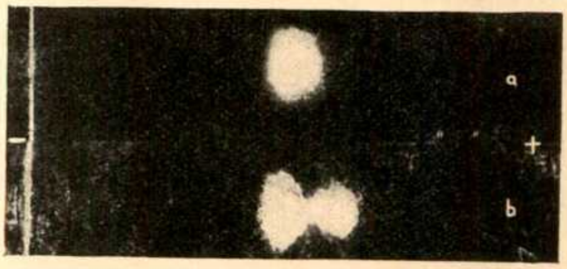


Fig. 2. Differential effect of resin on adenosine and formaldehyde-treated adenosine; ultra-violet absorption curves: 1, adenosine; 4 mgm./ml. (diluted 1–200 for analyses); 2, adenosine after exposure to formaldehyde: 4 mgm./ml. (diluted 1–200 for analyses); 3, solution as in 1, after treatment with resin '*IRC* 50 (*H*)' (undiluted); 4, solution as in 2, after treatment with resin (undiluted)





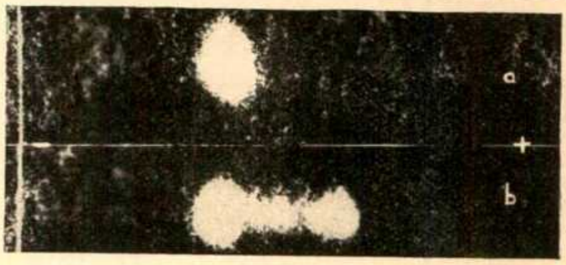


Fig. 3 (top). Electrophoresis of adenine (*a*) before; (*b*), after treatment with formaldehyde. 0·1 *M* acetate buffer, *p*H 4·0, 4 m.amp. per 5 cm. strip, 3 hr.

Fig. 4 (centre). Electrophoresis of adenosine-3′-phosphate (*a*) before; (*b*), after treatment with formaldehyde. Conditions as for Fig. 3

Fig. 5 (bottom). Electrophoresis of adenosine-5′-phosphate (*a*) before; (*b*), after treatment with formaldehyde. Conditions as for Fig. 3

The resolution of the nucleotides allowed a computation of the proportions of the components to be made. First, from the dried papers the areas denoted by the positions of the spots on the photographs were cut out, extracted with 4 ml. 0·1 *N* sulphuric acid and the content estimated by ultra-violet spectrophotometry.

From the figures for adenosine 3′-phosphate electrophoresis strips given in Table 2 it can be calculated that some 77 per cent of the nucleotide deposited on the paper was present as unchanged ribotide. Since it was evident from the absorption curves of the

Table 2. OPTICAL DENSITY OF SOLUTIONS OF SEPARATED COMPONENTS OF FORMALDEHYDE-TREATED ADENOSINE-3′-PHOSPHATE

mμ	Control adenosine phosphate spot	Formaldehyde-treated	
		Companion adenosine phosphate spot	Subsidiary-component spot
252	0·497	0·398	0·115
254	0·529	0·408	0·122
256	0·558	0·433	0·127
258	0·543	0·418	0·125
260	0·518	0·398	0·119
262	0·484	0·372	0·114

Extracted after drying paper at 110° C. with 4 ml. 0·1 *N* sulphuric acid, using extract of blank paper for comparison

Table 3. OPTICAL DENSITY OF SOLUTIONS OF SEPARATED COMPONENTS OF FORMALDEHYDE-TREATED ADENOSINE-3′-PHOSPHATE

mμ	Formaldehyde-treated	
	Main spot	Subsidiary spot
254	0·288	0·128
256	0·31	0·137
258	0·313	0·143
260	0·310	0·150
262	0·30	0·153
264	0·283	0·152
266	0·26	0·144

Extracted from undried paper with 4 ml. 0·1 *N* sulphuric acid.

relevant solutions that during drying the formaldehyde combination was reversed to a considerable extent, extraction from an undried paper was also carried out. In this case it is difficult to be sure that the positions for the separated components are selected accurately since they must be judged by the photograph of a dried paper run in parallel. However, the substance which had a greater mobility than the original ribotide then gave an absorption maximum at 363 mμ compared with 258 mμ for adenylic acid under the same conditions (Table 3).

This work was carried out with the support of the Birmingham Branch of the British Empire Cancer Campaign.

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GLUTAMYL PEPTIDASES IN RAT AND GUINEA PIG KIDNEY SLICES

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Histochemical Demonstration of γ-Glutamyl Transpeptidase-like Activity

UNTIL now, only the purely hydrolytic character of the amidase group of enzymes has been demonstrated histochemically. Recent reports[1,2] have described methods for the colorimetric estimation in serum and tissues of enzyme-catalysed cleavage of γ-L-glutamyl peptides possibly mediated by γ-glutamyl transpeptidase[3]. In order to localize this enzymic activity to tissue sections at a histological level a potentially chromogenic substrate *N*-(γ-L-glutamyl)-β-naphthylamide was synthesized for use in a simultaneous azo-coupling reaction.

The synthesis of *N*-(α-benzyl-N^{α}-carbobenzoxy-γ-L-glutamyl)β-naphthylamide was performed from N^{α}-carbobenzoxy-1-glutamyl α-benzyl ester[4] and β-naphthylamine using the mixed anhydride technique with isobutyl chlorocarbonate in dioxane. The product was crystallized from ethyl acetate–petroleum ether, and had a melting point of 142–44° C. *N*-(γ-L-glutamyl)β-naphthylamide was obtained by catalytic hydrogenation in the presence of palladium black in 80 per cent acetic acid as solvent. The product was crystallized from dilute acetic acid ; its melting point was 228° C. (Analysis found : C, 66·4 ; H, 5·9 ; N, 10·2 ; carboxyl-N, 4·9 (ref. 5). Calc. for $C_{15}H_{16}O_3N_2$: C, 66·2 ; H, 5·9 ; N, 10·3 ; carboxyl-N, 5·1). $[\alpha]^{20}_{D} + 2{\cdot}0°$ (*c*, 1·2 in dimethylformamide made 2 *N* with concentrated hydrochloric acid).

Kinetics of the release of naphthylamine from *N*-(γ-L-glutamyl)β-naphthylamide in a biochemical system using previously reported methods were investigated with rat kidney homogenates. The substrate, which was dissolved in 0·1 *N* sodium hydroxide and then neutralized, was used at a concentration of 0·001 *M* in 0·1 *M* *tris*(hydroxyamino)methane buffer at *p*H 8·5 with 0·0164 mgm. wet weight kidney/ml. No spontaneous hydrolysis of the substrate was noted. Pronounced activation by 0·003 *M* glycylglycine was noted with inhibition by γ-L-glutamyl-L-alanine but no inhibition by α-L-glutamyl-L-valine. There was complete inhibition by disodium phenoltetrabromophthalein sulphonate ('Bromsulphalein')[1]. It was of interest that release of naphthylamine from the naphthylamide substrate was enzymically catalysed many times faster than release of aniline from an analogous DL-anilide compound previously reported[1].

Histochemical work on the characteristics of enzymic release of naphthylamine from *N*-(γ-L-glutamyl)β-naphthylamide was performed predominantly on slide-mounted freshly frozen 10μ sections of guinea pig kidney using an incubating solution consisting of 0·5 ml. of a 4 mgm./ml. (0·013 *M*) stock suspension of substrate in acetone, 0·5 ml. of a 20 mgm./ml. (0·150 *M*) aqueous solution of glycylglycine in 10 ml. of 0·1 *M* *p*H 7·2 *tris* buffer, 28 ml. of water and 1 ml. of a solution containing 15 mgm. of the stable diazonium salt of *o*-aminoazotoluene (fast garnet *GBC*) at 37° C. for 20 min. A sub-optimal *p*H for transpeptidation[3] was used to facilitate simultaneous azo-coupling of the naphthylamine enzymically released. No effect on enzymic hydrolysis was noted after the addition to the incubating medium of 0·01 *M* L-glutamine, 0·01 *M* α-L-glutamyl-L-asparagine, 0·001 *M* magnesium chloride, 0·005 *M* *p*-chloromecuribenzoate, 0·001 *M* sodium iodoacetate, 0·01 *M* and 0·0005 *M* potassium cyanide, 0·01 *M* disodium ethylenediamine tetraacetic acid, 0·001 *M* sodium pyruvate, 0·0001 *M* di-iso-propylphosphorofluoridate or 0·004 *M* sodium taurocholate. 'Bromsulphalein' (0·0001 *M*) completely inhibited enzymic activity as did 0·005 *M* oxidized glutathione. No evidence of enzyme activation was noted using 0·01 *M* phosphate buffer at *p*H 7·2. When acetone-fixed paraffin embedded or acetone-washed frozen sections were incubated in the presence of glycylglycine as the γ-glutamyl acceptor, an estimated 50 per cent increase of activity was noted over incubation in its absence.

From the above histochemical and biochemical evidence it can be concluded that the enzyme which releases naphthylamine from *N*-(γ-L-glutamyl)-β-naphthylamide cannot be identified with glutaminase I since there was no inhibition by either *p*-chloromercuribenzoate or L-glutamine and phosphate did not activate the system. Moreover, it is unlikely that a sulphydryl-dependent enzyme, such as glutaminase I, would be demonstrated by a simultaneous azo-coupling reaction since the sulphydryl group would either be oxidized by, or coupled to, the diazonium reagent. Aminopeptidases described histochemically do not appear to be implicated since potassium cyanide failed to affect the reaction. In view of the above findings and the fact that enzymic release of naphthylamine is inhibited by oxidized glutathione and 'Bromsulphalein' and activated by glycylglycine[1], it would appear that the enzyme demonstrated has many of the characteristics of γ-glutamyl transpeptidase as defined biochemically[1,3].

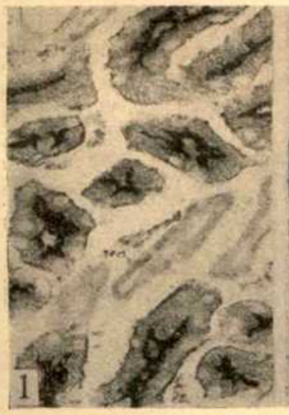

Fig. 1. Enzymic activity in brush border of proximal convoluted tubule

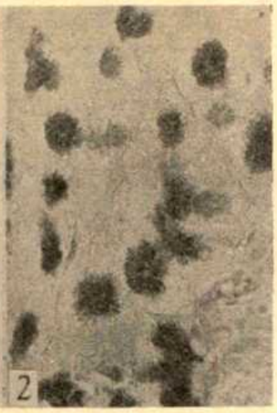

Fig. 2. Enzymic activity in acinar cells of pancreas. No activity evident in islet on the right

With freshly frozen tissues, acetone-washed or acetone-fixed and freeze-substituted paraffin-embedded tissues, γ-glutamyl transpeptidase-like activity was localized in the guinea pig kidney to the brush border of the proximal tubules in the outer medulla and the medullary rays (Fig. 1), and in the pancreas in acinar cells with no reaction evident in islets (Fig. 2). Although transferase reactions have been demonstrated histochemically previously by localizing the synthesized product of the reaction, this report is the first published description of a histochemically demonstrated transferase reaction in which kinetics of the reaction could be investigated during incubation.

Histochemical Characterization of a Unique α-Glutamyl Peptidase Activity

The use of histochemical substrates as a means of localizing known enzymic activities to specific cells or groups of cells in tissue sections has been well established. Histochemical techniques have also been used to distinguish selectively previously undescribed enzymic activity in single cells or groups of cells. The purpose of this communication is to report the identification and characterization of an enzyme(s) which catalyses the hydrolysis of *N*-(α-L-glutamyl)β-naphthylamide in the β-cells of the islets of Langerhans and the interlobular duct epithelium of the guinea pig pancreas.

The intermediate, *N*-(γ-benzyl-N^{α}-carbobenzoxy-α-L-glutamyl)β-naphthylamide, was prepared from *N*-carbobenzoxy-L-glutamyl-γ-benzyl ester[6] and β-naphthylamine using the mixed anhydride technique with isobutyl chlorocarbonate in dioxane. The product was crystallized from ethyl acetate, and had a melting point of 170° C. The substrate, *N*-(α-L-glutamyl)β-naphthylamide, was obtained by catalytic hydrogenation of the carbobenzoxybenzyl ester intermediate in absolute methanol. The substrate was crystallized from 90 per cent methanol-ether and had a melting point of 174–75° C. (Analysis found: C, 62·3; H, 6·2; N, 9·3; weight loss on drying at 100° C. under vacuum, 5·8; amino-N, 4·9 (ref. 7); Calc. for $C_{15}H_{16}O_3N_2.H_2O$; C, 62·1; H, 6·2; N, 9·7; weight loss, 6·2; amino-N, 4·8 $[\alpha]^{20}_{D}$ + 42·5° (*c*, 1·0 in dimethylformamide made 2 *N* with concentrated hydrochloric acid).

Initial investigations using a simultaneous coupling technique revealed localization of enzymic activity in the guinea pig pancreas to the β-cells of the islets of Langerhans (Fig. 3) and the epithelium of the interlobular ducts (Fig. 4). The *p*H optimum with *tris*(hydroxyamino)methane buffer was 6·2. Histochemical work was performed on slide-mounted fresh-frozen 10μ sections of guinea pig pancreas employing an incubating solution containing 0·105 mgm. of substrate and 0·375 mgm. of the stable diazonium salt of *o*-aminoazotoluene (fast garnet salt *GBC*) per ml. of 0·2 *M tris* buffer, *p*H 6·2 for 30 min. at 27° C. Substrate was added as a stock solution (4·2 mgm./ml.) in acetone in order to facilitate its dissolution.

Complete inhibition of enzymic activity was obtained with 0·01 *M* disodium ethylenediamine tetraacetic acid, 0·01 *M* citrate, 0·01 *M* phosphate or 0·001 *M* cadmium chloride, while marked activation of hydrolysis was evident on the addition of 0·001 *M* calcium chloride. The addition of 0·001 *M* potassium cyanide or 0·001 *M* cobaltous chloride resulted in a moderate activation. No significant effect on enzymic activity was evident with 0·001 *M* magnesium or manganous chloride nor with 0·001 *M* iodoacetate, 0·005 *M* *p*-chloromercuribenzoate, 0·001 *M* zinc sulphate, 0·0001 *M* di-iso-propylphosphorofluoridate or 0·001 *M* nickel chloride. The peptide α-L-glutamyl-L-phenylalanine caused pronounced inhibition of activity at 0·01 *M* concentration while no inhibition was observed with the same concentration of L-isoglutamine, L-asparagine, L-leucinamide, benzoyl-DL-arginine amide, L-glutamine, L-glutamic acid or glycylglycine.

From the above histochemical evidence it would appear that the enzyme(s) responsible for the hydrolysis of *N*-(α-L-glutamyl)β-naphthylamide in the β-cells of the islets of Langerhans and the interlobular duct epithelium of the guinea pig pancreas is activated by calcium, and inhibited by disodium ethylenediamine tetraacetic acid; in other words, it is a metal-activated enzyme. The fact that activity is not inhibited by leucinamide and the lack of effect of magnesium on the hydrolysis-rate of the histochemical substrate would tend to exclude the significant participation of the enzyme leucine aminopeptidase[8] in a hydrolytic reaction in the above sites. No other histochemical peptidase substrates including an *N*-(γ-L-glutamyl)β-naphthylamide (see previous communication) have been reported to be hydrolysed by an enzyme(s) having the activity characteristics or localizations of that reported here.

Many new potential peptidase substrates have been synthesized in attempts to characterize further this enzymic activity. Among these are the β-L-aspartyl,

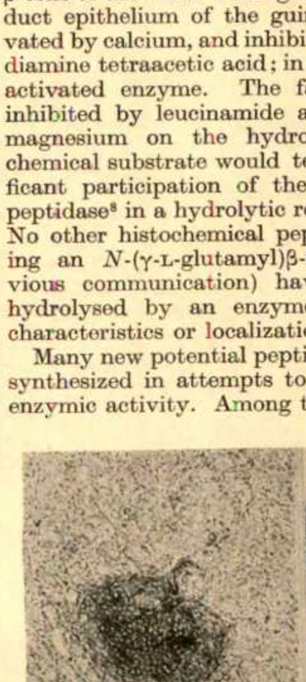

Fig. 3. Enzymic activity in β-cells of guinea pig pancreatic islet.

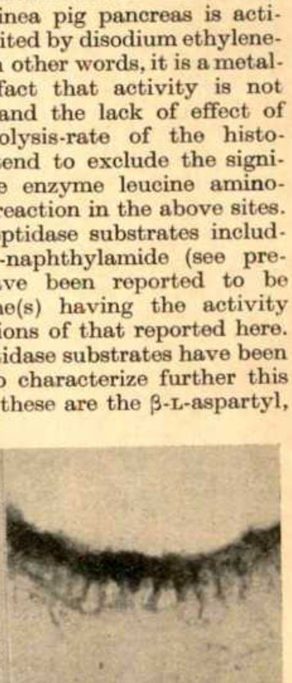

Fig. 4. Enzymic activity in epithelium of interlobular pancreatic duct

α-L-aspartyl, α-L-asparaginyl and α-L-glutaminyl-β-naphthylamides. None of the foregoing compounds was hydrolysed enzymically in the duct epithelium or β-cells of the islets of Langerhans of the guinea pig pancreas.

Attempts to purify the enzyme(s) responsible for the hydrolysis of *N*-(α-L-glutamyl)β-naphthylamide are in progress and a more detailed report of the kinetics and tissue localization of this activity will be the subject of a future communication.

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ANTIBIOSIS AMONG LACTOBACILLI

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IN an investigation of lysogenicity in lactobacilli, 199 strains of lactobacilli were examined. One hundred and eighty-nine of these strains were isolated from as many different sources of human saliva, and many have previously been used in attempts to isolate lytic phage[1]. They comprised 140 homofermentative and 49 heterofermentative species. The remaining ten strains are type-cultures kindly supplied by Dr. M. Elizabeth Sharpe, of the National Institute for Research in Dairying, Reading, England. The composition of the tomato dextrose broth and agar used has been mentioned in ref. 1 and all cultures were incubated at 37° C. in an atmosphere of carbon dioxide. Lysogenicity was investigated as follows: supernatants of 10-day-old broth cultures were sterilized by shaking with a 1 per cent volume of chloroform. After all traces of chloroform had been evaporated, drops of these supernatants were spotted on lawns of all remaining strains. Plates were then incubated overnight. The supernatants of twelve cultures produced clear areas of inhibition of growth. On occasions a few colonies composed of resistant variants of the indicator strain were present in the zone of clearing. Sub-cultures of pieces of agar cut from the clear areas never showed growth. The maximum dilution in which supernatants are active is 1 : 10. Afterwards these twelve supernatants were tested against a further 269 homofermentative and 86 heterofermentative salivary strains of lactobacilli. Three of the supernatants, Nos. 253 *A*, 713 *A* and 838 *A*, possess identical spectra of activity. The remainder show very little overlapping and a total of 127 strains of lactobacilli are killed by the twelve supernatants. Results are shown in Table 1. Cultures from which active supernatants are derived are also inhibitory as indicated in Fig. 1.

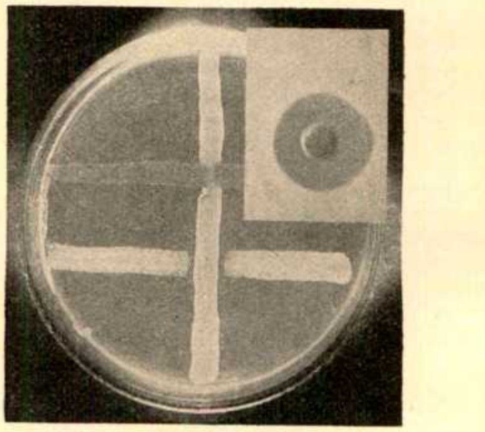

Fig. 1. Fermenti strain 466 washed and streaked on agar. Strain *F* 1 (Reading fermenti) and fermenti 206 cross-streaked. Plates incubated anaerobically overnight. Strain *F* 1 inhibited by strain 466

Inset: 0·2 ml. of a resuspended ammonium sulphate precipitate (*p*H 6·8) of supernatant 466 deposited in sealed hole in agar. After 8 hr. surface covered with sloppy agar containing strain *F* 1 and incubated. Area of inhibition of growth due to diffusion of antibiotic

Table 1

Supernatants producing zones of inhibition	Number of susceptible strains*				
	A (215)	*B* (1)	*S* (43)	*H* (1)	*F* (121)
Strain No. 35 *A*	2	1	1	1	1
Strain No. 93 *A*	6	1	1	1	15
Strain No. 124 *A*	0	0	1	0	1
Strain No. 185 *A*	6	1	1	1	9
Strain No. 253 *A*	15	1	1	1	20
Strain No. 303 *A*	14	1	1	1	19
Strain No. 496 *A*	11	1	1	1	17
Strain No. 558 *A*	20	1	1	1	21
Strain No. 571 *A*	3	1	0	1	3
Strain No. 713 *A*	15	1	1	1	20
Strain No. 838 *A*	15	1	1	1	20
Strain No. 466 *F*	2	0	0	0	7
Total number of different susceptible strains	51	1	1	1	73

* No activity demonstrated on 49 strains of *Lactobacillus casei*, 95 strains of *L. casei* var. *rhamnosus*, 13 strains of *L. plantarum* and 16 strains of *L. brevis*.

Figures in brackets indicate number of strains tested.

A, *L. acidophibus*; *B*, *L. bulgaricus* (Reading strain); *S*, *L. salivarius*; *H*, *L. helveticus* (Reading strain); *F*, *L. fermenti*.

The properties of active supernatants differ from those of phage suspensions in that no individual plaques are ever produced, activity is diffusable in agar (Fig. 1) and bactericidal titres are not affected by boiling for 15 min. The *p*H of the supernatants varies between 3·6 and 4·1, but activity is not affected by adjusting to *p*H 7 and/or conducting the tests on agar buffered to *p*H 7 with 1 per cent phosphates. In subsequent work the *p*H of supernatants was adjusted to 6·8. Microscopical observation of sensitive cultures treated with active supernatants demonstrated that the bactericidal action is not due to bacteriolysis and may correspond to the mode of action of colicins Particular attention was paid to avoiding the pitfall of mistaking this inhibition of catalase-negative organisms for the mere action of hydrogen peroxide which may have accumulated in the supernatants Using the methods of Coulthard *et al* [2], the hydrogen peroxide sensitivity of various strains was determined In no case is an inhibitory strain more resistant to hydrogen peroxide than its indicator lactobacilli and it is sometimes more sensitive Activity is not diminished by pre-incubating the supernatants with 0 1 per cent catalase (British Drug Houses) or using indicator strains seeded on agar supplemented with 10 per cent fresh rabbit serum Furthermore, it is possible to increase the titre of supernatants considerably by dialysis against 30 per cent polyethylene glycol Activity is not extractable with ether but is precipitated by saturated ammonium sulphate and also by ethanol The antibiotics often attain maximal bactericidal titres in the supernatants of overnight cultures Titres may decline on further incubation, and the detection of twelve antibiotic-producing cultures with the use of 10-day-old supernatants must be regarded as a minimal finding The antibacterial spectrum of the twelve supernatants (concentrated by ammonium sulphate precipitation) was tested on other members of the family Lactobacteriaceae, various enterobacteria and staphylococci A few strains of *D pneumoniae* and many isolates of enterococci are susceptible to a number of these antibiotics Enterobacteria and staphylococci appear to be immune The action of these antibiotics thus appears restricted to certain members of the family Lactobacteriaceae in much the same way as colicine activity is confined to the family Enterobacteriaceae

Apart from the spectrum of activity, no other differences have been noted between antibiotics derived from the eleven homofermentative strains and the single heterofermentative species Reactions to these antibiotics may serve as a useful complement to phage-typing schemes of lactobacilli[1]

Vincent *et al* [3] have considered the claims of workers who reported antibiotic effects produced by various homofermentative lactobacilli They conclude that the hydrogen peroxide effect can only be excluded in the report of Grossowics *et al* [4] and in their own particular case The antibiotic reported by Grossowics *et al* is produced by an unidentified homofermentative lactobacillus and is soluble in ether It is active on a large series of organisms but no activity against lactobacilli is indicated The antibiotic described by Vincent *et al* is produced by strains of *L acidophilus* It is only produced by aged agar cultures, is inactivated by ethanol and possesses a wide spectrum, including activity on certain *L acidophilus* strains

The antibiotics described here differ in many respects from the two just mentioned How their activity influences the ecology of natural populations of lactobacilli remains to be determined

This work is submitted with the permission of Prof C L de Jager, honorary director of the Nutrition and Dental Health Research Group of the South African Council for Scientific and Industrial Research

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AGENTS WITH HIGH ACTIVITY AGAINST TYPE 2 POLIOVIRUS

By Dr D G. O'SULLIVAN and Dr P W SADLER

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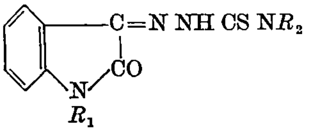
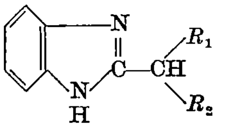
ISATIN-β-thiosemicarbazone (I , $R_1 = R_2 =$ H) and some derivatives in which the terminal amino-group remains unsubstituted (that is, I ; $R_2 =$ H) have high selective activity against neurovaccinia[1], alastrim[2], rabbitpox (in tissue culture)[3] and smallpox[4], but show no inhibiting propensities towards ectromelia[5]. When, however, both hydrogen atoms at the end of the side-chain have been replaced by alkyl groups (I, $R_2 =$ alkyl), marked activity against ectromelia is introduced, but the compounds now have no inhibiting action against vaccinia[5] Following these observations on the effect of isatin derivatives on pox viruses containing deoxyribonucleic acid (DNA), assays have been carried out using enteroviruses containing ribonucleic acid (RNA) Here again an interesting difference is observed Most compounds were inactive against type 1 poliovirus, but compounds possessing two substituents on the terminal amino-group (I , $R_2 =$ alkyl) exhibited high activity against type 2 poliovirus



(I)



(II)

Tamm and his co-workers[6] have recently studied and surveyed activities of promising compounds (benzimidazoles and benzotriazoles) towards poliovirus type 2 and have concluded that, although many compounds show purely inhibitory action, only 2-(α-hydroxybenzyl)-benzimidazole (II ; $R_1 =$ Ph, $R_2 =$ OH) and its 5-chloro derivative are potentially useful, in view of their selective action on the virus As 2-hydroxymethylbenzimidazole (II , $R_1 =$ H, $R_2 =$ OH) and the 2-(α-hydroxyethyl)-compound (II; $R_1 =$ Me, $R_2 =$ OH) are both inactive, and as the 2-benzyl derivative shows slight activity, the presence of both the benzyl group and a suitably sited hydroxyl group are probably necessary for useful activity in this particular series

As 1-methyl-4′·4′-dibutylisatin-β-thiosemicarbazone (III) at 30 μM concentration and 2-(α-hydroxybenzyl)-benzimidazole at 100 μM concentration have similar effects on poliovirus type 2 in tissue culture (Fig 1), the structures were examined for comparable features The infra-red spectrum[8] shows the presence of intramolecular hydrogen bonding in the isatin derivative (III), and intramolecular hydrogen bonds are also possible in the benzimidazole (IV)

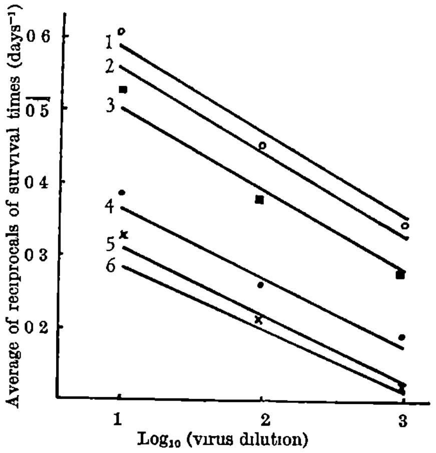


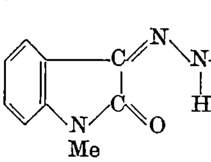
Fig 1 Poliovirus type 2 assays Variation of average reciprocals of survival times (in days) with logarithms of virus dilutions (in presence of 50 per cent of maximum tolerated dose of different compounds) (1) Control line without antiviral compound (2) 2-(*o*-Hydroxyphenyl)-benzimidazole (10 μM) (3) 2-(*p*-Hydroxybenzyl)-benzimidazole (100 μM) (4) 1-Methyl-4′ 4′-dibutylisatin-β-thiosemicarbazone (30 μM) (5) 2-(*o*-Hydroxybenzyl)-benzimidazole (50 μM) (6) 2-(α-Hydroxybenzyl)-benzimidazole (100 μM) For clarity, experimental points have been included only for line 3 (squares), line 4 (dots), line 5 (crosses), and for the control line (circles)

In the latter case, however, this bonding will be weak in view of the strain present in the small ring and in view of the strong tendency of benzimidazole derivatives to form intermolecular hydrogen bonds[9]

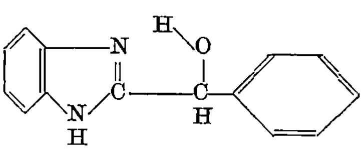
To shed further light on this, some related benzimidazoles were synthesized and tested. 2-(*o*-Hydroxybenzyl)-benzimidazole (V), which possesses the benzyl substituent and a hydroxyl group in the neighbourhood of the nitrogen atom, should form a similar, but slightly stronger, intramolecular hydrogen bond to that in compound (IV) Tissue culture experiments, described later, show that the *o*-hydroxy compound (V) has a similar selective action against poliovirus type 2 to that exerted by the α-hydroxy compound (IV) (see Fig 1)

The hydrogen-bonding hypothesis was further examined by assaying the *p*-hydroxybenzyl- and the *o*-hydroxyphenyl-benzimidazoles (VI) and (VII) respectively

The *p*-hydroxy compound (VI) is incapable of forming any intramolecular hydrogen bonds, but can associate by intermolecular hydrogen bonds Bearing in mind the low activity of 2-benzylbenzimidazole itself, some activity might be expected in the *p*-hydroxy compound, but, if the existence of an intramolecular hydrogen bond is of importance, then the activity should be low Fig 1 shows that the activity of this compound is considerably less than that of the α-hydroxy compound (IV)



(III)



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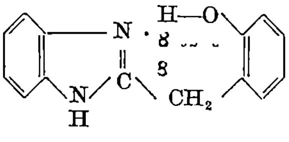
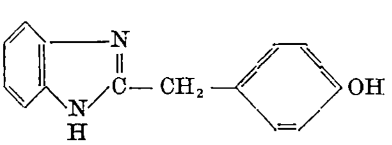
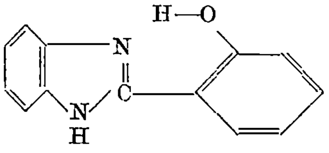


(V)

If the benzyl substituent were important for high activity in this series, then its absence from the *o*-hydroxyphenyl compound (VII) would affect the potential activity This compound can, of course, form intramolecular hydrogen bonds of reasonable strength Fig 1 shows that the activity is less than that of the *p*-hydroxybenzyl compound (VI) This result indicates that replacement of the benzyl group by a phenyl group reduces activity, and, consequently, conjugation between the benzimidazole and benzene rings may be detrimental to the antiviral action



(VI)



(VII)

Eggers and Tamm[10] have shown that the α-hydroxy compound (IV) acts by inhibiting virus multiplication Now the presence of intramolecular hydrogen bonding is a feature common to all antiviral agents of high activity in both the isatin-β-thiosemicarbazone and the benzimidazole series Thus it is probable that an active compound either chelates with a trace metal or forms intermolecular hydrogen bonds with some key molecule involved in the inclusion of nuclear DNA or perinuclear RNA into incomplete virus particles Many simple benzimidazoles[9] and benzotriazoles[11] of lower activity[6] cannot form intramolecular hydrogen bonds, but have been shown by their infra-red spectra to form intermolecular N—H N links Strong, but non-specific, bonding to protein is possible with these compounds

2-(*o*-Hydroxybenzyl)-benzimidazole was prepared by heating under reflux *o*-phenylenediamine (1 mole) and *o*-hydroxyphenylacetic acid (1 mole) with 2 *N* hydrochloric acid (2 moles) for 20 hr The hydrochloride, melting point 130°, crystallized on cooling After dissolving in water and making alkaline, 2-(*o*-hydroxybenzyl)-benzimidazole was obtained which, on crystallizing from aqueous ethanol, had a melting point of 229° (found C, 74 7, H, 5 2, N, 12 1, $C_{14}H_{12}N_2O$ requires C, 75 0, H, 5·4, N, 12 5 per cent)

2-(*p*-Hydroxybenzyl)-benzimidazole was prepared in a similar way using *p*-hydroxyphenylacetic acid After crystallizing from aqueous ethanol, it had a melting point of 270° (found C, 74 8, H, 5 2; N, 12 1 $C_{14}H_{12}N_2O$ requires C, 75 0, H, 5 4, N, 12 5 per cent)

2-(*o*-Hydroxyphenyl)-benzimidazole, melting point 231°, was prepared by the method of Hubner[12], and 2-(α-hydroxybenzyl)-benzimidazole, melting point 201°, as described by Bistzycki and Przeworski[13].

Antiviral assays were determined in tissue culture experiments Monolayers of *ERK* 1 cells[3] were grown in a number of tubes using *ELP* medium[3] After three days, the media were replaced by otherwise identical media, but containing half the maximum tolerated dose of the test compound Test compounds were absent from control tubes Different dilutions (ten-fold) of the virus were added to the tubes, which were then rolled continuously at 37° and examined twice daily for signs of cytopathic effects The end-point was taken when half the cells were affected Average of reciprocals of survival time was plotted against the logarithm of the virus dilution (Fig 1) Relative activities of compounds are given by displacement (to the left) of the line from that for the standard[14]

The results obtained indicate that both 1-methyl-4′ 4′-dibutylisatin-β-thiosemicarbazone (III) and 2-(*o*-hydroxybenzyl)-benzimidazole (V) have activities against poliovirus type 2 which compare favourably with those observed with other compounds and 2-(*o*-hydroxybenzyl)-benzimidazole shows considerable promise as an agent against poliovirus type 2

We thank Dr A P. Goffe, who kindly supplied the viruses (type 1 strain *Sc*, 2 *ab*, type 2 strain *P* 712 *Ch*, 2 *ab*) used in the assays, and Dr F W Sheffield for his generous advice.

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RESPONSE OF CATFISH BARBELS TO TASTE STIMULI

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TASTE buds were first discovered and their function correctly interpreted by Weber[1] on carp, and it was not until 1867 that Schwalbe[2] and Loven[3] discovered independently similar taste organs on the tongues of mammals Such taste-buds are found over certain areas of the skin of some fish, and are innervated by branches of the facial, glossopharyngeal and vagal nerves (Herrick[4]) In the catfish, these buds are abundant on the barbel, though they are also scattered over the whole body surface Using behavioural techniques, Herrick[4] and Parker[5] studied the effect of applying chemical stimuli to the barbel, and confirmed that these buds are chemosensory organs Later Hoagland[6] attempted to study chemoreception in the catfish electrophysiologically, but although he recorded activity from tactile fibres of the facial nerve innervating the barbel, he could record no spikes from the chemosensory fibres However, he did detect an increase of neural activity through the audio-system This suggests that, with the more refined techniques now available, responses from these fibres might be recorded, and in the present work this was attempted

The freshwater catfish (*Ameiurus melas*) used weighed 200–300 gm The fish was first anæsthetized by dipping into 0 03 per cent tricaine methanesulphonate solution for a few minutes, then transferred into 0 015 per cent tricaine solution and its head stabilized by a holder In order to stimulate the nasal barblet and record electrophysiologically, the dorsal region of the head was exposed to air (Fig 1) Only nasal barblets were used, since no response to chemical stimuli could be elicited from the maxillary barblet After removal of the skin near the basal region of the barblet, the branch of the facial nerve to the barblet was isolated from the surrounding tissue and severed centrally The nerve bundle was usually divided into small bundles, and one of them was placed on platinum electrodes to record the neural response Both nerve and electrodes were covered with mineral oil, and the electrodes connected with an a c pre-amplifier, followed by a cathode-ray oscilloscope and electronic summator to quantify the neural response (Beidler[7]) The stimulating solution was allowed to flow slowly into a special chamber which surrounded the barblet The barblet was washed with water after

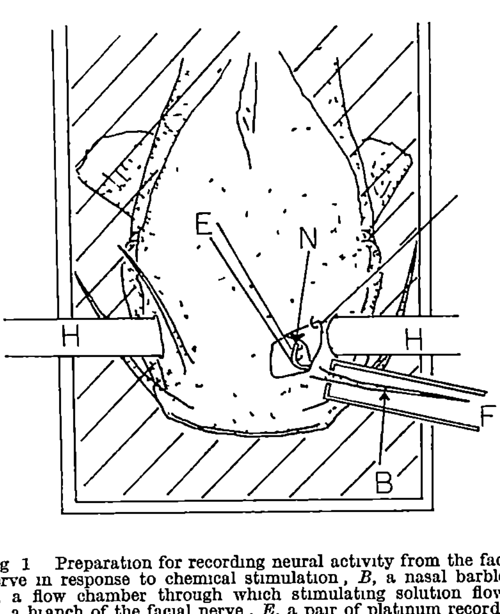

Fig 1 Preparation for recording neural activity from the facial nerve in response to chemical stimulation, *B*, a nasal barblet, *F*, a flow chamber through which stimulating solution flows, *N*, a branch of the facial nerve, *E*, a pair of platinum recording electrodes, *H*, animal holder

each stimulation The responses to adequate concentrations of chemicals were reproducible for several hours, if the blood supply were intact

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Hoagland[6] assumed that spikes from the tactile fibres were larger than those from the chemosensory fibres However, the present results show that many spikes caused by rapid flow of solution are of the same order of magnitude as those recorded from chemosensory fibres (Fig 2), although some of them are larger Since the rapid flow of solution causes a short burst of activity of such tactile fibres, only a gentle flow of solution was used

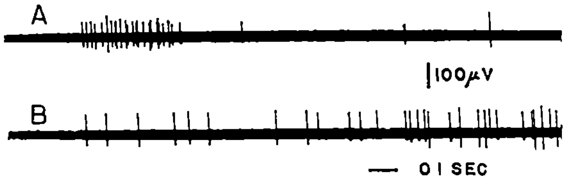

Fig 2 Spikes from the facial nerve, which includes a few intact nerve fibres *A*, responses of tactile fibres to rapid flow of tap water, *B*, responses of chemosensory fibres to 1 *M* sodium chloride The record shown in *B* was obtained after cutting the tactile fibres in the nerve used for record *A*

Application of sodium chloride resulted in a train of spikes which continued throughout stimulation The summated responses to various concentrations of sodium chloride are shown in Fig 3, while in Fig 4*a*, the responses are plotted against concentrations of various salts sodium chloride, potassium chloride, lithium chloride and ammonium chloride The maximum height of the integrated response to each stimulus was used as a measure of the magnitude of response It is interesting to note that potassium chloride is more effective for catfish than is sodium chloride, which agrees with results obtained from carnivores, but is in contrast to those obtained from rodents (Beidler *et al* [8]) The mean value of the ratio

$$\frac{\text{response to 0 25 } M \text{ sodium chloride}}{\text{response to 0 25 } M \text{ potassium chloride}}$$

is 0 36 Thresholds for these salts are about 0 1 *M* (Fig 4*a*) This value is comparable with that of 0 1 *M* obtained behaviourally by Stieck[9] for the minnow and that of about 0 1 *M* obtained by Koketsu and Kimura[10] for the frog, but is higher than that of 0 002 *M* for the rat obtained by Beidler[7] Divalent alkali salts of greater concentration than 0 5 *M* produce an irreversible reduction of activity, but the maximum magnitude of their response is not as large as that to potassium chloride The effect of different anions was tested by using the sodium salts, sodium chloride, sodium bromide, sodium nitrate, sodium sulphate and sodium citrate The magnitude of response to equi-normal concentrations of these salts was almost identical except the response to sodium sulphate, which was smaller than that to the other salts (Fig 4*b*) Application of hydrochloric acid elicited a large initial burst of impulses which declined rapidly until, within 10–20 sec after application, almost no spike activity remained The threshold for hydrochloric acid was about 0 0005–0 001 *M*, which is much higher than that of 0 00001 *M* obtained behaviourally by Allison and Cole[11] for the killifish, but is comparable with that of 0 001 *M* or less for the rat's tongue obtained electrophysiologically by me Hydrochloric acid, 0 01 *M* or higher in concentration, caused an irreversible reduction of response Maximum response to 20m*M* quinine hydrochloride was reached in ten or more seconds after application of the stimulus, and spike activity continued for a short time after washing the barblet with water, although no irreversible change in receptor activity was observed

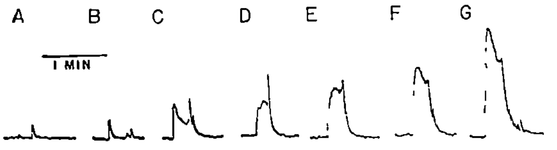

Fig 3 Summated response to tap water (*A*) and to sodium chloride of 0 1 *M* (*B*), 0 2 *M* (*C*), 0 25 *M* (*D*), 0 4 *M* (*E*), 0 5 *M* (*F*) and 1 0 *M* (*G*)

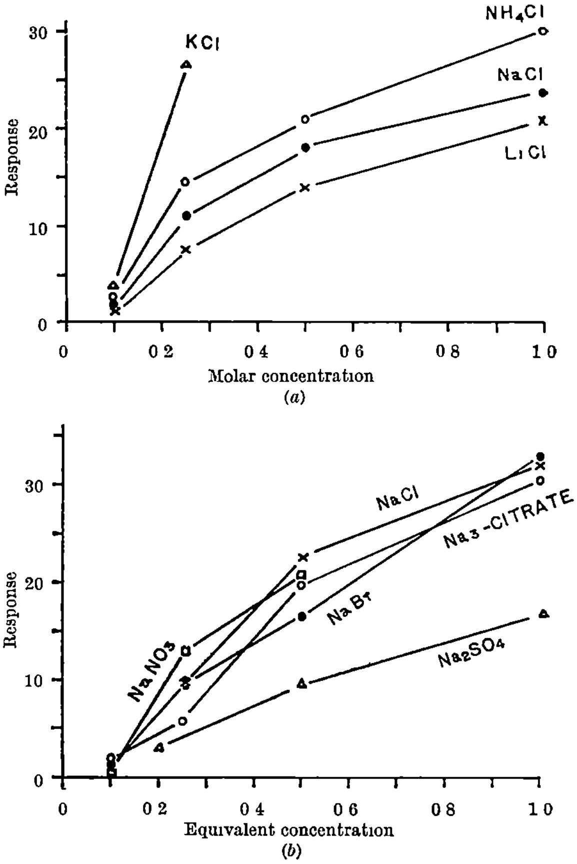

Fig 4 *a*, relationship between summated response and concentration of chloride salts, *b*, relationship between summated response and concentration of sodium salts

I am grateful to Dr Lloyd M Beidler for his kind suggestions and criticisms

This work was supported by a contract between the Office of Naval Research and Florida State University.

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LETTERS TO THE EDITORS

GEOPHYSICS

Diurnal Variation of High-latitude Auroral Frequency on Magnetically Quiet and Disturbed Days

DURING the winters of 1957–58 and 1958–59 an all-sky camera was operated by the Danish Meteorological Institute at Nord in Greenland (geographical co-ordinates 81 6° N, 343 4° E, geomagnetic co-ordinates 80 9° N, 134 5° E)

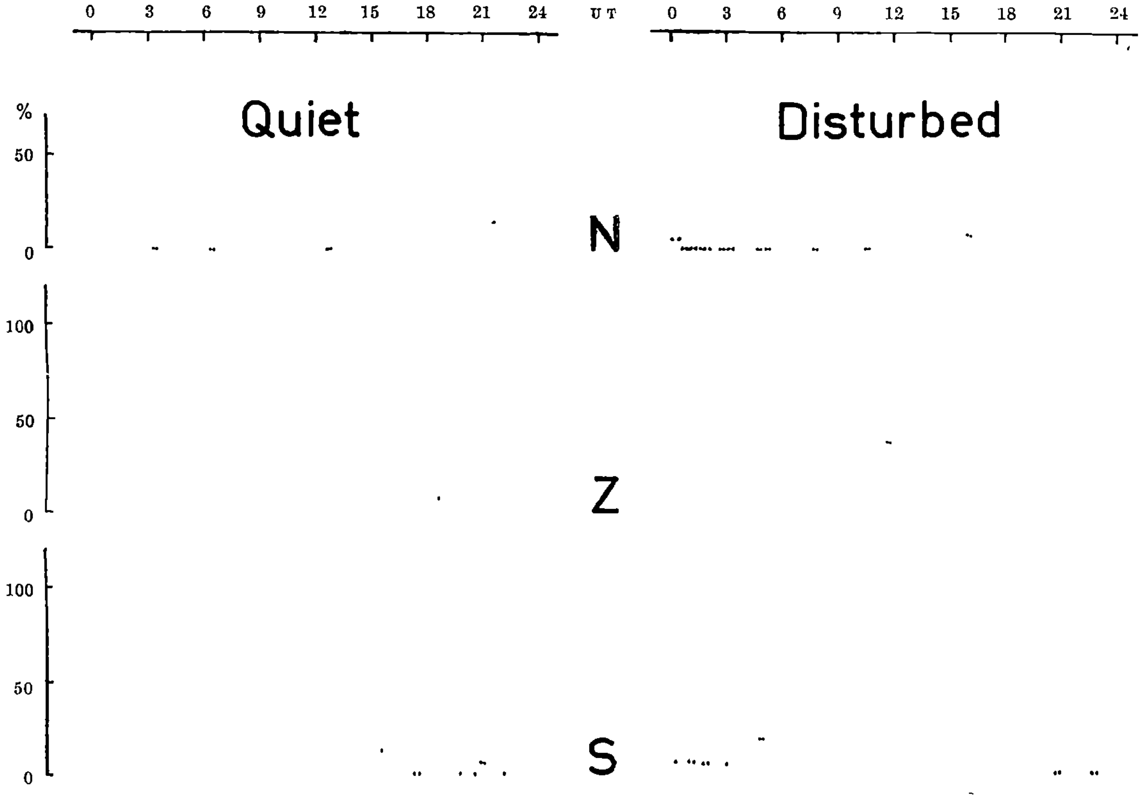

Fig 1 Diurnal variation auroral frequency, Nord, 1957–59

Following a proposal by Stoffregen[1] the sky was divided into sectors north (N), between 80° and 60° northern zenith distance, zenith (Z), between 60° northern and 60° southern zenith distance, and south (S), between 60° and 80° southern zenith distance, north being defined here as the direction perpendicular to the auroral isochasm[2] through the station, geographically this direction is approximately west-north-west

Fig 1 shows the diurnal variation of auroral frequency in the three sectors on international quiet and disturbed days, respectively It is seen that zenithal auroræ are most frequent in the day hours of international quiet days, with two principal maxima at about 5 and 13–14 hr U T On international disturbed days these auroræ are less frequent In S the quiet day variation similarly shows two distinct maxima, at about 6 and 11–12 hr U T, whereas the variation on disturbed days is of a different shape, with a single maximum at about 9 hr U T, that is, near magnetic noon in the Mayaud system[3] Smaller peaks at 9 hr may be seen in the diurnal variation on disturbed days at Z as well as at N

It is proposed that the observation should be interpreted as follows

At magnetic noon the auroral zone is situated not far to the south of the station When on magnetically disturbed days the auroral incidence near the zone increases, the zone broadens and zonal auroræ are observed from the station near magnetic noon

The auroræ which are observed above the station on quiet days become less frequent on disturbed days, possibly due to some sort of modulation effect They may be said to belong to a special polar population (inner auroral zone), or what may be the same in other words, to form a quiet-day appendix to the day-time part of the main auroral zone.

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ASTROPHYSICS

Megashatter Cone Hypothesis of the Origin of Lunar Volcanoes

I BELONG to the school that interprets the main observable lunar topographical features as the result of impacts with asteroids and comets. To proponents of this belief, a stunning blow was delivered in 1959 when Kuiper[1] exhibited, during a space symposium in Washington, his beautiful pictures of small features that just could not be impact explosion craters. The discovery of release of carbon dioxide gas also strengthened the picture of some internal lunar feature through which gas could be released.

Now another possibility occurs that the lunar volcanoes are not volcanoes at all, but extremely large shatter cones (which will be referred to as megacones or megamegacones) produced by the shock-waves resulting from impact of an asteroid. These would appear as stumps in the central uplifts of very ancient eroded lunar craters, namely, lunar astroblemes. Dietz[2] has found terrestrial shatter cones as large as 6 ft long in the Kentland, Indiana, 'cryptovolcanic' structure. He proposed the term 'astrobleme' for features of the Kentland type.

It is proposed that megacones of extremely large size exist and that these are composed of the smaller groups of shatter cones discussed in refs. 3 and 4. It is proposed that the roots of a central uplift in a crater formed by a large impact explosion produced by a nickel–iron asteroid consist of a megamegacone in turn composed of many concentrically arranged megacones all originally pointing upwards and inward toward the centre of impact. Most of these will have been almost instantly blown outward leaving a centre region filled with explosion breccia and/or devitrified impact glass. If glass is present, it will undoubtedly contain small spherulites of nickel–iron vapour.

The rebound after impact will lift up this root structure a considerable distance, however, it would always remain below the original lunar surface. Hydrodynamic forces resulting from the melting of the original ground-mass under extreme pressure and plastic flow of breccia dust produced would also tend to force the cone structure upward and outward. This megamegacone structure would then consist of somewhat tilted megacones with vertically up-ended ground-mass and breccia filling the voids between megacones during the original uplift.

After a couple of billion years one would expect that erosion of the uncompressed shattered matter around the stronger megacones would be removed as dust by methods as proposed by Gold[5] for a few craters at least. Shock of later impacts might also tend to unearth the buried compressed megacones by scattering the enclosing dust and exposing the volcano-shaped structure. A crater-like centre might be present in the original megamegacone because of the upward and outward thrust of its megacone components and the shape charge type of ejection of matter from the final rest point of the asteroid. If the volcano-like lunar structures are megacones, then their geometry may be a clue to their mineralogical composition and to the general selenology of the Moon. If the subsurface features of the Moon are largely strained, compressed, cone-like buried structures, then the shallow seismic effects of these regions may be predicted by a study of similar terrestrial features. Sharp, shattered rocks would be expected to be present near megacones because of gradual flaking from the compressed material resulting in the well-known small shatter-cone shapes and dust. The shattered nature of lunar astroblemes would probably promote faster dust formation by action of the extreme variations of temperature during lunar day and night than by the radiation damage mechanism proposed in ref. 5.

This work is supported by National Aeronautic and Space Administration research grant *NsG*-57-60.

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Sun–Earth Relations and Associated Periodicities

PERIODICITIES of less than 27 days in geophysical disturbance data have been investigated for many years, with inconclusive results, due to inherent difficulties in determining the statistical significance of the computations. However, there is now an increasing amount of evidence, arising from the examination of independent data samples, to support the existence of such periodicities. Some of the recent evidence is summarized here.

Auto-correlation of the 3-hourly magnetic planetary index a_p, during short moderately disturbed intervals in 1952, disclosed periods of from one to four days[1]. Moreover, the times of occurrence of magnetic impulses seemed to be closely connected with the amplitude of these periodicities, and with changes from one periodicity to another.

Ward[2] computed the variance spectrum of the daily C_i, K_p, and A_p indices for some portions of 1941–56, and found periods of $27/n$ days, with $n = 1, 2, 3, 4, 5$ and 6. No interpretation of the overtones of the 27-day period was given.

An examination has been made of the time separation, ΔT, between 817 magnetic impulses occurring during 1949–59. Intervals of up to 70 days were tabulated. The declining portion of the sunspot cycle, 1949–54, shows maxima in the number of occurrences $N(\Delta T)$ at about the period of solar rotation. Similar maxima do not occur in $N(\Delta T)$ during 1955–59. The same data were examined for higher frequencies, using a one-day running average. During 1949–54, in addition to the solar rotation maxima, other maxima in $N(\Delta T)$ occur at multiples of about $3\frac{1}{2}$ days. Similar maxima during 1955–59 occur at multiples of about 3 days (Fig. 1). This periodicity in $N(\Delta T)$ was

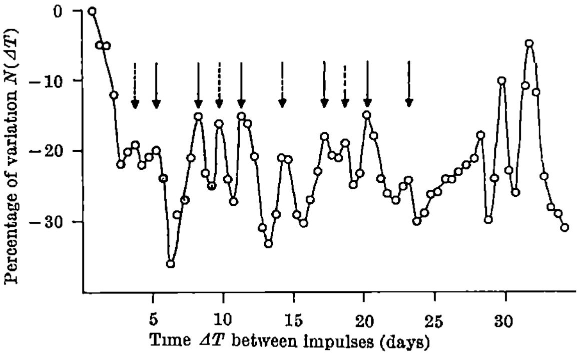

Fig. 1. The time separation ΔT between magnetic impulses, 1955–59

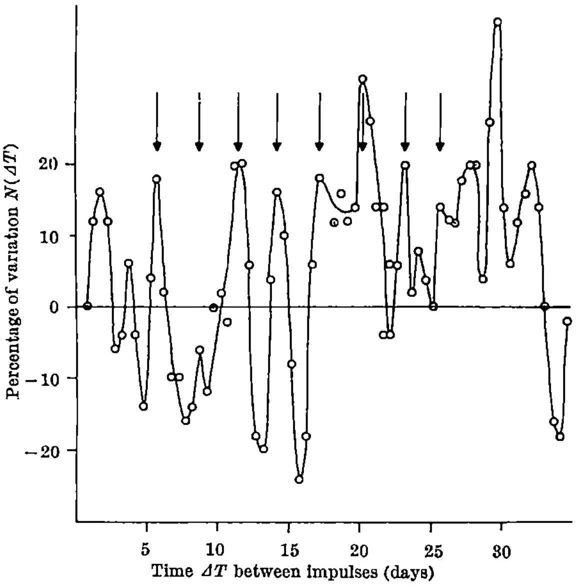

Fig 2 The time separation ΔT between magnetic impulses during the International Geophysical Year

particularly strong during the International Geophysical Year, and the mean period was 2⅞ days (Fig. 2) The data were insufficient to investigate periods of less than one day.

A separate investigation[3] was made of the time separation, Δt, between the occurrence of solar flares and magnetic *SSC* impulses during the International Geophysical Year All flares listed, from 1^{-} to 3^{+}, were given equal weight in the analysis Intervals of up to 14 days were tabulated to the nearest ¼ day, from *SSC* to flare ($-\Delta t$), as well as from flare to *SSC* ($+\Delta t$) The $N(+\Delta t)$ curves show a 1¾-day periodicity which is not present in $N(-\Delta t)$, and which could conceivably arise from the transit time of a disturbance from Sun to Earth Removal of the 1¾-day component leaves a 3-day periodicity in $N(+\Delta t)$ of about the same amplitude as a 2½-day periodicity in $N(-\Delta t)$ It could not be determined from this analysis whether the above difference between 2½ and 3 days is significant A one-day periodicity in the International Geophysical Year flare listings[4] made it impossible to decide whether the $N(\Delta t)$ contain a non-spurious one-day component.

The above periodicities in the time separation between geophysical disturbance data may be significant in the interpretation of statistical examination of upper atmospheric disturbances It was previously suggested[1] that the 1–4-day periodicities in a_p were consistent with repeated Sun–Earth transits of a hydromagnetic wave Although this is still a possibility, it now seems probable that the observed periodicities are more directly related to the timing of solar disturbance events The solar flare to *SSC* time analysis indicates that such solar disturbance events may occur within ¼ day of flare times, and this investigation is continuing

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Possible Solar Flare Effects in the *F* Region of the Ionosphere

I HAVE read with interest the recent communication by Knecht and Davies on this subject[1] which reports a series of large solar flares accompanied by marked effects in the *F* region of the ionosphere A particular feature is that the ionospheric effects and the flares are simultaneous only at isolated locations

In this connexion, I should like to direct attention to a communication by Martyn, Munro, Higgs and Williams[2] In that communication a correlation appeared to be established perhaps even more clearly than in the communication by Knecht and Davies. I write, however, not to claim priority of discovery but to report subsequent developments which considerably modified the conclusions in that communication Continuation of that work brought to light two very significant new facts It was found that the ionospheric disturbances occurred much more frequently than solar flares, and, even more important, that they almost always appeared at Canberra and Sydney with an appreciable time difference They were, in fact, travelling disturbances It becomes obvious, therefore, that there is a high probability, at any single point, of a disturbance occurring close to the time of a flare and an even higher one of a coincidence somewhere

When the War began in 1939, much research work, including the above, ceased Moreover, publication of scientific results was also largely inhibited until after the War, and ionospheric literature and history tend to start from that epoch, after a gap of some seven years

These results, therefore, though reported locally to the Radio Research Board, were never published When ionospheric work was resumed at this Laboratory in 1947 attention soon became directed to ionospheric travelling disturbances[3], and it was realized they were the same phenomena observed earlier.

The characteristics of such disturbances have now been very fully investigated here[4], and their manifestations are becoming more familiar in a variety of ionospheric investigations throughout the world, particularly as a result of work done during the International Geophysical Year Their effects on ionosonde records are similar to those shown in Fig 1 of Knecht and Davies's communication and they can also cause changes in frequency of the signals received by an ionospheric path as in their Fig 2

It would seem that since the ionospheric effects of a solar flare are due to ultra-violet radiation they should all be observed simultaneously over the sunlit surface of the Earth

I would urge the authors, in their further work, to give very careful consideration to these aspects For a correlation to have scientific value it must be significant both statistically and physically.

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Dr. Munro's scepticism regarding our recent suggestion[1] that certain large flares are associated with effects in the *F* region of the ionosphere is understandable As he points out, a solar photon phenomenon of this type would be expected to be evident over the entire sunlit hemisphere and not only at one or two isolated stations However, since the submission of our original communication, our work has revealed several new facts which may help to account for this apparent non-uniformity

First, from further examination of vertical soundings data, it appears that *F* region flare effects sometimes do occur simultaneously at a number of sunlit hemisphere stations The difficulty, of course, is to be able to determine with any degree of certainty whether or not an effect actually occurred in the presence of the normal variability of the *F* region and the flare-associated increase in *D* region absorption which usually is responsible for the loss of several crucial *F* region observations These factors, coupled with the relative infrequency of large flares, are undoubtedly responsible for the fact that the *F* region flare effect phenomenon has been so infrequently recognized in the past

The second new point concerns the height of the *F*2 layer just prior to the beginning of the flare We have found a strong tendency for the *F* region solar flare effect to occur only at those locations where the height of the *F*2 maximum electron density level ($h_{\max}$) at the time of the flare is relatively low (less than about 315 km)

The flare of November 12, 1960, has been chosen to illustrate these points Fifteen-minute values of *F*2 layer penetration frequency (*foF*2) are shown for six sunlit sounding stations in Fig 1 The stations are arranged in order of increasing $h_{\max}$ as determined by the manual 10-point method The time at which the flare begins (1323 U T) is shown by an arrow on the time-scale Considering pre-flare and post-flare trends, it seems clear that a flare-associated increase in penetration frequency, which is related to maximum electron density, occurred at at least four of the stations (San Salvador, Reykjavik, Belvoir and Talara) Flare-associated increases probably did not occur at Huancayo and Concepcion Note that the magnitude of the flare effect discontinuity seems to vary inversely with $h_{\max}$ This is what would be expected on the simple picture where the flare radiations have peak ionization-rates at a level significantly below the *F*2 layer, the rates falling off exponentially with increasing height above this level

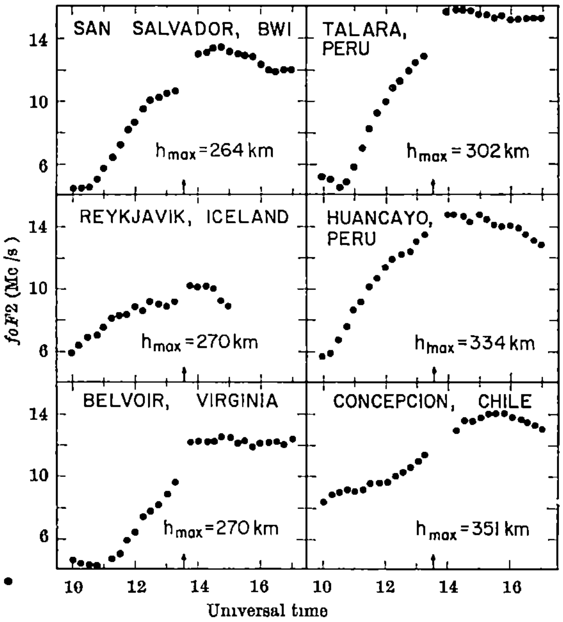

Fig 1 Variations in *F*2 layer penetration frequency at six sunlit sounding stations during the large solar flare of November 12, 1960 Arrow marks flare beginning

Because of the simultaneity of the increases over distances of 10,000 km or more, the possibility that the effects shown in Fig 1 are due to travelling ionosphere disturbances is, it seems to us, extremely remote. Moreover, travelling disturbances can be rather conclusively eliminated as the cause of even some single station occurrences, like that shown in our earlier communication[1] for Adak, Alaska, during the flare of November 15, 1960, since, during the 2½-year period from July 1957 until December 1959, on only one day were occurrences of discontinuities in *foF*2 of this magnitude (1 5 Mc /s increase in 15 min) observed during the corresponding period (1200–1800 L S T) To have one of the largest *F*2 layer travelling disturbances that occur at Adak pass over the station at almost exactly the same time as the occurrence of a large and relatively rare type of solar flare would seem fortuitous indeed Also, according to the earlier work by Munro and Heisler[2], travelling disturbances always take the form of first a reduction in penetration frequency and then an increase whereas in the present phenomena the increase occurs first Lastly, on the ionograms travelling disturbances are generally seen as additional cusps near the penetration frequency Cusps of this type have not been observed on the ionograms following the solar flare events.

Miss R E McDuffie has contributed substantially to the work discussed in this communication.

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Interstellar and Interplanetary Communication by Optical Masers

In *Nature* of April 15, 1961, p 205, reference is made to seven stars of nearly the same luminosity and spectral characteristic as our Sun within 10 light years. Apparently this erroneous statement is derived from an earlier general statement in *Nature*, September 19, 1959, p 844, except that 15 light years was changed to 10 light years

Even within 15 light years, there is only one star with "nearly the same luminosity and spectral characteristic as our Sun", namely, the brighter component of the Alpha Centauri system, while possibly one other may be considered to qualify, namely Tau Ceti, with a luminosity of 36 per cent of the Sun and spectral type *G*4 (instead of *G*0 for the Sun)

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Where the stars listed by Cocconi and Morrison are referred to in our article, 10 light years was erroneously written for 15 light years as noted by

Dr van de Kamp Even this is not strictly correct, since one of the distances is as large as 16 5 light years But these differences are not crucial for our purposes

The question whether certain stars are of "nearly the same luminosity and spectral characteristic as our Sun" is a more interesting one A term such as 'nearly' can have no absolute meaning and must depend on a scale of measurement relevant to the discussion at hand For example, it would not seem out of order for an astronomer to refer to stars within 15 light years distance as 'nearby' But such description may be shocking to a pedestrian when he learns that their distance is many trillions of miles In the case of our discussion, nearness or similarity should properly be measured in terms of what might be approximately equivalent for the possible development of life on planets somewhat similar to those we know On such a scale, ranges of about two magnitudes in luminosity and with spectral types *G* and *K*, which cover the stars in question, seem fairly small But it is understandable that Dr van de Kamp, accustomed to contexts where a different scale of measurement may be appropriate, does not find our statement a natural one

We hope that the actual list of stars, to which reference is given, leaves readers in no serious doubt about the facts

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PHYSICS

Dependence of Thermo-stimulated Current Curves on Heating Rate

The use of thermo-stimulated current and thermoluminescent ('glow') curves, to determine the energy distribution and population of traps in insulators and semi-conductors, has recently been severely criticized[1] because of the interactions which are now known to occur between energy-levels in the forbidden gap Nevertheless, it has so far been assumed that the shape of such curves is only slightly dependent on the heating-rate in the specimen

During observations of thermo-stimulated currents in a number of cadmium sulphide single crystals following irradiation at 90° K with cobalt-60 γ-rays, we have noted in one specimen a current peak, the shape of which is critically dependent on the rate of temperature increase As shown in Fig 1, the thermo-stimulated current curve generally exhibited two peaks corresponding to trapping-levels at 0 09 eV and 0 13 eV determined using the formula of Grossweiner[2] For rates of rise in temperature between approximately 17° C per min and 19° C per min a very marked increase occurred in the amplitude of the peak corresponding to the deeper trapping-level, the increase appearing as a secondary peak of high resolution No significant changes in the shapes of the two main peaks were observed when the secondary peak was present

If Grossweiner's formula[2] is applied to the secondary peak, the derived energy-level is 1 0 eV, which is below the steady-state Fermi-level calculated from the dark current at room temperature

It seems clear that monomolecular and simple bimolecular models on which theories of 'glow' curves have been based in the past[2-4] do not account for the present observation, but it is reasonable to assume that the high peak is associated with a particular energy-level of traps, which is initially filled and may empty either directly into the conduction band or indirectly through a second energy-level which is initially empty

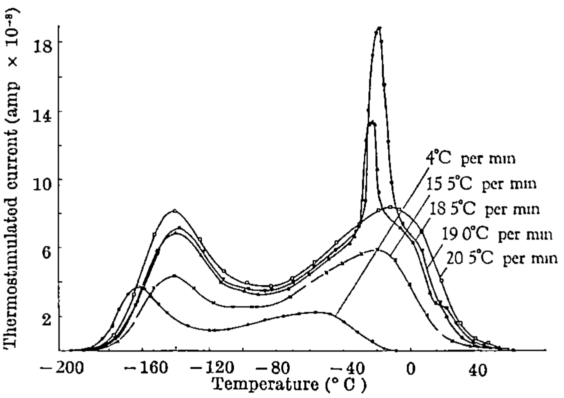

Fig 1 Typical thermo-stimulated current curves for a cadmium sulphide single crystal exhibiting a secondary peak highly sensitive to heating-rate

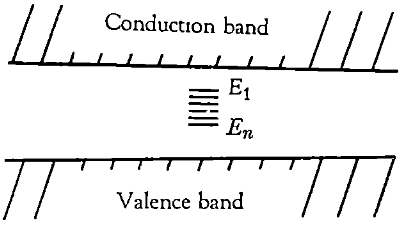

Fig 2

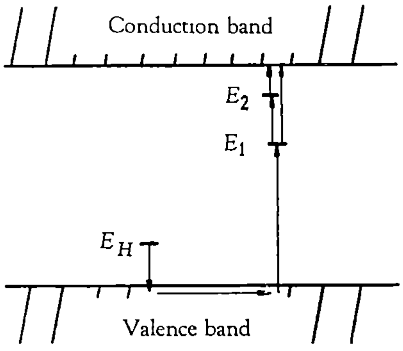

Fig 3

To account for the observation two possible models, shown in Figs 2 and 3, are suggested

In the first model, Fig 2, electrons are assumed to be held in traps of increasing depth E_1 to E_n It is also assumed that the escape frequency factor increases with depth of trap At low heating rates electrons are released directly into the conduction band from E_1 to E_n in turn, giving a broad thermo-stimulated current peak At high heating rates electrons are released from E_n to E_1 in turn, again giving a broad peak For particular values of trap depths and escape frequency factors, a high degree of simultaneous release may occur for a narrow range of intermediate heating rates resulting in a current peak of the type observed

In the second model, Fig 3, electrons associated with the appearance of the anomalous peak are assumed to be held in traps at the level E_1, and may be released either directly into the conduction band or indirectly through the second level E_2 with which E_1 is spatially associated The probabilities of direct or indirect release from E_1 to the conduction band depend on the heating rate and the escape frequency factors for the two levels

If the temperature of the crystal is raised slowly, the peak corresponding to E_1 does not appear since the electrons are all released indirectly through E_2

At some higher rate of rise of temperature the values of the escape frequency factors allow electrons from E_1 to be released directly into the conduction band giving a peak of the type observed

To account for the disappearance of the peak at higher heating rates, a hole trap, E_H, is introduced and E_1 is also assumed to act as a recombination centre

The escape frequency factor for holes in E_H is such that, for heating rates below that corresponding to the appearance of the anomalous peak, the holes are released after the release of the electrons from E_1 At higher heating rates, the holes, which are now released first, recombine with electrons at E_1 so that the anomalous peak no longer occurs.

The accurate evaluation of energy-levels from thermo-stimulated current and 'glow' curves depends on a satisfactory model being available Observations such as the present, and those reported by Hoogenstraaten[1] and by Halperin and Braner[4], indicate the limitations of the method and the care which must be exercised in the interpretation of results

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Electrical Conductivity of Calcium Oxide at High Electric Fields

Interest in the study of the electrical properties of the alkaline earth oxides as an aid to understanding the physics of the oxide-coated cathode has led to an extension of work previously carried out on the electrical conductivity of calcium oxide at low electric fields[1] In order to obtain activation energies for the conduction processes[2], it is necessary to determine the conductance at zero applied field as a function of temperature, but information of some value can be obtained from a study of the conductance of the cathode material at higher electric fields than those commonly employed Reports of investigations at high fields are much less numerous than those of conventional measurements, but the work of Danforth and Goldwater[3] and of Nakai, Inuishi and Tsung-Che[4] may be referred to Of interest is the recent investigation by Adams and Jacob[5] on strontium oxide the behaviour of its high-field conductance appears to be dissimilar to that of barium oxide, the form of the graph of conductance versus applied voltage depending strongly on the temperature of the oxide The present work on calcium oxide may be of interest in a general comparison with results from the other alkaline earth oxides

Two types of experimental tube have been used in the present work the calcium oxide samples were contained between nickel or between platinum cathode bases The construction and processing of the tubes have been described in a previous communication[1] Conductivity currents were measured on a galvanometer for applied potentials of up to 100 V d c , and characteristics of conductivity current versus applied potential were traced at various values of the sample temperature The conductance of the calcium oxide sample at an applied potential V was determined by taking the gradient of the characteristic at the relevant value of V

Fig 1 shows a typical series of current-voltage characteristics for a nickel-based sample of calcium oxide for the range of temperature T of 598–926° K , and Fig 2 shows the variation of the conductivity with the applied voltage for these curves Currents in the voltage- and temperature-ranges shown were stable, but at temperatures greater than about 1,000° K for this sample the application of potentials greater than 10 V was sufficient to cause gross instabilities due to breakdown Curves of similar form were obtained with platinum-based samples, although conductivity currents were smaller as the activation process was less effective For the sample giving the results in Figs 1 and 2, the conductivity plot obtained by plotting log σ_0 (where σ_0 is the zero-field conductivity) versus $1/T$ shows two approximately linear portions intersecting at about 700° K , above this temperature the Loosjes–Vink hypothesis[2] predicts that the current-voltage characteristics should be curved towards the applied potential axis, and below it they should be linear Such a difference in the form of the characteristics is, in fact, observed The general form of the variation of conductivity with applied potential in the high-temperature region is broadly the same as that found by Danforth and Goldwater[3] in pulsed current measurements on barium oxide, strontium oxide and the mixed oxide the

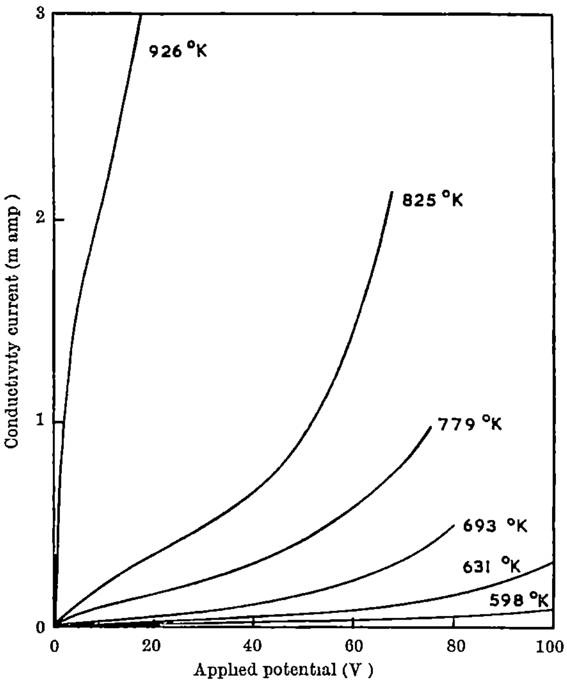

Fig 1 Conductivity current–voltage characteristics for a sample of calcium oxide

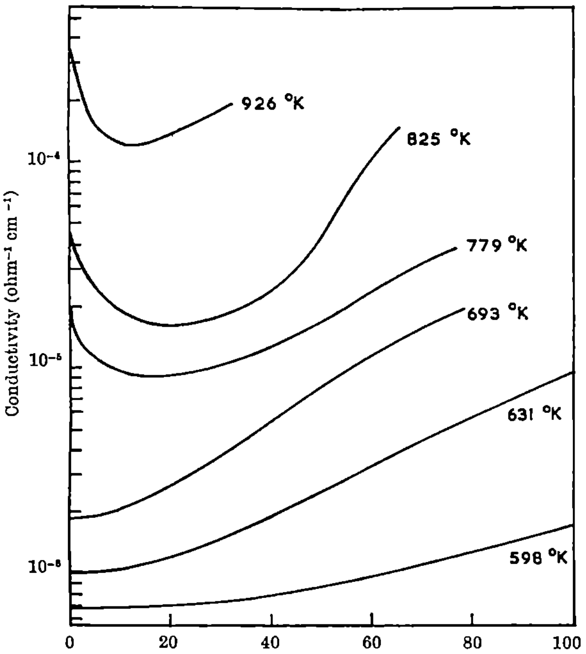

Fig 2 Conductivity as a function of applied voltage for calcium oxide

conductivity decreases to a minimum and then increases with increasing field An interesting feature of the characteristics is the increase in conductivity at high fields, which is observed throughout the temperature range Curvature of the current-voltage characteristics in this sense might be expected from a consideration of the Joule heating effect of the conductivity current, but an estimate of the orders of magnitude of the quantities involved shows that the curvature cannot be attributed solely to a heating effect In the range of temperatures above 700° K, where for this sample the conduction process is predominantly one of thermionic emission across the pores of the oxide, the increase of conductivity current with applied voltage is too rapid to be completely accounted for by the Schottky effect The fact that similar curves are obtained for nickel- and platinum-based samples indicates that the form of the characteristics is a property of calcium oxide rather than of any interface compound which may be formed between calcium oxide and the nickel cathode base

A detailed comparison between the results of different workers using the various oxides is not possible Among the difficulties involved is a satisfactory definition of the field across the porous specimen[5], and the possibility also exists of differences in the porosities of samples used by different workers, which may cause considerable differences in the shape of the characteristics[3]. The general conclusions reached as a result of the present work are that at low electric fields the shape of conductivity current-voltage characteristics for calcium oxide agrees with the predictions of the Loosjes–Vink hypothesis, at high fields an increase in conductivity is observed, similar to that previously observed in the other alkaline earth oxides and in the mixed oxide[3], and the field- and temperature-dependent effects obtained with strontium oxide[5] are not found with calcium oxide in the temperature-range investigated.

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A Difference Band of a Fundamental and a Lattice Mode in the Infra-red Spectra of Lead and Other Carbonates

Lead carbonate, $PbCO_3$, crystallizes in the potassium nitrate structure, space group D_{2h}^{16}, with four molecules per unit cell[1] From crystallographic considerations, it follows that the carbonate ion occupies a site in the lattice of which the symmetry is C_s The degeneracies of ν_3 and ν_4 are removed by the perturbing crystalline field, and all the vibrational modes are active in the infra-red

'Vaseline' mulls of lead carbonate were prepared and the spectra were recorded with a Perkin–Elmer model 21 spectrometer fitted with sodium chloride optics The frequencies and assignments are given in Table 1 and the infra-red spectrum is shown in Fig 1

Table 1 FREQUENCIES AND ASSIGNMENTS OF THE INFRA-RED BANDS OF LEAD CARBONATE

Frequency (cm $^{-1}$)	Relative intensity	Assignment
2,405	vw	$\nu_1 + \nu_4$
1,731	w	$\nu_1 + \nu_3$
1,437	vs	ν_4
1,406	vs	
1,053	m vsp	ν_1
849	s sp	ν_2
824	vw	ν_2-lattice mode
690	sh	ν_3
678	s	

In agreement with the prediction based on the crystal structure, the degeneracies of ν_3 and ν_4 have been removed and the infra-red inactive ν_1 has become infra-red active

A small band occurs on the low-frequency wing of ν_2 of the spectra of the following salts

Lithium	carbonate	(work in this Laboratory)
Sodium	,,	,, ,,
Potassium	,,	(ref 2)
Lead	,,	
Calcium	,,	(ref 2)

Sodium	nitrate	(ref 2)
Lithium	,,	(work in this Laboratory)
Potassium	,,	,, ,,
Calcium	,,	,, ,,
Barium	,,	,, ,,
Strontium	,,	,, ,,

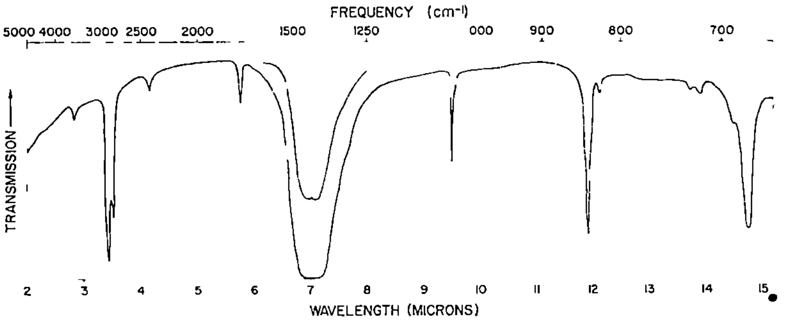

Fig 1 Infra-red spectrum of lead carbonate ('Vaseline' mulls) ν_4 is shown in detail with the 'Vaseline' bands compensated for by a 'Vaseline' sample in the reference beam of the spectrometer

The frequency difference between ν_2 and the small band is always of the order 10–30 cm $^{-1}$ An explanation of this phenomenon can be given on the following lines The ν_2 mode of planar XO_3^- ions can be described by a motion in which the central X atom moves perpendicular to the plane of the oxygen atoms This corresponds to movements in two directions in the crystal if the crystal contains a two-fold axis, and in one direction if a three-fold axis is present This directional movement is only present in the case of ν_2 It is evident that this situation greatly facilitates the transfer of energy between the ν_2 vibration and a translational lattice mode The result of this coupling is that a difference band appears of ν_2 and a translational lattice mode From these considerations it can be predicted that this difference band occurs in the infra-red spectra of all crystals containing flat XO_3 ions

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[2] Haas, C , thesis, Amsterdam (1956) Shultin, A A , *Dokl Akad Nauk S.S S R* ,125, 767 (1959) assigns the band in $NaNO_3$ to a component of Davydov splitting of the ν_2-mode This assignment is not in agreement with the temperature-dependence of the intensity which he finds

CHEMISTRY

Products of the γ-Radiolysis of Aqueous Cystine Solutions

RECENTLY, Markakis and Tappel[1] determined the yields of cysteine, alanine, ammonia, hydrogen sulphide, sulphur and sulphate in hydrochloric acid solutions of cystine irradiated with γ-radiation from spent fuel elements However, their results indicate that especially at low doses ($< 10^6$ rads) not all the radiolytic products were identified The present communication describes the identification of products in cobalt-60 γ-irradiated unbuffered and acidified cystine solutions at relatively low doses

All solutions were made using doubly-distilled water and L-cystine (Mann-assayed grade from Mann Research Laboratories, Inc [α] = –216° in M hydrochloric acid) Oxygenation and deoxygenation of solutions were effected by bubbling oxygen and nitrogen respectively through the solutions during irradiation All irradiations were carried out at ambient temperature with a dose-rate of about 670 rads/min In order to inhibit cysteine oxidation 1 ml of 0 1 M N-ethyl-maleimide (NEM) was added to each solution (50 ml) after irradiation Cystine solutions (3×10^{-4} M) were concentrated (25 fold) at 30–32° C prior to spotting on paper for descending chromatography or electrophoresis Radiolytic products were detected on developed chromatograms and electrophoretograms using the polychromatic copper nitrate–ninhydrin reagent[2]

Table 1 R_A (R_F REL TO ALANINE) VALUES OF RADIOLYTIC PRODUCTS FROM CYSTINE

Compound	R_A* and (colour)†	R_A* and (colour)† of spot from irradiated solution
Cystine disulphoxide	0 41 (g–b)	0 38 (g–b)
Cystine	0 54 (g)	0 54 (g)
Cysteic acid	0 65 (p)	0 65 (p)
Cysteine sulphinic acid	0 80 (p)	0 78 (p)
Alanine	1 00 (p)	1 00 (p)
Cysteine (uncoupled with NEM)	0 94 (g)	0 94 (g)
Cysteine (coupled with NEM)	1 05 (r)	1 04 (r)

*, in $EtOH/H_2O/H_2SO_4$ (80 20 0 5)
†, g = grey, b = brown, p = purple, r = rust

In most cases excellent separation of ninhydrin-positive products was obtained using ethanol/water/sulphuric acid (80 20 0 5) for chromatography Although development with ethanol/water (80 20) and butanol/acetic acid/water (4 1 5) also yielded fair separation of products, the use of a strongly acidic solvent seemed desirable in light of the ease of hydrolysis of cystine disulphoxide The chromatographic data and spot colours (Table 1) suggest that cystine disulphoxide, cysteic acid, cysteine sulphinic acid, alanine and cysteine are radiolytic products

Although the reaction between NEM and thiols is known[3] to proceed more slowly the lower the pH, the 'uncoupled cysteine' spot was not observed in the chromatograms of acidified cystine solutions provided they were allowed to react with NEM for at least one day prior to spotting The presence of cysteic and cysteine sulphinic acids in unbuffered solutions was confirmed by paper electrophoresis at a number of pH values Some confirmation of the identification of cystine disulphoxide in certain irradiated solutions follows from the observation that the 'disulphoxide spot' was absent in chromatograms of aliquots (containing no NEM) to which cysteine had been added prior to chromatography This is to be expected since it has been shown[4] and confirmed in the present work that cysteine reacts with cystine disulphoxide to form cystine and cysteine sulphinic acid

No precise quantitative analysis of the products was attempted and the very approximate G-yields listed (Table 2) were deduced from visual comparison of the spots from irradiated solutions with those from appropriate amino-acid solutions

In hydrochloric acid solutions estimation of the amounts of cysteic acid on chromatograms developed with the ethanol/sulphuric acid solvent was not possible owing to interference from a grey-blue spot with R_A of 0 7 However, fair separation of the cysteic acid/cysteine sulphinic acid fraction from other species present was effected with ethanol/water (80 20) When this solvent was used, the irradiated solutions were first dried to remove hydrochloric acid and redissolved in 10^{-2} M acetic acid prior to spotting on paper

Table 2 PRODUCTS OF IRRADIATED 3×10^{-4} M CYSTINE SOLUTIONS (DOSE OF 8×10^4 RADS)

[Acid]	Initial degree of oxygenation*	Products† and (approx G-yield)				
Unbuffered	O	RSO_2SR‡	RSO_3H (1 1),	RSO_2H‡	RSH‡	RH‡
Unbuffered	A		RSO_3H (1 2),	RSO_2H‡	RSH (0 9),	RH‡
Unbuffered	D		RSO_3H‡	RSO_2H (0 1),	RSH (0 9),	RH‡
0 04 M HCl	O	RSO_2SR‡	RSO_3H (0 3)	RSO_2H‡	RSH (0 5),	RH‡
0 04 M HCl	A		RSO_3H (0 3),	RSO_2H‡	RSH (0 6),	RH‡
0 04 M HCl	D			RSO_2H‡	RSH (0 7),	RH‡
0 02 M H_2SO_4	O	RSO_2SR (0 4),	RSO_3H (1 5),	RSO_2H‡		RH‡
0 02 M H_2SO_4	A	RSO_2SR‡	RSO_3H (1 5),	RSO_2H‡	RSH (0 4),	RH‡
0 02 M H_2SO_4	D		RSO_3H‡	RSO_2H‡	RSH (0 5),	RH‡

* O, Oxygenated, A, aerated, D, deoxygenated
† RSO_2SR, Cystine disulphoxide, RSO_3H, cysteic acid, RSO_2H, cysteine sulphinic acid, RSH, cysteine, RH, alanine
‡ G-yield < 0 1

Discussion of the results is simplified by assuming that the following reactions occur

$$\begin{array}{lcll} RSSR + OH & \rightarrow & RSOH + RS & (1) \\ RSSR + H & \rightarrow & RSH + RS & (2) \\ RS + RS & \rightarrow & RSSR & (3) \\ RS + X & \xrightarrow{O_2} & RSO_2SR + Y & (4) \\ H + O_2 & \rightarrow & HO_2 & (5) \\ RSH + RSO_2SR & \rightarrow & RSO_2H + RSSR & (6) \\ RSH + RSOH & \rightarrow & RSSR + H_2O & (7) \end{array}$$

The sulphenic acid formed in (1) is assumed to be oxidized by hydrogen peroxide (or HO_2) through the sulphinic acid to cysteic acid Reactions (2) and (7) would explain the low yields of cysteic acid in deoxygenated solutions In aerated solutions apparently oxygen is depleted to such an extent (preliminary experiments indicate that in unbuffered aerated solutions 18 per cent of the initial oxygen content remains after 80,000 rads) that some hydrogen atoms are available for reaction (2) Reaction (6) helps to maintain high yields of cysteic acid in aerated solutions and, of course, ensures that high yields of cysteine and cystine disulphoxide are never detected in the same irradiated solution The low yields of cystine disulphoxide and cysteic acid in hydrochloric acid solutions is presumably in part due to chloride ion-inhibition of reaction (1) However, the mechanism of the inhibition must remain obscure until the grey-blue spot observed on chromatograms of irradiated hydrochloric acid solutions of cystine is identified The low yield of cystine disulphoxide in unbuffered solutions is not unexpected owing to rapid hydrolysis of the disulphoxide in these solutions Although the accuracy of the yield-estimates is poor, the results seem to indicate that yields may depend on the *p*H of the solutions It is possible that the observed increase in yield of cysteine and the decrease in yields of cysteic acid in unbuffered solutions is due to participation of the O_2^- ion which could conceivably reduce cystine to cysteine

Regarding the possible effect of dilute hydrogen peroxide on cystine, it has been found that chromatograms of cystine (3×10^{-4} *M*)–hydrogen peroxide (2×10^{-4} *M*), which had been concentrated in the normal manner, exhibited only one faint spot with R_A characteristic of the disulphoxide

We are indebted to Mr A Casselman and Dr R Greenhalgh for advice on electrophoresis and chromatography

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Inhibition of the Formation of 3,4-Benzpyrene during Hydrocarbon Combustion

It is well known that polycyclic hydrocarbons including the carcinogen 3,4-benzpyrene occur in association with the carbonaceous deposits or smokes resulting from the incomplete combustion of a wide variety of organic materials Thus 3,4-benzpyrene has been identified in soots, carbon black, certain smoked foods, cigarette- and tobacco-smoke and in the exhaust smoke of Diesel engines The mode of formation of 3,4-benzpyrene has been reviewed by Badger *et al* [1], who have suggested possible intermediates during the pyrolysis of organic compounds, and have pointed out that the observed products can be accounted for by secondary reactions involving initial or primary free radicals A recent examination of the products from the pyrolysis of petroleum hydrocarbons by electron spin resonance supports the view that free radicals participate, the phenalenyl radical[2] has been identified

The subject of formation of carbon in flames has attracted attention in recent years, the mechanism is still obscure but polycyclic hydrocarbons are often associated with the carbonaceous material formed The present work was undertaken to determine whether the formation of 3,4-benzpyrene can be inhibited during hydrocarbon combustion It is well established that nitric oxide exerts a remarkable inhibiting effect on the chain-decomposition of some hydrocarbons[3] A substance was sought which would give rise to nitric oxide as a product of pyrolysis It is known[4] that the carbon zone in a diffusion flame is a region where the hydrocarbon is decomposed thermally in the absence of oxygen (although OH radicals do reach this zone) and it seemed plausible that the production of nitric oxide within this zone might lead to inhibition of free-radical reactions leading to polycyclic hydrocarbons Grey *et al* [5] have investigated the thermal decomposition of nitroalkanes and have found nitric oxide in the products (with formaldehyde and some nitrogen dioxide, etc)

In the present work, known amounts of nitroethane were introduced into a stream of commercial propane ('Bottogas') which was burned as a laminar diffusion flame in air The air supply was reduced to give heavy soot formation in the absence of additive and the soot was collected, mainly on a brass target placed just above the flame, but also from the walls of the surrounding 'Pyrex' chimney A weighed amount of

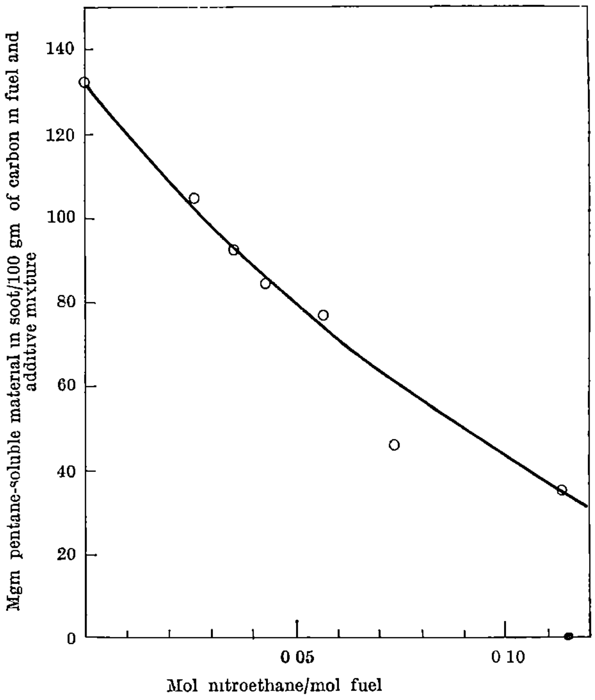

Fig 1

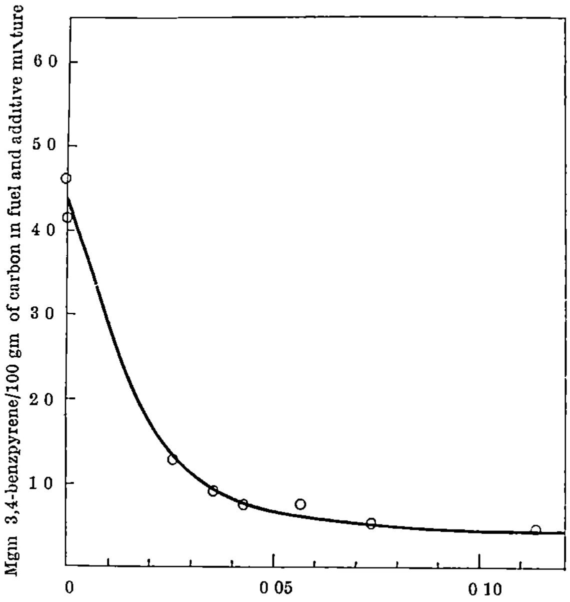

Fig 2

soot was extracted with A R chloroform in a Soxhlet apparatus for about 3 hr The solvent was removed by distillation in an atmosphere of nitrogen and the residue was extracted repeatedly with *n*-pentane The extract was filtered and the *n*-pentane removed in an atmosphere of nitrogen, the residue was weighed and reported as mgm *n*-pentane-soluble material per 100 gm of carbon input in the fuel + additive mixture (Fig 1) The 3,4-benzpyrene content of the *n*-pentane-soluble material was determined by the method described by Sawicki *et al* [6] This depends on the fact that 3,4-benzpyrene in a concentrated solution of sulphuric acid exhibits a characteristic peak in its fluorescence spectrum at 545 mμ when irradiated by ultra-violet light of activating wave-length 520 mμ This peak is unaffected by the presence of other polycyclic hydrocarbons The fluorescence spectrum was obtained by means of an Aminco-Bowman spectrophotofluorometer In order to eliminate the effect of concentration quenching, the concentration of total polycyclic aromatic hydrocarbons was kept constant in each determination at 50 μgm/ml Calibration was effected using a pure sample of 3,4-benzpyrene It can be seen (Figs 1 and 2) that small additions of nitroethane lead to a marked reduction in the formation of *n*-pentane-soluble material in the soot and in the amount of 3,4-benzpyrene

Nitro-compounds (including nitroethane) are known to raise the cetane number when added in small amounts to Diesel fuels[7] Although the present work has not been extended to engine studies we suggest that a reduction in emission of 3,4-benzpyrene from Diesel engines might be expected when nitroethane and similar additives are employed

It has been found by a number of workers and recently confirmed by Pichler and Deschner[8] that no 3,4-benzpyrene is present in soot-free Diesel exhaust gases but under conditions of low revolutions and high load it does occur in the exhaust smoke Although Diesel engines are probably relatively minor contributors to atmospheric pollution compared with industrial and domestic fuel-burning equipment[9], any attempt to reduce the emission of 3,4-benzpyrene in Diesel exhaust smoke, either by attention to engine design and maintenance or by chemical means, would be valuable

We are indebted to the Coal Tar Research Association for the gift of a pure sample of 3,4-benzpyrene and to the Department of Surgery of the Medical School for permission to use the Aminco-Bowman spectrophotofluorometer

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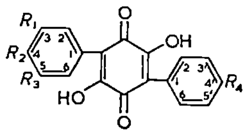
A Novel Type of Antioxidant related to Polyporic Acid

IN the inhibition of the autoxidation of 1,4-dienes, for example, linoleic acid and its esters, it is generally assumed that polyphenolic compounds act as chain breakers in accordance with the mechanism $RO_2 + AOH \rightarrow ROOH + AO$, where RO_2 is the chain propagating peroxy radical and AOH is the antioxidant The efficiency of the latter is determined not only by the rate of the inhibitory reaction but also by the stability of the intermediate AO radical[1]. Recently it was shown that certain polyhydroxy flavones[2] exhibit a remarkably high antioxidant efficiency In this work, which was carried out with the view of finding better antioxidants for fat-containing foods, attention was concentrated on a group of compounds which were related to certain terphenyl quinones occurring in some edible fungi They can be represented in terms of the following structure



The compounds which were investigated include polyporic acid (R_1, R_2, R_3, R_4 = H), atromentin (R_1, R_3 = H, R_2, R_4 = OH) and leucomelone (R_1, R_2, R_4 = OH, R_3 = H), which occur in certain fungi and lichens (A detailed discussion concerning the structure of leucomelone will be presented elsewhere)

In addition, a number of related compounds which do not occur naturally were synthesized They include trihydroxypolyporic acid (R_1, R_2, R_3 = OH, R_4 = H) and its *tert*-butyl derivative (R_1, R_2, R_3 = OH; R_4 = *tert*-butyl)

The synthesis of leucomelone has been described by Akagi[3], and in this investigation a similar method was used for the synthesis of the compounds mentioned above

In the preparation of the new trihydroxypolyporic acids the reaction of 2,5-dichlorobenzoquinone with the appropriate *N*-nitrosoacetanilide gave 2,5-dichloro 3-phenyl 1,4-benzoquinone and 2,5-dichloro 3-(4-*t*-butylphenyl) 1,4-benzoquinone, which were then treated with diazotized 3,4,5-trimethoxyaniline The dichloroterphenyl quinones thus prepared were hydrolysed to the corresponding dihydroxyquinones Demethylation of the dihydroxy terphenyl quinones with a mixture of hydrobromic acid and acetic acid gave the pentahydroxyquinones directly, reductive demethylation followed by oxidation was not attempted

The antioxidant activity of the compounds was tested in pure linoleic acid and in pure methyl linoleate at a temperature of 25° C Uptake of oxygen was followed with conventional Warburg apparatus and this was correlated with peroxide formation, which was determined by means of an iodometric method[4] A special test was used for the determination of antioxidant efficiency in the presence of metal catalysts, using ferrous phthalocyanine in ethyl benzoate solutions as standard The results indicated that polyporic acid had no significant antioxidant activity, while atromentin and leucomelone were comparable with conventional antioxidants, for example, propyl gallate, for the inhibition of fat autoxidation The new antioxidants 3,4,5-trihydroxypolyporic acid and 4′-*tert* -butyl 3,4,5-trihydroxypolyporic acid showed outstanding activity In linoleic acid these compounds gave protection factors which were, respectively, about ten and twenty-five times that obtained with propyl gallate It should be noted that the *tert* -butyl substituent substantially increases the solubility of the trihydroxypolyporic acid in higher fatty acids and their esters

Anti-leukæmic activity of unsubstituted polyporic acid was reported recently[5], it would be interesting to compare the polyhydroxypolyporic acids which show greater antioxidant activity

The work was supported by the U S Department of Agriculture *P.L* 480 counterpart funds

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Ionic-Covalent Bonding in Crystals

An attempt[1] to resolve misunderstandings regarding ionic-covalent bonding in solids has been criticized in a recent article by Mooser and Pearson[2] It should be pointed out, however, that their discussion of formal ionicity is limited to the simple and well-known case of atoms which each contribute one electron to a two-electron bond, and cannot be applied as it stands to 'dative bond' systems (such as are found in the zinc blende structure and in many other types of crystal, as well as in co-ordination complex molecules) In such systems formal covalency, defined as a state in which bonding electrons are equally shared between bonded atoms, can only be achieved by a donor–acceptor relationship with associated charge separation

The main point in my communication[1] was the importance of the neutral-bond state (defined as that with zero charge separation) as the logical zero for the scale of electronegativity (It should be noted that this is generally applicable, thus it holds for bonds in diatomic molecules, since for such molecules neutral-bonded and formally covalent states are identical) This suggests that 'ideal covalency' (so termed for want of a better name) is associated with zero charge separation rather than with equal sharing of electrons If this is so, the difficulty pointed out by Mooser and Pearson regarding the zero of the scale of bond ionicity used in my communication[1] can readily be resolved It may well be that some revision of the accepted definition of the covalent bond on these lines is needed However, in terms of the definitions used, the conclusions regarding charge separation presented in ref 1 are still valid In this connexion the concluding statement by Mooser and Pearson that "any attempts such as have been made to determine effective charges from formal bond ionicities are bound to fail" is certainly not a dogmatically required belief It is my opinion, on the contrary, that useful qualitative information regarding charge separations in crystals can be obtained, although, for obvious reasons, some of which are referred to in my earlier communication, no quantitative estimates are as yet possible

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Goodman's comments on our recent article[1] in no way affect our views on the subject of ionic-covalent bonding as outlined there Since it is obvious, however, that Goodman is misinterpreting these views, we should like to stress once again the following points

(1) The 'effective bond ionicity' is defined in terms of the actual charges associated with the constituents of a compound It is, therefore, a physical quantity which can, at least in principle, be measured

(2) The 'formal bond ionicity' is a purely theoretical concept It is, in fact, an abbreviation with which to indicate to what extent various arbitrarily chosen valence structures are mixed to describe the bonding in a molecule or a solid Contrary to Goodman's remark, the formal bond ionicity is defined also for 'dative' bonds

(3) The covalent bond is defined as arising from the sharing of two electrons between two bonded atoms It does not specify the charges associated with these atoms and a covalent valence structure cannot, therefore in general, be taken as the zero of the bond-ionicity scale

(4) All the definitions given by us[1] and repeated here are in agreement with chemical tradition The intention of our article was merely to point out that two different meanings are currently attached to the term 'bond ionicity' To distinguish between them, we introduced the names effective and formal bond ionicities

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BIOCHEMISTRY

Lipase Activity in the Slow- and Fast-contracting Leg Muscles of the Cockroach

THE recent work of Becht and Dresdon[1] and Smit[2] has brought to light certain histological and physiological features of the slow- and fast-contracting mesothoracic leg muscles of the cockroach, *Periplaneta americana* Smit[2] has shown that the slow-contracting muscle fibres contain more lipids and possess a stronger reducing capacity for methylene blue than the fast-contracting ones Histochemical investigation of these two types of fibres[3] has shown that there is more lipase activity in the slow-contracting muscles Here we report the results of a quantitative investigation of lipase activity in the slow- and fast-contracting leg muscles of the cockroach

Only the metathoracic and mesothoracic leg muscles of the male and female cockroaches were used According to Becht and Dresdon[1] the muscles 136, 137 and 135*a* and *c* (Carbonell's numbering) are fast-contracting and 135*b*, *d* and *e* are slow-contracting The slow muscle in this case is the tergo-trochanteral muscle, which is an exceptionally large muscle passing down the coxa to be attached to the trochanter The fast muscles which are the extensors, and act antagonistically to the above muscle, arise from the coxal rim and are attached on the coxo-trochanteral joint These two types of muscles were carefully dissected and removed separately from the coxa of the leg into separate ice-cold watch-glasses and weighed Separate aqueous extracts of the above samples of muscles were prepared at 4° C The extract was centrifuged at 2,500 r p m for 5 min and the supernatant was used as the enzyme material in each case The method used for the assay was adopted from Martin and Peers[4] using a Warburg apparatus Tributyrin was used as substrate with a bicarbonate–carbon dioxide buffer system of *p*H 7 4 at 37° C The reaction flask contained 1 5 ml 0 025 *M* bicarbonate solution and 1 ml enzyme solution in the main chamber and 0 5 ml substrate (4 per cent v/v tributyrin in 0 0148 *M* bicarbonate solution emulsified by shaking with a drop of 'Tween 80') in the side arm The flask and manometer were gassed for 3 min with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide After equilibrating the flask in the constant temperature water-bath of the apparatus for 10 min the substrate was tipped and the initial readings were taken after 3 min The subsequent readings were taken after a 30-min interval for 1 hr Lipase activity was calculated on the basis of the protein content of the enzyme solution used and is expressed as µl carbon dioxide / mgm protein / hr Protein was estimated by the micro-Kjeldahl steam-distillation method[5]

Table 1 LIPASE ACTIVITY IN THE SLOW- AND FAST-CONTRACTING LEG MUSCLES OF THE COCKROACH

Tissue	Lipase activity* (µl CO_2/mgm protein/hr) Male	Female
Slow muscle	129 31	81 43
Fast muscle	75 65	68 47

*Average of 12 experiments

From the results obtained it is evident that the slow-contracting muscle has a higher lipase activity than the fast-contracting muscle It is also seen that the activity of the enzyme in the same muscle is considerably lower in the case of the female than that in the male Again the difference between the concentrations of the enzyme in the two types of muscle is more in the male than in the female A histochemical study[3] of lipase and certain oxidative enzymes in these muscles also indicated a correspondingly sharp difference in the amount of these enzymes present in these two types of muscles, the slow muscles having more of them The greater concentration of the enzyme, lipase, in the slow- and fast-contracting muscles could be well correlated with the higher lipid content of these muscles, the slow muscle fibres being more pigmented and showing considerably greater ability to stain for fat than the fast muscle fibres The higher concentration of lipase and the greater fat content of the slow muscle, which could indulge in sustained contractions for considerably longer periods than that of the fast one, is suggestive of more fat being split for utilization for energy in the former The higher lipase value in the muscles of the male could also be regarded as an index of the greater capacity for sustained activity involving greater fat utilization in the male

One of us (N M G B) is indebted to the Ministry of Education, Government of India, for the award of a senior research scholarship

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Comparison of the Serum Protein Fractions of the Developing Chicken Embryo by the Technique of Starch-Gel Electrophoresis

SEVERAL workers have investigated the serum protein composition of the developing chicken embryo, by moving boundary[1-3] and paper electrophoresis[4,5] The ultracentrifugal analysis of the serum proteins of the early stage of embryonic development of chicken, as investigated by two groups of workers, are not in agreement in regard to the complexity of the molecular structure in that stage[1,2] Electrophoretic investigation does show, however, the multiplicity of the components in the early stage, although no complete agreement exists in regard to the number of the components and their relationship to those found in newly hatched and adult birds[6] Thus Hradec and Lemez[4] reported that the serum proteins of chicken embryo undergo considerable qualitative transformation throughout the development The technique of starch-gel electrophoresis suggested by Smithies[7] enables one to detect in the serum pattern fractions that cannot be observed by other methods of electrophoresis The pooled sera of five different age-groups of chicken embryo, incubated for 10, 13, 16, 19, and 21 days were examined by the technique of starch-gel electrophoresis, run simultaneously on the same gel The electrophoresis was performed on a vertical tray[8] for 18 hr , using borate buffer of *p*H 8 6 and a field-strength of 4 V /cm Twelve fractions could be distinguished on the pattern of the serum from the embryo aged 21 days as is shown in Fig 1 (two pre-albumins are not shown), as was also indicated in ref 6 At least nine components (except the pre-

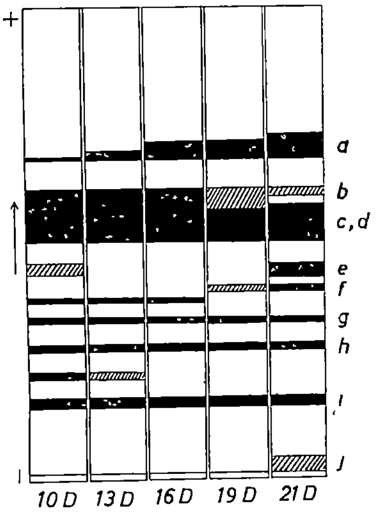

Fig 1 A schematic representation of the starch-gel electrophoretic pattern of serum of the developing chicken embryo The letters *a* to *j* refer to albumin and γ-globulin The numbers 10, 13 16, 19 and 21 indicate the days of incubation The striped bands represent the areas that could scarcely be distinguished on the original patterns

albumins) could be distinguished on the pattern from the 10-day old chicken embryo, of which at least seven components corresponded in mobility to those found in the day-old chick The patterns from the sera of 13, 16, and 19 days of incubation also showed similarity in pattern to that of the day-old chick A component present in the sera of the 10-day and 13-day old embryo and situated somewhere between components *h* and *j* of 21-day old embryo was not distinguishable in the sera of the older embryos The results presented here indicate that apparently with the exception of γ-globulins and some other fractions, as demonstrated in Fig 1, almost all other components present in the day-old chick are present in the serum of 10-day old embryo, as is revealed by the method of starch-gel electrophoresis, and that the transformation occurring throughout this period of development is apparently of a quantitative rather than qualitative nature

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A Zinc-requiring Alkaline Phosphatase in the Ventral Lobe of the Rat Prostate

Zinc has been shown to play an important part in the activity of phosphatases$^{1-7}$ We have now found evidence for the existence of a zinc-dependent alkaline phosphatase in the ventral lobe of the rat prostate The hydrolysis of 0 002 *M* adenosine monophosphate in 0 065 *M* *tris*-(hydroxymethyl)-amino methane buffer at *p*H 9 4 by homogenates of the gland was progressively inhibited by increasing concentrations of zinc (Fig 1)

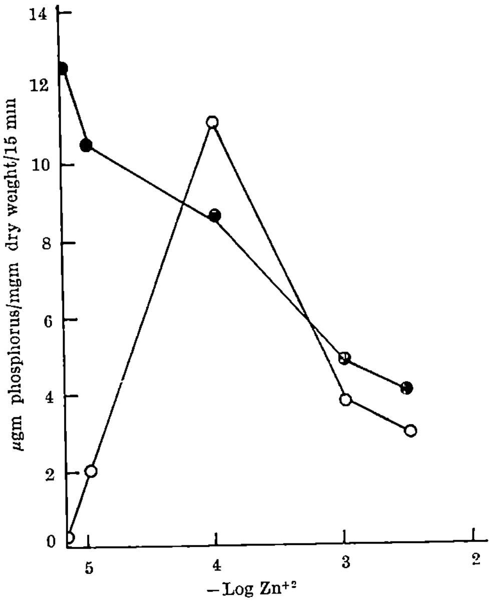

Fig 1 Effect of zinc on the hydrolysis of adenosine monophosphate by prostatic homogenates from the rat ventral lobe The effect of the addition of zinc to a homogenate dialysed for 3 hr against *tris* buffer at *p*H 7 0 at 0° (●) and the addition of 10^{-3} *M* EDTA to the homogenate followed by dialysis for 3 hr (○) Phosphatase activity was measured by the method of Fiske and Subbarow (ref 9) Incubation mixture contained 0 5 ml 0 25 *M* *tris* buffer at *p*H 9 4, 0 1 ml 0 04 *M* adenosine monophosphate, 0 9 ml water, 0 5 ml homogenate, the mixture was incubated for 15 min at 37°

The addition of ethylenediamine tetraacetic acid (EDTA) at concentrations greater than 10^{-3} *M* to homogenates inhibited the phosphatase activity by 30–50 per cent When EDTA treatment was followed by dialysis against *tris* buffer at *p*H 7 4, the inhibition was practically 100 per cent

The addition of zinc acetate restored the activity of the inhibited enzyme to about 80 per cent of its original value Sodium acetate had no effect After reactivation of the enzyme, increasing the concentration of zinc caused normal inhibition (Fig 1)

In contrast to zinc ions, magnesium and calcium ions reversed to a slight extent the inhibition caused by EDTA and dialysis Since the logarithms of the stability constants for EDTA complexes of zinc, calcium and magnesium are in the ratio 16 3 10 6 8 7 (ref 8), the greater activity of zinc did not depend on the binding capacity of EDTA

We wish to thank the British Empire Cancer Campaign for a grant to support the work

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Demethylation of the Plant Œstrogen Biochanin *A* in the Rat

GENISTEIN and biochanin *A*, 4′-methylgenistein, have been isolated from red clover and have been found to account for the œstrogenic activity of this plant[1] Though their œstrogenic potency is very weak, the substances may nevertheless be of importance in animal physiology because they are ingested in considerable amounts by grazing animals

Very little is known about the metabolic transformations of these compounds Biochanin *A* was found to be demethylated to genistein by the influence of rumen micro-organisms[2]. The present investigation has been undertaken with the aim of studying the metabolism of biochanin *A* in rats The compound was synthesized by known methods[3] and labelled with tritium[4]

Each of twenty rats was injected intraperitoneally with 25 mgm of a fine suspension of biochanin *A* in saline Urine and fæces were collected separately An ether extract of the urine was prepared and fractionated on a 'ChroMax' preparative paper column (LKB, Stockholm) with butanol/2 *N* ammonia (1 1) as eluant Radioactive biochanin *A* (12 7 mgm) and genistein (3 5 mgm) were isolated from the eluates Their melting points and ultra-violet and infra-red absorption curves coincided with corresponding values of authentic test samples Both these isoflavones were isolated in the same way from an ether extract of fæces

The demethylation of biochanin *A* to genistein was demonstrated by incubation experiments with rat liver slices The experiments were performed as follows liver slices, 1–1 2 gm dry weight, were prepared by hand and incubated with 6 μmoles of tritium-labelled biochanin *A* (specific activity 2 μc per μmole) in 20 ml *p*H 7 4 Krebs–Ringer phosphate buffer The incubation was performed at 37° C for 2 hr To the volume of the incubation medium was added 4 times this volume of alcohol The mixture was centrifugated The slices were homogenized and extracted with 80 per cent alcohol. The solvent was evaporated in a partial vacuum under nitrogen The dry residues were dissolved in 5 per cent sodium bicarbonate solution and extracted with ether. The sodium bicarbonate solution contained conjugates of the isoflavones The ether extracts were evaporated and subjected to paper chromatographic analysis on Whatman No 3 paper, using benzene/acetic acid/water (165 34 1) as the solvent system The isoflavones were located by their absorption in ultraviolet light The chromatograms were cut into strips, eluted with 80 per cent alcohol, and the eluates analysed for radioactivity in a liquid scintillation counter Ekco *N* 612 at a temperature of $-20°$ C (Fig 1 *A*) The radioactive eluates from the genistein spots on the chromatograms were rechromatographed in different solvent systems[2] They were found to coincide with genistein in all the systems Incubation experiments were also performed as above with intestine contents and labelled biochanin *A* No radioactivity except from the biochanin *A* spots could be found on these chromatograms (Fig. 1 *B*) Traces of the radioactivity remained at the starting point on the chromatograms both with material from incubation experiments and controls, probably due to insufficient elution

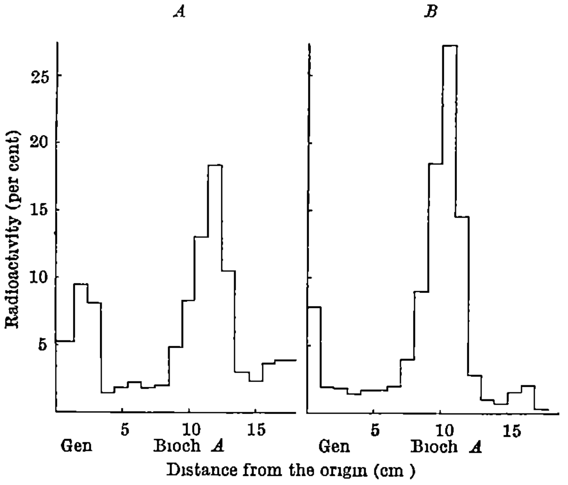

Fig 1 The distribution of radioactivity on paper chromatograms with biochanin *A*, incubated with liver slices (*A*) and intestine contents (*B*) The radioactivity of the eluates is expressed as percentages of the sum of recovered activity from the chromatogram and indicated on the abscissa The positions of the test substances are indicated below the diagrams

These preliminary experiments show that a demethylation process occurs in the liver Several other aromatic ethers have been found to be split in the liver[5] It is known that methylation of the hydroxyl groups of many synthetic œstrogens retards or decreases their œstrogenic action[6] It is, therefore, generally believed that these molecules have to be demethylated before becoming active It is not known if the presence of free —OH groups is also necessary for the action of the isoflavones However, genistein with three free —OH groups is the most active one of the four œstrogenic isoflavones[7].

Work on the demethylation of methyl ethers of other œstrogens and with different experimental animals is now in progress

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Enhancement of Steroidogenesis by Rat Adrenal Slices *in vitro* with Œstradiol-17-β

RECENT experiments have shown that the concentration of corticosterone in adrenal vein blood of female rats is 2 4 times that in male rats[1] The possibility that this difference is due, at least in part, to the direct action of œstrogen on the adrenal cortex is the subject of the present study.

Male or female rats of the Sherman strain, weighing 120–150 gm , were maintained in a constant-temperature room for at least one week prior to use. Female rats were bilaterally oophorectomized at 21–30 days of age and used about 6 weeks later Adrenal slices were prepared according to a modification[2] of the technique of Saffran and Schally[3] The final incubation medium was modified by addition of U S P adrenocorticotrophic hormone (5 milliunits/100 mgm adrenal tissue) and œstradiol-17-β 7 5 × 10^{-6} *M* In each experiment, comparable tissue pools were treated by addition of the appropriate diluent alone (saline and propylene glycol respectively). Corticoid yields were determined by ultraviolet absorption and expressed as μgm./100 mgm. adrenal tissue.

The effects of œstradiol addition are shown in Table 1. Significant increments in corticoid production were obtained on addition of œstradiol to male adrenal slices in the absence or presence of adrenocorticotrophic hormone. No effects were observed with female adrenal slices. However, increased corticoid yields, comparable with those obtained with male tissue, were found when adrenal tissue from oophorectomized female rats was employed.

Table 1. EFFECTS OF ŒSTRADIOL ON STEROIDOGENESIS *in vitro*

Adrenal source	No. of observations	ACTH (m.U.)	Corticoid yields* (μgm./100 mgm.) Control	Œstradiol
Male	4	0	9·2 ± 0·6	16·4 ± 1·1†
	4	5	14·9 ± 1·7	20·3 ± 1·8†
Female	4	0	19·1 ± 1·6	20·1 ± 2·0
	4	5	24·8 ± 1·4	25·9 ± 1·0
Oophorectomized female	4	0	11·8 ± 0·8	17·8 ± 0·5†
	4	5	15·5 ± 0·9	19·8 ± 0·9†

* Mean ± standard error.
† Significant at the 1 per cent level.

Administration of adrenocorticotrophic hormone or a standardized stress to adult female rats has been shown to result in significantly higher peripheral plasma corticosterone concentrations than those obtained in male animals[1]. Yates *et al.*[4] have demonstrated that the capacity of liver homogenates from female rats to reduce the *A* ring of corticosterone is significantly greater than that of male animals. These authors have proposed that this more rapid metabolism of steroid is the principal mechanism responsible for greater adrenal secretory capacity in the female rat. The present findings indicate that œstrogen may also affect steroidogenesis directly and independently of its action on liver metabolism. A comparable degree of stimulation was found when œstradiol was added to either male or oophorectomized adrenal tissue. The failure to demonstrate a stimulatory effect on adrenal slices from intact female animals suggests that such tissue has already been subjected to maximal œstrogenic stimulation. The significantly higher control corticoid-levels obtained with intact female adrenal tissue compared to tissue from the other sources are consistent with this hypothesis.

Utilizing an essentially similar assay system, McKerns[5] found that the addition of œstradiol-17-sodium sulphate *in vitro* to intact male or female adrenal tissue resulted in no change in corticoid production. The discrepancy between his findings and those of the present work perhaps may be explained by differences in either the form or the dose of œstrogen added.

These results will be discussed in greater detail in a subsequent publication. This study was supported by a research grant (*A*-3370) from the U.S. Public Health Service.

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Female Rat Preputial Glands as a Source of β-Glucuronidase

THE preputial gland of the adult female rat has been shown by Beyler and Szego[1] to be an extremely rich source of β-glucuronidase. However, it may not generally be realized that laboratories needing a steady supply of this enzyme and having access to a rat colony have a cheap and readily available source. Adult female rats which are being discarded can be used, and the two glands, each weighing approximately 50 mgm., are easily dissected free from the surrounding tissue (Fig. 1).

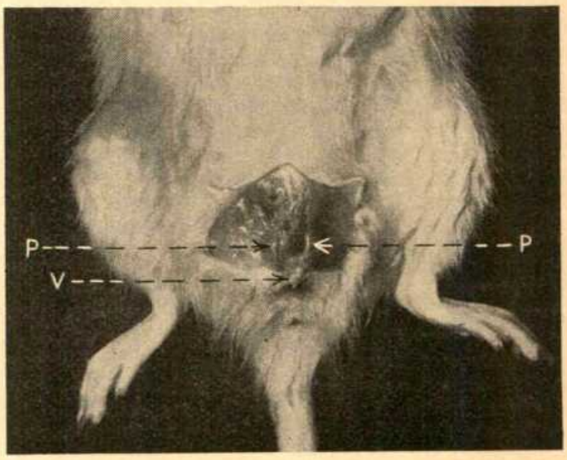

Fig. 1. Adult female rat dissected to show preputial glands. *P*, Preputial gland; *V*, vagina

Homogenization and extraction are then carried out as described by Levvy, McAllan and Marsh[2], though only one ammonium sulphate precipitation is necessary. An equal volume of saturated aqueous ammonium sulphate solution is added to the supernatant obtained, at the end of stage 2 (ref. 2). The ensuing precipitate, after separation from the aqueous phase, is then dissolved in a suitable quantity of 0·1 *M* sodium acetate buffer, *p*H 5·2, so that the resulting solution contains 0·5–1 × 10^6 Fishman units of β-glucuronidase per ml. The enzymic activity of the preparation is assayed by the method of Talalay, Fishman and Huggins[3]. The enzyme solution is stored in the deep freeze and deteriorates only slowly. It does not contain phenolsulphatase, as determined by the method of Roy[4].

The total working time involved in the preparation and assay of one batch is about 2 hr.

Extracts of β-glucuronidase, prepared in this manner from adult female rat preputial glands, have been used for more than four years in the enzymic hydrolysis of steroid glucuronides, notably those of pregnanediol and pregnanetriol.

I am indebted to Mr. A. J. Bailey, of Chelsea Hospital for Women, for the photograph.

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Use of Phenol in determining Chromatographic Purity

WATER-SATURATED phenol is a well-known solvent for the paper chromatographic separation of polyphenols and was, for example, one of the solvents used by Roberts and Wood[1] for the two-dimensional paper chromatographic separation of tea-leaf polyphenols. This solvent was, however, later discarded in favour of water[2], or preferably 2 per cent acetic acid[3].

The use of 75 per cent (w/v) aqueous phenol (or *m*-cresol saturated with water) has proved to be of inestimable value in determining the chromatographic purity of various flavonoid compounds isolated in this laboratory. A fraction isolated from rooibos tea (*Aspalathus acuminatus*) and suspected of consisting solely of isoquercitrin was found by chromatography in phenol to consist of two major flavonoid components even though the material was chromatographically homogeneous in *n*-butanol/acetic acid/water (4:1:2·2 v/v), in water, in 15 per cent acetic acid, in ethyl acetate saturated with water, in propan-2-ol/water (1:3·5 v/v), and in chloroform/butan-2-ol/water (2:4:4 v/v). Separation also took place in 60 per cent acetic acid but was incomplete and not very convincing. The identity of the second component is still under investigation.

In the isolation of quercetin-4′-monoglucoside from the inner scales of the onion (*Allium cepa* L.)[4], difficulty was found in reproducing the melting point of the crystalline material obtained on successive recrystallizations from various solvents. The material was chromatographically homogeneous in all the more commonly employed solvent systems, only phenol and *m*-cresol resolving it completely into two components. The material was therefore purified by chromatography on Whatman 3*MM* paper sheets with 75 per cent (w/v) aqueous phenol as the developing solvent. The contaminant, which had a higher R_F in phenol than quercetin-4′-monoglucoside, crystallized from aqueous ethanol as fine yellow needles with melting point 259–261°.

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Trypsin Inhibitor in Wholewheat Flour

PRELIMINARY work on a trypsin inhibitor in wholewheat flour was reported by Shyamala[1]. A crude concentrate containing the trypsin inhibitor was prepared by the 'ammonium sulphate' method of Borchers *et al.*[2] modified by Lyman[3] as follows:

(1) A homogenate of the wholewheat flour was prepared by mixing the flour with ten volumes of water in a blender. The *p*H was adjusted to 4·2 with acid and the mixture was allowed to stand overnight.

(2) The 'ammonium sulphate precipitate' was obtained by adding ammonium sulphate, 30 per cent of the volume of the extract. The precipitate containing the inhibitor was re-precipitated with ammonium sulphate. The material obtained was dialysed overnight and then lyophilized. Approximately 3 gm. of material was obtained from 300 gm. of flour.

The activity of trypsin was determined by the method of Anson[4] using the modification of Orringer *et al.*[5]. The degree of colour produced by the phenol reagent indicated the relative proteolytic activity of the trypsin. For testing the activity of the inhibitor, it was added to the enzyme at several concentrations and the degree of proteolytic activity inhibited was estimated. A logarithmic plot of this inhibition gave a straight line from which the point of 50 per cent inhibition could be readily obtained.

Crude material (6·5 mgm.) prepared as described here was needed for 50 per cent inhibition of the activity of trypsin while 55γ of a crude concentrate of soybean trypsin inhibitor prepared by the same method was needed for 50 per cent inhibition. The activity was lost when the preparation was heated in a boiling water bath for 20 min.

Table 1. TRYPSIN-INHIBITING ACTIVITY OF THE INHIBITORS FROM WHOLEWHEAT FLOUR AND SOYBEAN

Ammonium sulphate precipitation				Trichloroacetic acid	
Soybean inhibitor (γ)	Optical density at 765μ	Wholewheat inhibitor (mgm.)	Optical density at 765μ	Wholewheat inhibitor (mgm.)	Optical density at 765μ
0	0·480	0	0·470	0	0·480
13	0·412	1·0	0·440	1·0	0·395
26	0·355	2·5	0·375	2·0	0·320
52	0·270	5·0	0·285	3·0	0·270
78	0·180	10·0	0·170	5·0	0·172
50 per cent inhibition at 55γ.		50 per cent inhibition at 6·5 mgm.		50 per cent inhibition at 3·5 mgm.	

The crude concentrate prepared as described was used as the starting material for the extraction of the inhibitor with trichloroacetic acid by the method of Kalser and Grossman[6]. The inhibitor appeared in the trichloroacetic acid filtrate while the precipitate had no inhibitor in detectable amounts. The trichloroacetic acid filtrate was dialysed and lyophilized. 3·5 mgm. of this material was needed for 50 per cent inhibition. The appearance of the inhibitor in the trichloroacetic acid filtrate indicates a possible similarity in its behaviour to the pancreatic juice trypsin inhibitor. Further attempts to purify the material are under way.

Recently, Learmonth and Wood[7] reported the presence of a trypsin inhibitor in wheat flour using different assaying techniques.

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PHYSIOLOGY

Prevention of Freezing Damage to Living Cells by Pyridine *N*-Oxide

PYRIDINE, like dimethyl sulphide, forms a high-boiling, water-soluble oxide, pyridine *N*-oxide (PNO). It was surmised that PNO might protect living cells against freezing damage in the same way as dimethyl sulphoxide[1] and some other aliphatic compounds[2]. The particular interest attached to PNO is that it would be the first aromatic compound to be used in this connexion.

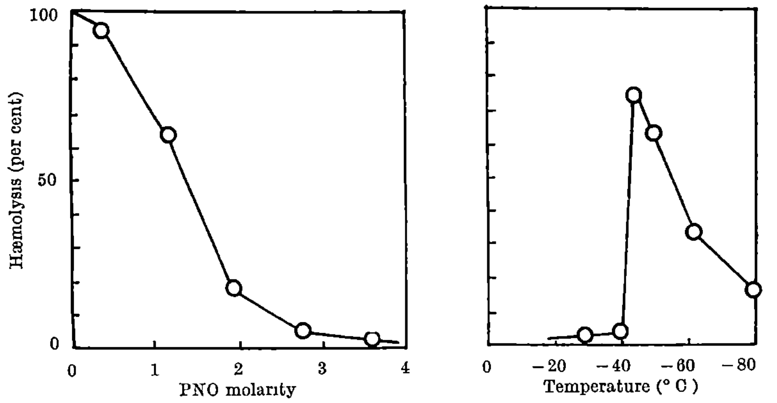

Fig 1 Hæmolysis of red cells suspended in a solution of pyridine N-oxide (PNO) and frozen for 15 min , as a function of PNO concentration at -40° (left), as a function of temperature with 3 2 M PNO (right)

Washed human red blood cells were suspended in buffered saline containing different amounts of PNO and frozen for 15 min at various temperatures between - 20° and - 80° C They were then thawed and spun down, and the amount of hæmolysis estimated colorimetrically in the supernatant Some of the results are shown in Fig 1, hæmolysis being expressed as a percentage of that found in controls without PNO, at each temperature

The sharp rise in hæmolysis at - 40°, and slower fall, recalls the behaviour of acetamide at - 51°, and is probably due to a rise in solute concentration to a toxic level[2] The protective effect is known to depend on permeation of the cells by the solute, and possibly more important than the limited protection found is the fact that PNO is both penetrating and physically non-toxic up to high concentrations On intraperitoneal injection into mice, PNO had a delayed toxicity (LD_{50} 1 5 gm /kgm) rather less than that of pyridine[3], to which it is probably reduced *in vivo* (could be smelt in the viscera) The high solubility of PNO in water is believed to be due to localization and enhancement of the three lone-pairs of electrons on the oxygen, by mesomerism with the aromatic ring It is hoped to discuss the structure of such highly soluble compounds in a later communication

I am indebted to Mr M J Ashwood-Smith for carrying out the toxicity experiments

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Influence of Anaerobic Glycolysis on Release of Histamine in the Guinea Pig and Rat Anaphylactic Reaction *in vitro*

An increase of oxygen uptake has been observed during the anaphylactic reaction *in vitro*[1], and it has been shown that anaphylactic release of histamine is inhibited by lack of oxygen[2-4] It has also been shown that the anaphylactic reaction is inhibited by uncouplers of the oxidative phosphorylation[5] It would be expected that the energy-rich compounds formed during the glycolytic cycle would be available for release of histamine, as are those generated in aerobiosis The present investigation was undertaken in order to see if the anaphylactic reaction depends on oxidative metabolism, or if it could also proceed in the presence of an anaerobic metabolism The experiments were carried out with both guinea pig and rat tissues, in order to verify any possible difference in their behaviour, since it has recently been suggested that anaphylactic reaction in rat tissues is not affected by lack of oxygen[6,7]

Sensitized guinea pig and rat lung-slices were incubated in Ringer-phosphate or Ringer-bicarbonate medium with or without glucose, in nitrogen or oxygen atmosphere, in Warburg flasks in which release of histamine was determined by the addition of antigen from the side arm Nitrogen was purified by passing it through melted phosphorus, and its purity verified in an Orsat apparatus[4] Rats were sensitized with horse serum treated by kaolin, according to the method in ref 8 The technique of histamine dosage and other experimental details were as described in ref 5

It has already been shown that a pure nitrogen atmosphere completely inhibits anaphylactic release of histamine in guinea pig lung-slices[4] In the presence of glucose the sensitized guinea pig lung-slices, maintained in anaerobic conditions, showed as would be expected a glycolytic activity, while histamine was released on tipping the antigen Addition to the glucose anaerobic medium of mono-iodoacetate, well known as an inhibitor of the anaerobic glycolysis, completely inhibited this release Table 1 shows the results obtained in typical experiments

Table 1 Effect of Glucose and Mono-iodoacetate on Metabolism and on Antigen Histamine Release from Sensitized Guinea Pig Lung-Slices under Anaerobic Conditions

Experimental conditions		Metabolism		
Atmosphere	Additions	Oxygen uptake Q_{O_2}	Glycolysis Q_{CO_2}	Histamine release (μgm /gm w w)
Nitrogen + 5 per cent carbon dioxide	—		0 85 + 0 09	0 0 ± 0 1
Nitrogen + 5 per cent carbon dioxide	Glucose		5 69 ± 0 34	3 2 ± 0 7
Oxygen	—	5 44 ± 0 30		3 9 ± 0 3
Oxygen	Glucose	5 25 ± 0 36		4 9 ± 0 3
Nitrogen + 5 per cent carbon dioxide	Glucose		6 90 ± 0 54	10 7 ± 0 6
Oxygen	—	5 90 ± 0 40		4 1 ± 0 1
Oxygen	Glucose	6 00 ± 0 19		4 7 ± 0 5
Nitrogen	Glucose	0 11 ± 0 10		5 3 ± 0 5
Nitrogen + 5 per cent carbon dioxide	Glucose		7 98 ± 0 45	17 5 ± 0 4
Nitrogen + 5 per cent carbon dioxide	Glucose + monoiodoacetate, 1 mM		0 45 ± 0 10	0 0 ± 0 1
Nitrogen + 5 per cent carbon dioxide	Glucose		7 06 ± 0 27	11 3 ± 0 4
Nitrogen + 5 per cent carbon dioxide	Glucose + monoiodoacetate, 0 5 mM		0 73 ± 0 09	0 4 ± 0 3

Medium was Ringer-phosphate, or Ringer-bicarbonate when atmosphere contained carbon dioxide Glucose, 0 01 M Spontaneous release of histamine was calculated from these data Each figure is the mean of results from three flasks ± $S E$

In sensitized rat lung-slices, anaerobiosis completely inhibited the histamine released by antigen and small quantities of oxygen were sufficient to remove this inhibition Addition of glucose to the medium also removed the anaerobiosis inhibition (Table 2)

Table 2 EFFECT OF TOTAL AND PARTIAL ANAEROBIOSIS AND OF GLUCOSE IN ANAEROBIC CONDITIONS ON METABOLISM AND ON ANTIGEN HISTAMINE RELEASE FROM SENSITIZED RAT LUNG-SLICES

Experimental conditions		Metabolism		
Atmosphere	Additions	Oxygen uptake Qo_2	Glycolysis Qco_2	Histamine release (μgm/gm w w)
Oxygen	—	7 23 ± 0 35		2 9 ± 0 3
Nitrogen	—	0 65 ± 0 05		0 0 ± 0 0
Oxygen	—	6 34 ± 0 11		2 3 ± 0 3
Nitrogen	—	0 70 ± 0 14		0 0 ± 0 0
Oxygen	—	7 75 ± 0 12		1 4 ± 0 15
Nitrogen + 2 per cent oxygen	—	1 58 ± 0 07		1 0 ± 0 1
Oxygen	—	8 10 ± 0 20		3 9 ± 0 2
Nitrogen + 4 per cent oxygen	—	2 15 ± 0 15		3 6 ± 0 3
Oxygen	—	7 30 ± 0 14		3 5 ± 0 3
Nitrogen + 5 per cent carbon dioxide	Glucose		6 52 ± 0 14	3 5 ± 0 0

Same experimental conditions as in Table 1 Each figure is the mean of results from two flasks ± *S E*

These experiments show that in both guinea pig and rat-sensitized lung-slices the anaphylactic histamine release is not specifically dependent on oxygen This reaction is able to proceed in complete anaerobiosis, if energy is available to the cell, such as that produced by a carbohydrate glycolytic metabolism

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Effect of Monovalent Cations on the Blood Pressure of Intact and Hypertensive Rats

WE demonstrated in earlier experiments that it was possible to inhibit renal neurogenic hypertension in rats when potassium salts were mixed with their food[1]. It was likewise demonstrated that the degree of Grollman hypertension did not depend on the absolute quantity of sodium and potassium in the food but on the mutual proportions of the two electrolytes[2] Meneely's experiments[3] gave similar results rats, made hypertensive by excessive doses of sodium chloride, could be kept alive and the rise of their blood pressure stopped by feeding them potassium salts The blood pressure of hypertensive patients can be lowered by a high potassium diet[4]

Excessive consumption of sodium chloride is known to promote hypertension in human beings and animals alike Many workers ascribe this to the higher hydration of the sodium ion which leads to a swelling of the arterial walls[5] According to Pallman[6], an atom of sodium binds 4 3, and an atom of potassium only 0 9 mol of water If the hypertensive effect of sodium and the anti-hypertensive effect of potassium depend on hydration, it would be justified to expect that lithium, as the most hydrated monovalent cation, would produce higher blood pressure than sodium, and that rubidium and cæsium, which are less hydrated, would produce a stronger depressing effect than potassium (An atom of lithium binds 10, that of rubidium 0 5 and cæsium 0 2 mol of water)

In order to test this hypothesis we mixed rubidium and cæsium salts (Rb_2SO_4, CsCl) to the diet of male rats of 150–180 gm body-weight which had been made hypertensive by Grollman's method We also administered lithium salts (LiCl) to such animals since they refused the food which contained lithium it was given by means of intraperitoneal injection The daily doses of rubidium and cæsium were 15 mgm , those of lithium 4 mgm The basal diet contained 26 mgm. of sodium and 61 mgm of potassium per day, while there was 120 mgm. of potassium in the 'high-potassium diet' Figs 1 and 2 illustrate changes in the blood pressure of intact and Grollman-hypertensive animals after administration of different monovalent cations. We determined the blood pressure with our method[7] once a week It can be seen from Fig 1 that the blood pressure of the intact lithium-fed animals already showed an increase during the second week, while the blood pressure measured in rats which had been kept on a high-potassium diet or received rubidium or cæsium did not differ from that of animals kept on a normal diet

By keeping rats, operated by Grollman's method, on a high-potassium diet or by administering rubidium or cæsium to them, it was possible to arrest a further increase in their blood pressure (Fig. 2) Cæsium inhibited the development of hypertension almost completely after a small increase during the first weeks, blood pressure went back in the sixth week to the pre-operative (normal) level

Our experiments allow the conclusion that all monovalent cations are capable of influencing the blood pressure of rats Effect on blood pressure depends on the hydration of the atoms Cations of high hydrating power (lithium, sodium) promote hypertension This property of sodium ions has long been known Lithium has now been proved to be a substance which enables us to produce experimental hypertension in an easy and reliable manner This discovery seems to be of great significance in the field of experimental hypertension since it is now possible to study problems concerning the pathogenesis and treatment of hypertension promptly, without surgical intervention and without the administration of mineralocorticoids (in other words, under compara-

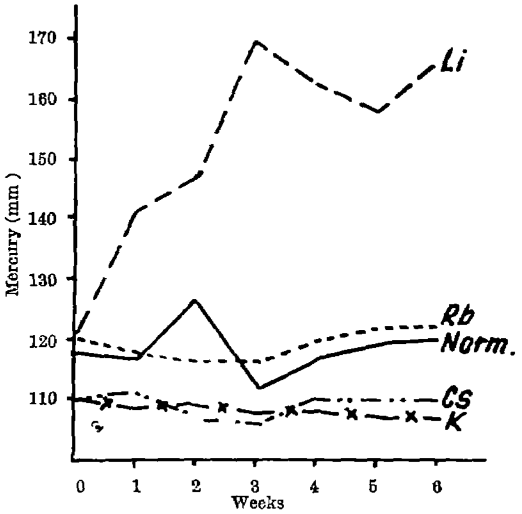

Fig 1 Action of Li, K, Rb and Cs on the blood pressure of intact rats Norm , normal diet, Li, normal diet plus 4 mgm Li, K, normal diet plus 59 mgm K, Rb, normal diet plus 15 mgm Rb, Cs, normal diet plus 15 mgm Cs

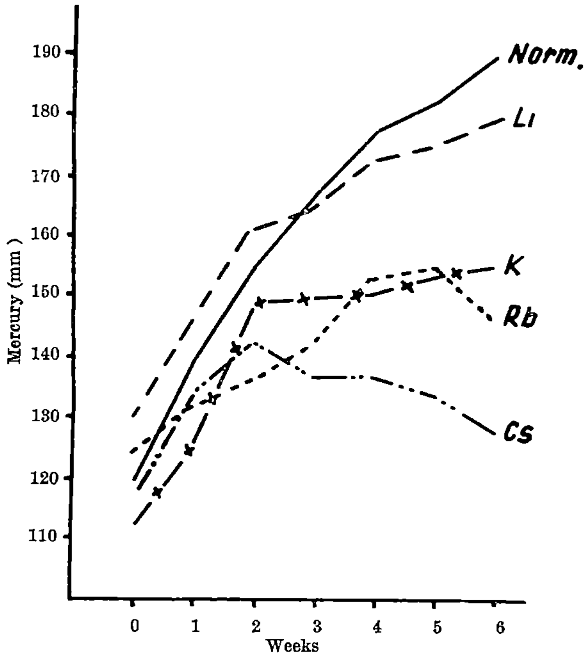

Fig 2 Action of Li, K, Rb and Cs on the blood pressure of Grollman-operated rats (key, see Fig 1)

tively more physiological conditions) The prevention of hypertension by means of rubidium and cæsium also supports (but does not definitely prove) our working hypothesis that the action of monovalent cations on blood pressure, as also the direction of the effect, depends on the degree of ionic hydration

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Ambient Temperature and Depot Fat Iodine Number in Children

The degree of unsaturation of depot fat, as measured by its iodine number, has been shown to depend on species, temperature of the environment, diet and site within the body of the fat[1] During the course of investigations in Bantu children aged up to 4 yr in South Africa it was found that as the child got older, so the iodine number of the depot fat (taken from the anterior abdominal wall) increased and the difference between the means of the group under 1 yr old and those aged 1 yr was significant ($P = 0·025 - 0·01$) and between the means of 1 yr and 3 yr. the significance was 1 in 20 Comparison with the 2-yr -old group did not show any significance owing to the small number in the group (Fig 1)

These findings, it is suggested, are consistent with the view that the degree of unsaturation of the depot fat in children is related to the temperature of the environment, as has been shown in animals[2] Of the factors influencing depot fat iodine number the site within the body and species variability need not be considered here The composition of the fat in the diet has been shown to alter the composition of adipose tissue, but relatively large quantities of fat have to be consumed in order to demonstrate this effect[3] These children have no excess of dietary fat, their highest fat intake probably being while breast fed (fat in breast milk = 4 per cent)

Furthermore, the fat consumed by the mother and which is to some extent reflected in the composition of her milk[4] is the same fat as would be consumed by the child when weaned

The difference with age in the South African series could be explained by differences in temperature of the environment to which the child is exposed When this was done the atmospheric temperature varied from 5 to 18° C During early life the child is not exposed to this temperature for long periods of the day as it is carried wrapped in blankets on its mother's back. As it gets older it spends longer intervals of time away from its close contact with the mother and thus the temperature to which the skin is exposed will have lower mean values The iodine number of the depot fat in adult Bantu in South Africa is 67 (ref 5)

In a second series of children in West Africa where the atmospheric temperature was 28–33° C the iodine values showed no rise with age and were similar to those of the 0–1 yr age group in South Africa (Fig 1) The atmospheric temperature in West Africa was therefore close to that which would exist when the child is tied to its mother's back Gradual 'weaning' from the mother's back would not be associated with any marked fall in temperature

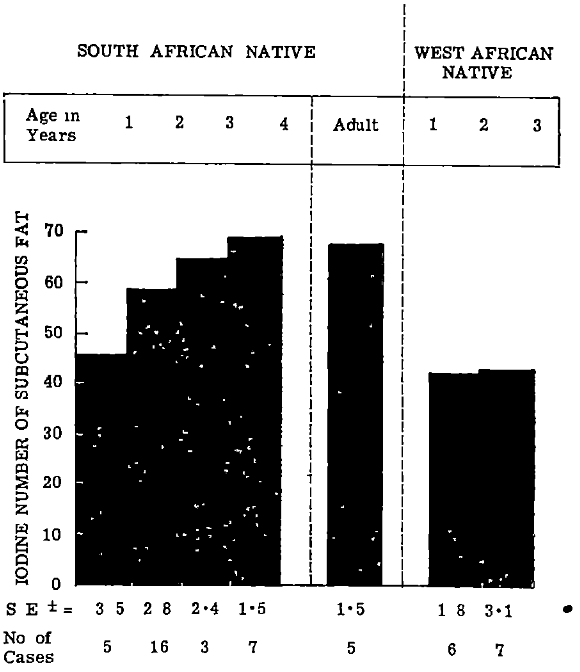

Fig. 1

exposed to the skin, and therefore no striking change in the degree of unsaturation of depot fat, and could account for these older children having the same iodine number as the very young age group in South Africa

It is therefore concluded that these results in children would be consistent with the hypothesis that the iodine number of depot fat is determined, to some extent, by the ambient temperature

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Seminal Degeneration induced in Merino Rams by the Administration of Stilbœstrol Dipropionate

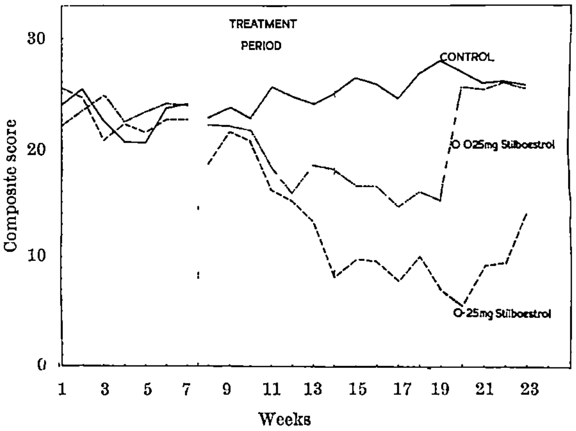
THE statement that rams are unaffected by the ingestion of œstrogenic pasture seems to be based on the failure to detect lowered fertility following mating[1], or metaplastic changes in the bulbo-urethral and prostatic glands of rams from properties where ewes exhibit the syndrome of 'clover infertility'[2,3] Substantiation has been drawn from the protection conferred by the administration of androgens to castrates treated at the same time with stilbœstrol[4] No reports have been noticed of the results of semen examination of rams fed œstrogenic pastures or treated with synthetic œstrogens, although it has been shown that injections of 35 mgm /week of œstradiol benzoate may alter semen characters of bulls in 3 weeks, these returned to normal within 5 weeks of the cessation of treatment[5]

In experiments conducted at the Ian Clunies Ross Laboratory, fifteen Merino rams, aged approximately 24 months, were divided at random within live weight classes into three groups, mean live weights were 45 kgm

Semen samples were collected from each ram by electro-ejaculation[6] at weekly intervals for 23 weeks beginning August 17, 1960 The samples were assessed for volume, sperm density and total sperm numbers, motility and for the percentages of morphologically normal and live sperm After a pre-treatment period of seven weeks, to allow each group to establish reasonably consistent base-lines, treatments were begun These consisted of intra-muscular injections of stilbœstrol dipropionate at daily dose-rates of 0 025 mgm /sheep for group 1 and 0 25 mgm /sheep for group 2 Peanut oil alone was injected into the control group 3 Treatments continued between the eighth and fourteenth weeks inclusive of the experiment, and were then stopped

Sperm motility was scored visually between 0 and 10, and the percentages of live and of normal sperm were converted to a scale of 0–10 by taking each 10 per cent as one point Because of the large variation that occurs in total sperm numbers in semen samples collected by electro - ejaculation (Mattner and Voglmayr, unpublished) this character was disregarded in the composite scores

Mean total scores for each week for each group of rams are shown in Fig 1, which reveals a depression in semen quality as the result of the treatments Group 2, which received 0 25 mgm stilbœstrol, had not completely recovered within 9 weeks of treatment being stopped, but group 1, which received 0 025 mgm stilbœstrol, showed substantial recovery within 6 weeks of treatment ceasing The control group remained unaffected



Of the individual semen characters examined, motility was most adversely affected, and in group 2 this fell to zero by the seventh week after stilbœstrol injections began The mean percentage of live sperm in semen samples from this group fell from 85 per cent before treatment to 40 per cent by the seventh week of treatment, and to 20 per cent by the twentieth week, that is, 6 weeks after treatment ceased The mean total sperm numbers of both groups 1 and 2 began to decline 5 and 7 weeks respectively after treatment commenced Four weeks after these declines became evident, mean total sperm numbers obtained from group 2 at each collection ranged between 100 × 10^6 and 300 × 10^6 and from group 1 between 500 × 10^6 and 1000 × 10^6 No upward trend occurred in group 2 until 9 weeks after treatment ceased, mean sperm numbers for group 1 rose to 2000 × 10^6 within 3 weeks and were maintained at this average until the ninth week after the administration of stilbœstrol stopped Mean total sperm numbers obtained from the control group during the whole of the experiment fluctuated between 4000 × 10^6 and 5000 × 10^6

Rams grazing highly œstrogenic pastures in Australia are unlikely to ingest the equivalent of 0 25 mgm stilbœstrol/day administered to group 2 Dose-rates of 0 03 mgm diethylstilbœstrol/sheep/day for 6 months adversely affected the breeding performance of ewes[7] Preliminary observations (Southcott, Braden and Moule, unpublished) indicate that Merino ewes grazing red clover (*Trifolium pratense*) pastures at Armidale (New South Wales) may have an œstrogen intake equivalent to 0 015 mgm stilbœstrol dipropionate/day, but bio-assay of collected subterranean clover (*Trifolium subterraneum*) fed to castrated guinea pigs has shown far higher œstrogenic equivalents[4] It is clear that the dose-rate of œstradiol benzoate/kgm live weight required to produce seminal degeneration in bulls[5] could be as much as twenty times the dose of stilbœstrol dipropionate/kgm live weight required to affect the semen of rams, thus it is unlikely that bulls could consume sufficient œstrogenic pasture to affect spermatogenesis The evidence available indicates that cattle are unaffected by grazing pastures that severely affect sheep[1]

It should not necessarily be concluded that grazing pasture possessed of high œstrogen equivalencies will induce seminal degeneration in rams. So far the activity of pasture in terms of stilbœstrol dipropionate has been estimated by the use of castrated male sheep and spayed ewes allowed to graze the pasture under test. On assimilation by the entire male, the isoflavones formononetin, genistein and biochanin A, and diadzein and the lactone coumestrol may not lead to the formation of substances likely to impair spermatogenesis. Furthermore, this experiment was conducted at a time of the year when the androgen output by the rams may have been low.

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PHARMACOLOGY

Repetitive After-discharge produced by Drugs in Nerves treated with Cetyltrimethylammonium Bromide

A WELL-ESTABLISHED pharmacological property of tetraethylammonium ion (TEA) is the production of repetitive nerve action potentials in response to single stimuli[1]. We have recently been examining the relationship between quaternary ammonium ion structure and this type of activity. After several experiments, however, it became apparent that the effects of TEA and other ethonium ions were not reproducible except at very high concentrations of 30 m*M* or more. Accordingly, we resorted to testing these drugs on nerves treated with the cationic detergent, cetyltrimethylammonium bromide (CTMB). Certain methonium ions which normally have little or no effect on nerve produce conduction block after CTMB treatment[2,3]. The experiments described here reveal that CTMB also increases the capacity of ethonium ions to produce repetitive firing.

All experiments were done on sciatic nerves isolated from cold-adapted winter and spring frogs (*Rana pipiens*). Except for brief stimulating and recording intervals in air, the nerves were bathed in a buffered frog Ringer solution (*p*H 7·2) at a temperature of 16–18° C. Monophasic action potentials were recorded with differential amplification, displayed on an oscilloscope, and photographed. A square-wave stimulus of 0·1 m.sec. duration was applied every 2 sec. Stimulus intensity was supramaximal for the rapidly conducting *A* fibre group. The nerve chamber contained a small centre well located between the platinum stimulating and recording electrodes. Drugs and CTMB were added to the Ringer solution in this centre well only. The so-called treated segment of the nerve was initially bathed in CTMB–Ringer for 60 min., then in CTMB–Ringer–drug solution. CTMB concentration was kept at 1 μgm./ml.

The compounds used in these experiments were TEA chloride, phenyltriethylammonium iodide and 3-OH phenyltriethylammonium iodide. With the appropriate concentration of each compound, two effects were consistently observed: (1) an increase in amplitude and time-course of the negative after-potential; (2) repetitive after-discharge. Summation of asynchronous and low-amplitude repetitive spikes of many individual axons prevents accurate measurement of the negative after-potential. Therefore, only the repetitive firing was used as an indicator of drug action, and this response was treated as a graded one. The frequency, number and amplitudes of repetitive spikes were concentration and time dependent.

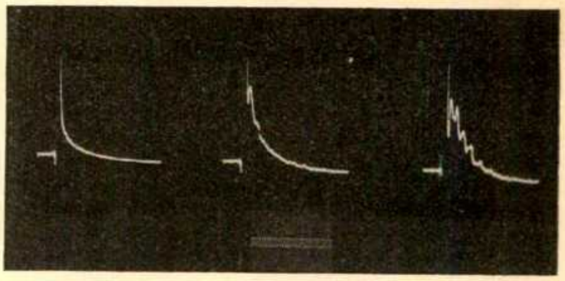

Fig. 1. Repetitive after-discharge produced by 5 μgm./ml. phenyltriethylammonium ion in presence of CTMB. Left, control record taken after 30 min. in Ringer; middle, threshold response obtained after 5 min. in CTMB–Ringer–drug solution; right, maximal response obtained after 30 min. in CTMB–Ringer-drug solution. Time line, 1,000 cycles/sec.

The approximate concentration of each ethonium ion required for a minimal response was determined on at least 4 normal and 4 CTMB-treated nerves. A typical response is illustrated in Fig. 1. For each ion, CTMB markedly lowered the approximate concentration required for threshold response (Table 1). The time between drug application and onset of effect was not altered by CTMB when using threshold concentrations; but when the threshold concentration as determined on a normal nerve was applied to a CTMB-treated nerve, the latent period was too short to measure. Our results might have been even more striking had we used a higher CTMB concentration. However, we wished to avoid the direct actions of CTMB on ion permeability which were recently observed in the giant axon of the squid[4].

Table 1. COMPARISON OF DRUG-INDUCED REPETITION IN NORMAL AND CTMB-TREATED NERVES

Drug	Normal nerves Threshold concentration* (m*M*)	Normal nerves Time to onset† (min.)	CTMB-treated nerves Threshold concentration* (m*M*)	CTMB-treated nerves Time to onset† (min.)
Tetraethylammonium	10–20	20	0·5–2	45
Phenyltriethylammonium	20–30	30	3–5	30
3-OH phenyltriethylammonium	30–40	10	3–10	13

* Range of approximate threshold concentrations on 4–7 nerves.
† Average of results obtained on 4–7 nerves.

Koketsu's experiments[5] indicated that TEA prolongs the negative after-potential in frog motor nerve terminals. The concentrations used by Koketsu approximated those needed in the present work to produce repetitive firing of CTMB-treated axons. The unmyelinated axon terminals are more sensitive than the rest of the axon to TEA, probably because the latter possesses a permeability barrier which impedes the access of quaternary ammonium ions to their sites of action. It is this barrier which a cationic detergent like CTMB appears to remove.

This investigation was supported by U.S. Public Health Service grant *B*–2605, National Institute of Neurological Diseases and Blindness.

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Cholinergic Transmission Mechanisms for both Excitation and Inhibition in Molluscan Central Synapses

WE have previously reported[1] that the application of acetylcholine by perfusion or local injection to different central neurones of the mollusc *Aplysia depilans*, impaled with intracellular microelectrodes, always produced one of the two following reactions: (*a*) cells of one type, conventionally designated by us as *H*-neurones, are hyperpolarized and inhibited by the drug (Fig. 1, *H*1), and (*b*) cells of another type, or *D*-neurones, are depolarized and excited (Fig. 1, *D*1).

Further microphysiological analysis revealed that *H*-neurones receive both excitatory and inhibitory inputs, but that *D*-cells receive only excitatory input. A pharmacological investigation enabled us to demonstrate that acetylcholine fulfils almost all the requirements necessary in order that it be accepted as the chemical transmitter of synaptic inhibition in *H*-neurones[1,2].

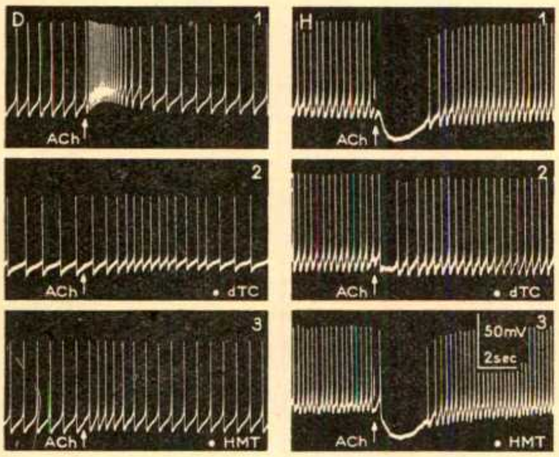

Fig. 1. (1) Effect of brief electrophoretical injections of acetylcholine (arrows) on the somatic membrane of a *D*-cell (left column) and an *H*-cell (right column) firing spontaneously. The drug depolarizes the *D*-cell and accelerates its firing, while it hyperpolarizes the *H*-cell and inhibits firing.
(2) Same amount of acetylcholine as in (1) is injected on the *D*-cell (left) and the *H*-cell (right) but in the presence, in the bathing fluid, of 10^{-4} gm./ml. of *d*-tubocurarine, which clearly inactivates both *D*- and *H*-cell membranes to the action of acetylcholine.
(3) The same electrophoretical injection of acetylcholine on *D*- and *H*-cells, as in (1), but in the presence, in the bathing medium, of 10^{-4} gm./ml. of hexamethonium bromide. This latter drug inactivates the *D*-cell membrane to the action of acetylcholine (left) but has no effect on the response of the *H*-cell membrane to acetylcholine

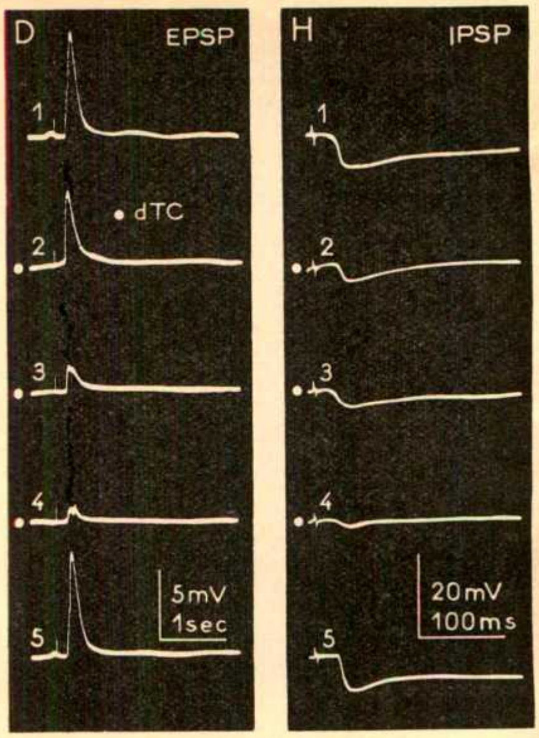

Fig. 2. Effects of *d*-tubocurarine in 10^{-4} gm./ml. concentration (white dots, 2–4) on the evoked excitatory postsynaptic potential in a *D*-cell (left column) and on the inhibitory postsynaptic potentials in an *H*-cell (right column). The synaptic potential in the *D*-cell is composed of two elementary excitatory synaptic potentials. In both cells the drug inactivates the postsynaptic membrane to natural excitatory and inhibitory transmitters respectively, as indicated by the gradual diminution (2–4) of the postsynaptic potentials. The removal of the curare restores the initial amplitude of the synaptic potentials (5)

Recent experiments brought out the surprising result that acetylcholine may also very likely be considered as the chemical transmitter for synaptic excitation in *D*-neurones. These neurones are highly sensitive to the drug; threshold effects on the cell excitability were observed with concentrations of less than 10^{-10} gm./ml. in absence of any anticholinesterase. Applied either by perfusion or locally injected, acetylcholine produces a spectacular drop in the *D*-cell membrane impedance, as in the neuromuscular end-plate of vertebrates[3] and the Purkinje fibres of vertebrate heart[4], where its transmitter action is well proved. Detectable increases of membrane conductance were observed for concentrations of acetylcholine down to 10^{-8} gm./ml. with a square pulse technique permitting the detection of impedance variations as small as 2–3 per cent.

Electrophoretical injections of acetylcholine on the *D*-cell membrane, following the technique of del Castillo and Katz[5], resemble the excitatory synaptic action (Fig. 1, *D*1). Like that of the excitatory synaptic potential, the equilibrium potential-level of this action is closer to the zero potential than the resting potential; if the membrane potential is artificially increased, the amplitude of both excitatory postsynaptic potential and depolarization produced by acetylcholine increases. The effects of electrophoretical injections of acetylcholine on the *D*-cell membrane (as well on the *H*-cell membrane) are diminished or abolished in the presence of a solution 10^{-4} gm./ml. of *d*-tubocurarine (Fig. 1, *D*2 and *H*2) or atropine. This block may probably be attributed to the inactivating effects caused by these drugs on the cell membrane cholinergic receptors It is highly significant that, in the presence of *d*-tubocurarine or atropine, the excitatory postsynaptic potentials in *D*-cells (and the inhibitory postsynaptic potentials in *H*-cells) also show rapid diminution of their amplitude or even disappear (Fig 2, *D* and *H*) This inactivating effect of curare and curare-like drugs on synapses constitutes an important argument in favour of the cholinergic nature of the synapses[6,7]

The action of hexamethonium was very remarkable (Fig 1, *D*3 and *H*3) In the presence of this drug, the *D*-cell membrane is inactivated to the action of the injected acetylcholine, whereas the *H*-neurone membrane is not affected and shows a normal hyperpolarizing response to acetylcholine Likewise, hexamethonium greatly diminishes the amplitude of the excitatory postsynaptic potentials in the *D*-neurones, but has no effect on the inhibitory postsynaptic potentials in *H*-cells This behaviour illustrates once more the pharmacological specificities of the *D*- and *H*-cell membranes, seen also with several other drugs[8]

The foregoing arguments seem to us to be a rather strong indication in favour of the cholinergic nature of the excitatory synaptic input in *D*-cells, especially if we remember that acetylcholine is normally present in *Aplysia* nervous system[9] and that a specific cholinesterase was demonstrated by both biochemical[10] and physiological[2] methods As in *H*-neurones, the extrasynaptic membrane of the cell body is highly sensitive to the natural transmitter

Recalling that the synaptic excitatory input of *H*-neurones is not at all affected by drugs which block cholinergic transmission[2], we presume that the excitatory synaptic input is conveyed by an unknown transmitter other than acetylcholine

Fig 3 illustrates the nature of the synaptic inputs on *D*- and *H*-cells. As the excitatory synaptic transmission in *D*-cells and the inhibitory one in *H*-cells appear to be cholinergic, the existence of interneurones capable of excitatory action through some of their synaptic endings and inhibitory actions through others at the same time is likely (dotted lines in Fig 3) This dual cholinergic mechanism emphasizes the importance of the postsynaptic membrane for the character of the transmission, the same transmitter in the same ganglion being able to produce opposite synaptic actions

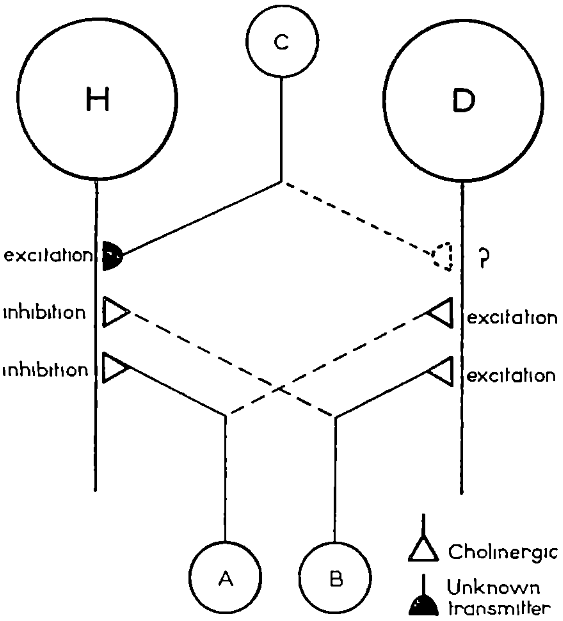

Fig 3 Diagram illustrating the nature of synaptic contacts on the *H*- and *D*-cells The inhibitory input to the *H*-neurone and the excitatory one to the *D*-cell are cholinergic A probable contact of a single presynaptic neurone (either *A* or *B*) with both *H*- and *D*-cells is indicated by the dotted lines The excitatory input on the *H*-cell is conveyed by an unknown transmitter different from acetylcholine A possibility of contact between the presynaptic cell *C* and the postsynaptic *D*-neurone is marked with a dotted line, no evidence exists concerning the effect of the unknown transmitter on the *D*-neurone

Conclusions from the above results may be summarized as follows (1) As was demonstrated elsewhere[1,2], acetylcholine, by its effects on *Aplysia depilans* neurones, enables one to distinguish two types of cells *D*-cells, depolarized and excited by the drug, and *H*-cells, hyperpolarized and inhibited The *D*-neurones only show excitatory synaptic input, whereas *H*-neurones present both excitatory and inhibitory synaptic input (2) In *D*-neurones, the synaptic excitatory input may be considered as cholinergic (3) In *H*-neurones, inhibitory input is also very likely cholinergic, but the excitatory input in these cells appears to depend on another, unknown chemical transmitter Hence, the character of synaptic transmission, excitatory in *D*-cells and inhibitory in *H*-cells, does not depend solely on the nature of the chemical transmitter—acetylcholine in both cases—but on physicochemical differences in the postsynaptic membrane that react in a specific way to the transmitter (4) Existence of interneurones which would simultaneously induce excitatory and inhibitory synaptic actions in different postsynaptic neurones is a real possibility

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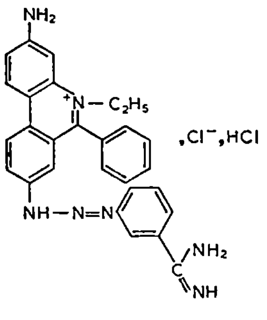
CHEMOTHERAPY

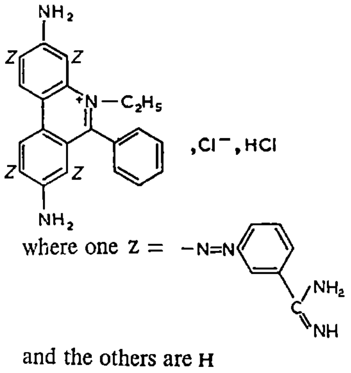
A New Prophylactic Trypanocidal Drug, 2,7-di-(*m*-amidinophenyldiazoamino)-10-ethyl-9-phenyl-phenanthridinium Chloride Dihydrochloride (*M & B* 4596)

THE properties of the trypanocidal drugs obtained from the coupling reaction of *m*-amidinobenzenediazonium chloride and homidium chloride have been described by Wragg, Washbourn, Brown and Hill[1], and Berg[2]. Metamidium was the mixture of the red (I) and purple (II) isomers (45 and 55 per cent respectively), and isometamidium was the pure red isomer (I)

In the study of the structure–activity relationships among this series of compounds, many similar coupling reactions have been investigated. In an attempt to prepare the 2,7-di-*m*-amidinophenyl-



(I)



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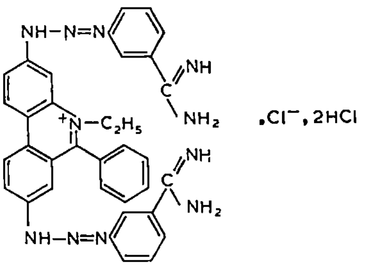

(III)

diazoamino derivative (III) of homidium, the coupling reaction of 2 mol. of *m*-amidinobenzenediazonium chloride and 1 mol. of homidium chloride was examined. Only (I) and (II) were present in the reaction product and none of the desired *bis*-compound (III) was obtained. The latter compound, however, was readily prepared by the coupling reaction of tetrazotized homidium chloride (1 mol.) and *m*-aminobenzamidine monohydrochloride (2 mol.). It separated from the reaction mixture as a brown granular solid, which was washed with aqueous sodium chloride and dried at room temperature. It usually contained approximately 10 per cent of sodium chloride and was submitted for biological examination without further purification as *M & B* 4596. It decomposed at 245°, and was approximately 1.5 per cent w/v soluble in water; the *p*H of a 1 per cent w/v solution being 4.7 at 20°. It could be stored in the solid state for at least two years without significant decomposition, but it began to lose nitrogen when heated above 70°. Aqueous solutions lost nitrogen when warmed above 40°. On heating (III) with cuprous chloride and 3 *N* hydrochloric acid for 7–10 min. the theoretical amount of nitrogen was evolved.

Table 1. TOXICITY AND THERAPEUTIC ACTIVITY AGAINST A LABORATORY STRAIN OF *T. congolense* IN MICE

Compound	*LD*50 (mgm./kgm.) subcutaneous	*CD*50 (mgm./kgm.) by subcutaneous injection
Homidium	75	0.03
Metamidium	210	0.0075
Isometamidium	260	0.002
M & B 4596	> 1,000	0.6

Table 2. PROPHYLACTIC ACTIVITY OF *M & B* 4596 AGAINST A LABORATORY STRAIN OF *T. congolense* IN DIRECT COMPARISON WITH METAMIDIUM CHLORIDE

Compound	Dose (mgm./kgm.) subcutaneously	No. of mice protected/No. treated Weeks after dosing 2	3	4	6	9	12	16	20	24
● Metamidium chloride	25		6/6		5/5	6/6	11/11	10/10	11/14	8/10
	5			4/4		4/6				
	1	6/6		19/19	9/14					
M & B 4596	25		6/6		5/5	6/6	20/20	19/21	15/15	10/10
	5			4/5		4/4				
	1	5/6		11/19	9/16					

The therapeutic and prophylactic activity of the compound against a laboratory strain of *Trypanosoma congolense* was determined by the methods described by Wragg *et al.*[1], and the results are summarized in Tables 1 and 2. The compounds were all examined as their chloride hydrochlorides, and the doses are given as weight of total salt.

Although *M & B* 4596 was much less active therapeutically than metamidium, it was no less active prophylactically. The local reactions produced by the subcutaneous injection of 10 per cent w/v aqueous suspensions of *M & B* 4596 into calves compared favourably with those caused by metamidium chloride and metamidium suraminate.

The prophylactic activity of *M & B* 4596 against *T. congolense* has been confirmed in cattle, and results of field-trials in Africa are very encouraging. The compound has also been presented for examination as its 12 per cent w/v water-soluble methane sulphonate (*M & B* 4596*B*), and its sparingly water-soluble β-naphthalene sulphonate (*M & B* 4596*A*).

We thank Dr. H. J. Barber, Dr. R. Wien and Dr. J. N. Ashley for their interest, and Mr. J. M. S. Lucas for the local tolerance studies.

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ANATOMY

Staining Properties of Stressed Bone

THE recent communication by Prof. C. J. Dreyer[1] on this topic is of great interest in relation to the general matter of the staining reactions of (presumably) collagen fibres. The change in staining reaction from green to reddish-brown with a corresponding change from optical isotropy to anisotropy, in unstressed and stressed bone, respectively, accord well with what has been observed in this Department in work on developing collagenous tissues. Using the Falg technique[2,3], it has been found that there are three stages in the development of this tissue which may be called the initial, the semi-mature and the mature. These three stages are distinguishable with the greatest constancy by the readiness with which they accept acid fuchsin, that is, by what has been earlier termed their erythrophilia. In the initial stage, presumptive collagen fibres are weakly erythrophile, in the semi-mature stage they are moderately erythrophile, and in the mature stage they are strongly erythrophile. The Falg technique, as well as others based on a similar chemical process, enables the consequent differences in monochromatic coloration (amplitude) to be converted into corresponding differences in colour (wave-length). Weakly erythrophile elements (of any kind) are coloured blue, the moderately erythrophile are coloured violet and the strongly erythrophile are coloured a deep red. In these reactions the acid fuchsin, which is an amphoteric and not an acid dye, behaves as an acid towards the basic elements of the tissues stained by it. Other acid dyes, for example, light green yellowish *SF* in the Falg technique, attach themselves to the acid fuchsin basic side-chains in a manner discussed in the papers cited.

The Masson stain used by Dreyer is a very complex staining mixture, for it includes not only acid fuchsin and aniline blue (an acid triphenylmethane behaving like the other acid triphenylmethane, light green) but also hæmatoxylin. Recent work in this Department shows that hæmatoxylin will attach itself to free acid groups of the acid dye which has attached itself to acid fuchsin as mentioned above. Nevertheless, in all cases mature collagen fibres retain the red colour. Accompanying the increased erythrophilia of developing collagenous tissues there is a corresponding increasing birefringence, particularly well shown by the compound polarizer, the principles of which have already been stated in *Nature*[4].

It would appear then that Prof. Dreyer's results are explainable as a demonstration of a special maturation of collagen fibres in those regions of stressed bone which he has studied. This is in accord with the general principle that collagen fibres are to tension fields as iron filings are to magnetic fields[5], a principle well established both by gross and microscopic anatomy on one hand and experiment on the other.

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PATHOLOGY

Experimental Atheroma in Budgerigars

Since Anitschkow and Chalatow[1] in 1913 first induced lesions simulating human atherosclerosis in rabbits by feeding a diet rich in cholesterol, many workers have used rodents for this purpose despite the fact that spontaneous atheroma, apart from microscopic lesions, rarely develops in these mammals. Experimental atheroma has been produced by various dietetic alterations in other animals, including monkeys[2,3], pigs[4], chickens[5,6] and geese[7], but the parrot family (Psittacidae) have rarely been used although the more aged, captive birds in this order develop spontaneous atheromatous-like arterial lesions of an intensity usually seen only in man[8,9]. This communication describes cholesterol atheromatosis induced in budgerigars (*Melopsittacus undulatus*), which are the least expensive and most easily reared and housed of the psittacines.

Twenty-eight adult female budgerigars, reputably aged between 2 and 5 years, were divided into four equal groups. Group 1, used as controls, were fed a diet of 60 per cent millet and canary seed and 40 per cent pin-head oatmeal. Group 2 received 60 per cent seed, 38 per cent oatmeal and 2 per cent cholesterol. Group 3 received 60 per cent seed, 23 per cent oatmeal, 2 per cent cholesterol and 15 per cent Danish lard. Group 4 were fed a diet of 60 per cent seed, 25 per cent oatmeal and 15 per cent Danish lard. Food, water and grit were available *ad libitum*. One bird in each group was killed at 30-day intervals over a period of six months. Three birds died during the experimental period. Tissues were examined by naked-eye and histologically, and in order to facilitate recognition of small lipid deposits in the gross aorta and brachio-cephalic arteries the opened vessels were treated with sudan IV.

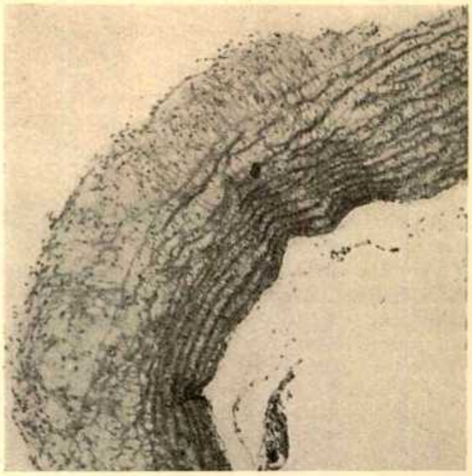

Fig. 1. Atheromatous plaque containing numerous foamy, lipid-laden histiocytes in aorta of a budgerigar fed on a diet containing cholesterol and lard for 4 months. Due to inversion the vessel lumen is at top left. Hæmatoxylin and eosin staining. (× 65)

Birds in groups 2 and 3 showed slight, sudanophilic lipid stippling of the inner walls of the aorta and brachiocephalic arteries after receiving their respective diets for two months. After three months, multiple, creamy white or yellow, lipid-containing nodules and plaques had formed in the intima and inner media of the arteries (Fig. 1). These lesions were more marked and more extensive at the end of the present experimental period of six months. Sub-endothelial accumulations of lipid narrowed several of the small coronary vessels (Fig. 2) and, in two instances, related small areas of myocardial infarction were seen. Vascular thrombosis was not observed. Blood cholesterol-levels were raised in all birds of groups 2 and 3; concentrations being greater than 300 mgm. per ml. as compared with cholesterol-levels

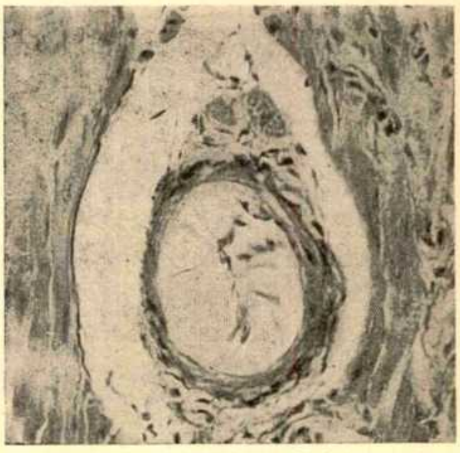

Fig. 2. Sub-endothelial lipid in small, intramural coronary vessel. Lumen distorted and narrowed. Weigert's elastic and van Gieson's stain. (× 300)

ranging from 150 to 200 mgm. per 100 ml. in group 1 (control) birds. The vascular changes were slightly more marked in group 3 as compared with group 2, but considering the small number of animals used this may well have been due to chance. No vascular lesions were observed in birds of group 1 and group 4, except for some light sudanophilic speckling of the thoracic aorta and brachio-cephalic trunks, associated histologically with small, focal lipid deposits in the non-muscular laminæ of the inner media of the arteries, of one bird in group 1.

The livers of birds in groups 2 and 3 were enlarged, pale and fatty. Initially the lipid infiltration was diffuse and largely within the cytoplasm of parenchymal hepatic cells; later the lipids accumulated more focally and appeared to be mainly within the cytoplasm of histiocytes. Lipid-laden histiocytes were seen also in the spleen, kidneys and ovaries. Birds in group 4 showed moderate fatty changes in the liver during the first three months of the experimental period. The liver and other viscera of birds in group 1 were normal.

Although experimentation has been limited, it would appear that diets containing added 2 per cent cholesterol or 2 per cent cholesterol and 15 per cent animal fat in the form of lard are highly atherogenic to young, adult female budgerigars, whereas a diet supplemented only by 15 per cent lard does not produce vascular lesions. Further work on naturally occurring and experimental atheroma in psittacines is being undertaken. While the pig is probably the most satisfactory animal for studies on atherosclerosis, it is suggested that the budgerigar may be of value where space and money are restricted.

One of us (R. F.) is indebted to Eli Lilly and Co., Ltd., for financial support. We thank Prof. J. W. S. Blacklock for advice; Mr. J. W. Miller, St. Bartholomew's Hospital, for technical assistance, and Mr. P. R. Crocker for photomicrography.

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Orosomucoid in Schizophrenia

Orosomucoid, a glycoprotein extremely rich in carbohydrate, is the principal constituent of the perchloric acid-soluble seromucoid fraction of human serum[1]. In contrast to the seromucoid fraction which contains several proteins and represents more than one biological entity, orosomucoid is homogeneous and behaves as a single protein. With the production of specific chicken antiorosomucoid sera[2,3], it has become possible to investigate the clinical significance of orosomucoid. Interest in doing so arises from the fact that elevations of seromucoid and total serum glycoprotein-levels occur in a number of diseases; for example, in malignancy, tuberculosis, rheumatoid arthritis, and multiple sclerosis[4]. Elevations in this chemically defined group of proteins also occur in response to a wide range of non-specific stressors such as fractures, epinephrine injection, and burns[5].

Serum-levels of orosomucoid have now been measured immunochemically[2,3] in a large number of cancer patients and to a smaller extent in patients with other organic pathologies. Quite marked elevations of the protein have been observed, particularly in advanced malignancy[3], suggesting that processes destructive of tissue play a key part in increasing its serum concentration. However, such processes may merely serve as non-specific stressors which then act through systemic mechanisms to increase the serum concentration of orosomucoid. This communication concerns orosomucoid in schizophrenia, a disease in which tissue pathologies have never been demonstrated.

Comparisons were made between 41 non-psychotic control subjects (laboratory personnel) and 40 schizophrenic patients with respect to serum-levels of orosomucoid and albumin. These proteins were determined as in refs. 3 and 6, involving the precipitin reactions of specific chicken antisera. In previous work with cancer and tuberculosis sera, it was observed that when the orosomucoid concentration was markedly elevated, the albumin concentration was depressed. Thus the ratio of orosomucoid to albumin seemed to provide an even more sensitive indication of a pathological process than the orosomucoid-level alone. It was for this reason that both orosomucoid and albumin were measured in the controls and schizophrenics and that the orosomucoid : albumin ratio was computed for each subject.

Both the control and patient groups were off drugs and were free of physical disease so far as could be determined by physical examination, chest X-ray, routine complete blood count, and urinalysis. All the patients had been in hospital with schizophrenia for more than 4 years. Two-thirds of the group were under careful dietary supervision in a metabolic ward at the Lafayette Clinic, while the remaining third came from Pontiac State Hospital. The data for these two patient groups were combined, since there were no significant differences between them in the variables under investigation.

Table 1. COMPARISON OF ALBUMIN- AND OROSOMUCOID-LEVELS IN SCHIZOPHRENIC AND CONTROL GROUPS

	Control group ($N = 41$)		Schizophrenic group ($N = 40$)			
	Mean	*S.D.*	Mean	*S.D.*	t	P
Albumin	48·3	3·4	45·7	4·5	2·90	< 0·01
Orosomucoid	0·842	0·185	1·005	0·196	3·81	< 0·001
Ratio of orosomucoid to albumin	0·0175	0·0041	0·0223	0·0055	4·34	< 0·001

The mean albumin (Table 1) was significantly lower for the schizophrenic group than for the control subjects ($P < 0·01$). In contrast, the mean orosomucoid was significantly higher for the schizophrenic subjects ($P < 0·001$). The difference between the two groups was also evident when the orosomucoid/albumin ratios were compared: 0·0223 in the schizophrenic group against 0·0175 in the control group.

These findings are of interest in two ways: (*a*) they suggest that orosomucoid may be a reactor to a variety of non-specific stressors not necessarily associated with tissue destruction or proliferation, and (*b*) they indicate that other than purely psychological factors appear in the chronic schizophrenic illness and that either primary or secondary metabolic changes have occurred In this connexion the hypothesis that schizophrenic patients are in a chronically stressed state would seem to be supported

This work was aided by a grant from the National Institute of Mental Health (grant No *MY*-2476)

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RADIOBIOLOGY

Activity of Deoxyribonuclease as related to Root-Growth

It has been repeatedly observed in the past few years that deoxyribonuclease (DNase) activity is increased in normal growing animal tissues[1-5]. As postulated by Brody[2], it is possible that the increase in acid DNase activity counterbalances the greatly enhanced activity of the deoxyribonucleic acid (DNA) synthesizing enzymes and indeed determines the normal character of the growth Cancerous tissues, on the contrary, have a lower DNase activity, at least when the activity is expressed per mgm DNA (ref 2), but not, apparently, when it is expressed per mgm nitrogen[6].

In plant roots, the mitotically active meristem can be easily separated from the upper regions where the mitotic activity is very much lower Pea seeds (*Pisum sativum*) were allowed to germinate on paper soaked with distilled water for 3 days at room temperature Roots of approximately equal length were then selected and cut in two fragments the tip, 1 5–3 mm long, and a longer fragment immediately above the tip The mitotic indices determined on sections of each fragment after Feulgen staining gave values of 35–45 a thousand in the tip and less than 1 in a thousand in the upper fragment

Fifty to hundred fragments of each kind of the same length are pooled and homogenized in cold distilled water in a Potter tube A sample of the homogenate is pipetted for total nitrogen determination by the Kjeldahl method The rest is used for measurements of DNase activity by viscosimetry at 37° C (ref 7) The activity of the acid enzyme is measured in acetate buffer 0 2 *M* *p*H 4 8 in the presence of sodium citrate (final concentration 0 017 *M*) and highly polymerized DNA (final concentration 0 4 mgm /ml) prepared from calf thymus[8] For neutral DNase, phosphate buffer 0 2 *M* *p*H 7 6 with magnesium chloride (final concentration 0 04 *M*) is used 0 02 ml 'Triton-*X* 100' 10 per cent is added just before introduction of the mixture into the viscosimeter The activity is expressed by the initial rate of decrease of the specific viscosity extrapolated to 10 min , or by the actual decrease in 10 min when a straight-line relationship with time is obtained For determination of the DNA content, at least one hundred root segments, cut exactly as for DNase assays, were homogenized in 70 per cent ethanol and processed according to the method in ref 9, a small modification was introduced and consisted in incubating the delipidated tissue in cold 2 *N* perchloric acid for 18 hr in order to get a better extraction of the ribonucleic acid (RNA) A diphenylamine reaction (Burton[10]) was performed on the hot perchloric extracts A sample of the alcoholic homogenate was pipetted for nitrogen determination the expression of DNase activity/mgm DNA rendered it indeed necessary to make a correction for any small differences in nitrogen content which might occur between the segments selected for DNA determinations and those selected for enzymic assays

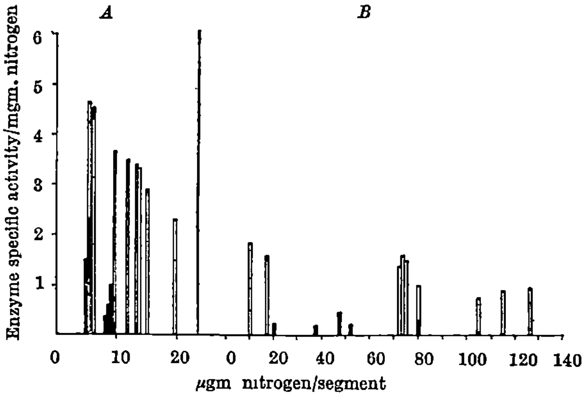

Fig 1 Specific DNase activity in two different segments of the pea root *A*, Tip of root, *B*, upper fragment (see text), white bars, acid DNase, black bars, neutral DNase

In Fig 1, the specific activity of DNases/mgm nitrogen is given for the tip of the root (part *A*) and for the upper fragment (part *B*) The length of the segment is indicated by the values of total nitrogen/segment, in μgm , on the abscissæ It is clearly seen that the specific activity of acid DNase is much higher in the tip than in the other part of the root, the shorter the tip fragment, the higher the specific activity Neutral DNase is also higher in the tip, but the difference between the two fragments is less marked than for the acid enzyme

In Table 1, the specific activity of acid DNase expressed per mgm nitrogen can be compared with the specific activity expressed per mgm DNA in four experiments

The ratio of enzyme activities between the tip and the upper part of the root ranges from 1 to 2 58 when the activity is expressed per mgm DNA and from 2 to 6 65 when it is expressed per mgm nitrogen

Table 1 DNA and Nitrogen Content and DNase Activity in Two Different Fragments of the Pea Root

	μgm DNA/ segment	μgm nitrogen/ segment	Acid DNase/ segment	Acid DNase/ mgm. DNA	Acid DNase/ mgm nitrogen
Tip	1 9	13 8	0 042	22 5	3 05
Upper part	6 7	75	0 124	18 5	1 53
Tip	2 8	19 8	0 045	15 6	2 27
Upper part	6 2	115	0 098	15 6	0 84
Tip	1 72	13 4	0 046	26 8	3 41
Upper part	10 5	126	0 109	10 4	0 86
Tip	1 32	9 8	0 036	27 3	3 66
Upper part	4 60	103	0 058	12 6	0 55

Thus, as in many animal tissues, the acid DNase seems also linked to the process of growth in pea roots Of course, many syntheses are going on at a high rate in the meristem, for example RNA, protein and DNA syntheses[11], but the metabolism with which the DNase is more probably connected is the DNA metabolism. It is known, however, from ref 12 that DNA synthesis goes on in the older cells above the meristem and that there is up to four times more DNA per cell during elongation, without mitosis. This fact has been confirmed on *Vicia faba* roots[13], it was observed by autoradiography that ^{3}H-thymidine was incorporated into the nuclei up to 7 mm behind the meristem, with decreasing intensity

Therefore, the decreased activity of acid DNase in the upper part may be a mere reflexion of a lower rate of DNA synthesis, or may indicate that DNase is also connected with the mitotic process and markedly decreases because the mitotic activity shows a dramatic drop above the meristematic part of the root.

The help of Dr Demalsy for the Feulgen staining is gratefully acknowledged

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Enterohepatic Circulation of Bilirubin

The enterohepatic circulation of bile pigment has been the subject of protracted debate Recirculation of bilirubin, and its bacterial reduction products, the urobilinoids, has been postulated and denied[1]. The availability of bilirubin labelled with carbon-14 in this Laboratory[2] has obviated many of the problems previously inherent in the study of bilirubin metabolism The following is a preliminary report of investigations of the enterohepatic circulation of bilirubin

Radioactive crystalline (unconjugated) bilirubin was prepared by methods described previously[2] Radioactive conjugated bilirubin (in bile) was prepared by infusing crystalline labelled bilirubin intravenously into rats with an external bile fistula Essentially all the injected label was excreted in bile as conjugated labelled bilirubin during the ensuing 3-hr. period

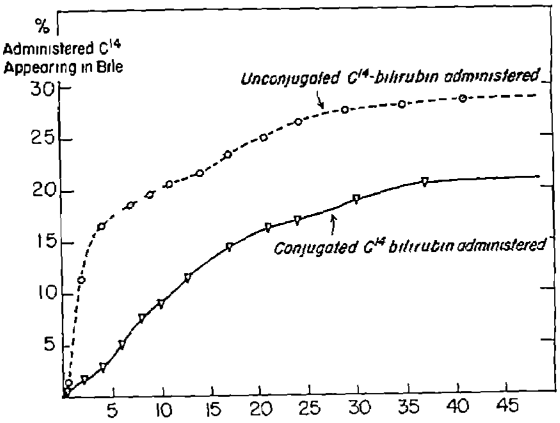

Fig 1 Appearance of radioactivity in bile following intraduodenal infusion of either conjugated bilirubin (▽——▽) or unconjugated bilirubin (○ – – – ○) labelled with carbon-14

For work on intestinal absorption, crystalline labelled bilirubin was dissolved in aqueous taurocholate, human albumin solution, or normal rat bile prior to administration, while conjugated labelled bilirubin was administered in bile The labelled bile pigments were infused through a transoral duodenal feeding tube into each of 9 rats with an external bile fistula The rats were allowed to recover from anæsthesia, and maintained, thereafter, in restraining cages permitting complete separation and recovery of excreta After infusing the labelled pigments into the duodenum, collections of bile, urine, and fæces were made at regular intervals for determination of total radioactivity Bilirubin was crystallized[2] from selected pooled samples of bile and the specific activity determined At the termination of each experiment animals were autopsied to ascertain the absence of operative complications and the persistence of the bile duct catheter *in situ*

Table 1 RADIOACTIVITY RECOVERED AFTER INTRADUODENAL ADMINISTRATION OF CONJUGATED AND UNCONJUGATED BILIRUBIN LABELLED WITH CARBON-14

	Infused radioactivity recovered in excreta (per cent) Conjugated ^{14}C-bilirubin	Unconjugated ^{14}C-bilirubin
Bile	21	28
Urine	2	trace
Fæces	40	32
Total	63	60

Data based on radioactivity recovered in two rats during the 72-hr period following intraduodenal administration of either 0 13 μc conjugated labelled bilirubin or 0 25 μc unconjugated bilirubin in a final volume of 2 ml at similar bilirubin concentrations

Representative results are given in Fig 1 and Table 1 In all instances, significant intestinal absorption and biliary excretion of the carbon-14 label were evident After the intraduodenal administration of both unconjugated labelled bilirubin and conjugated labelled bilirubin, much of the demonstrable absorbed radioactivity appeared in the bile as conjugated labelled bilirubin The initial phase of absorption was more rapid for unconjugated bilirubin than for conjugated bilirubin and the total absorption of the former appeared somewhat greater Little radioactivity was recovered in urine

This work was supported in part by U S Public Health Service grant *A*–1833

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BIOLOGY

Effect of Pupal Brain Surgery on the Rocking Response of *Automeris*

THE rhythmic rocking responses of Hemileucine moths may be released from insects which have been subjected to quite drastic surgical procedures[1], but they have never been observed in decapitated preparations[2] An attempt has now been made to investigate the role of the brain in mediating this response and in determining its quantitative strength, using the techniques of pupal surgery described by Williams[3] One hundred female pupæ of *Automeris cinerea* were chilled at 6° C. for eight weeks immediately following their reception from Argentina, and were then transferred daily (at 25° C) in batches of five in order to break diapause On the fifth to eighth day after transfer, they were chilled at 4° C for 1 hr , anæsthetized in carbon dioxide for 30 min while packed round with melting ice, and subjected to gross brain surgery through a window cut in the facial cuticle After surgery, the pupæ were sealed with the usual precautions[3] and returned to 25° C. The yield in terms of histologically acceptable lesions was approximately 50 per cent The surgical procedures, which were previously rehearsed on the large Indian *Antherea mylitta*, are summarized in Fig 1 It may be noted that while procedure 1 results in blind moths the optic glomeruli II and III of which remain intact, procedure 2 ablates the entire optic complex, while sparing the medial lobes of the protocerebrum Procedure 5 permits the development of a recognizable suboesophageal unit in isolation; while the resulting neuropil shows evidence of condensation, the normal features of its intrinsic organization may readily be recognized in favourably oriented sections, and moths so treated differ characteristically in their behaviour from those from which the suboesophageal ganglion has been extirpated.

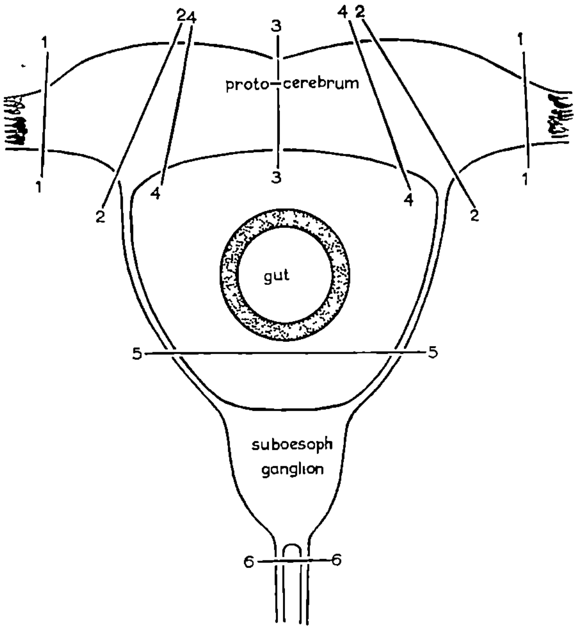

Fig 1 Diagram showing the pupal brain, and the six surgical procedures

Some 12 hr before emergence was due, and just after the absorption of the moulting fluid, each surviving moth was dissected from the pupal case, and its settling behaviour examined by the techniques which have been described in ref 2, some individuals were kept at 20° C and tested at intervals. The rocking response of *A cinerea* does not show the quantitative stability of that of *A aurantiaca*[2], and repeated testing at 5-min intervals typically yields a range of scores lying between 10 and 45 The brains of all moths were dissected out, fixed in Dubosq–Brasil solution, sectioned at 10μ, and impregnated by a modification of the Holmes[4] technique, similar to that of Larsen[5] but using 2,6-lutidine in place of pyridine

The results are summarized in Table 1; the left-hand column records the effective surgical procedure as scored at histological examination The highest

Table 1

No	Effective surgical procedures Left	Medial	Right	Score at dissection from pupa	6 ± 4 hr.	Adult scores 12 ± 4 hr	24 hr –5 days
1	1		1	(Spontaneous eclosion)	24 22 28*	—	—
2	1		1	26 33 38*	—	—	23 23
3	1		1	16 22 26 30*	—	—	22 23
4	1		1	12*	11 12 10	—	—
5	1		2	(Spontaneous eclosion)	24* 6	—	—
6	1		2	18 22*	—	—	—
7	2		2	41 42*	27 24 16 27 32	—	—
8	2		2	12 12 14 14 14	—	—	18 12 12 10 12 12 37*
9	2		2	33* 32	—	—	—
10	2		2	15	—	9 31 32*	—
11	2		2	(Spontaneous eclosion)	—	15 15 15 15 23 33* 18 6 15	—
12	2		2	33 34*	—	—	—
13	2		2	32*	—	—	—
14		3		30*	10 19 25	—	—
15		3		15 37*32	—	—	—
16	2	3	2	20 22 24 22 25 34 45*	16 38	9 8 9 (after oviposition)	—
17	2	3	2	10 10 14 10 26*	—	—	—
18	2	3	2	36* 12 10 12 10	—	—	—
19		4 (large part of medial lobes left intact)		10 10 10 12 13 5 5 5	—	—	—
20–31		4		Rocking absent	Rocking absent	Rocking absent	—
32–36		5 (Brain removed)		Rocking absent	Rocking absent	Rocking absent	—
37–41		5 (Brain left *in situ*)		Rocking absent	Rocking absent	Rocking absent	—
42–48		6 (Brain left *in situ*)		Rocking absent	Rocking absent	Rocking absent	—
49		Dummy		(Spontaneous eclosion)	18 24*	—	—
50		Dummy		10*	—	—	—
51		Dummy		42*	34 34	—	—

scores for each moth are marked with an asterisk, they may be compared with the highest response strengths scored for each of seven females at first settling after spontaneous hatching (38 , 36 , 43 , 34 , 17 ; 42 , 45), and with the scores yielded by 66 males aged 8–24 hr when settling for the first time following a disturbance[6] (mean = 23 39, σ = 11 53) Despite the striking abnormalities of behaviour characteristic of each lesion, the co-ordination of the rocking response was unaffected except by procedures 2 and 3 in combination, where the amplitude of the response was increased the only moth which gave a weak response after procedure 4 showed oscillations of shallow amplitude While this latter procedure necessarily ablates the medial protocerebral neurosecretory cells, and the corpora cardiaca and allata, procedures 5 and 6 eliminate the rocking response whether or not the brain and its associated organs are left *in situ* The role of the protocerebrum cannot, therefore, merely be that of providing endocrine priming for the lower neural structures Furthermore, while eserine potentiates the rocking response of intact moths, it is unable to cause its re-appearance in decapitated animals[6].

These results show that for *A cinerea* (i) protocerebral structures are necessary for the facilitation of the rocking response, which cannot be performed in their absence, (ii) within the limits of these experiments the quantitative strength of the response is independent of input from the optic complex, and (iii) it is probably independent of transverse connexions at the protocerebral level

I am grateful to Prof J B S Haldane and Dr Helen Spurway for sending pupæ of *A mylitta* from Calcutta The work was supported by grants from the University of London Central Research Fund, and from the United States Department of Health, Education and Welfare (Project *RG*-7109)

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Role of Cell Division in Gastrulation of the Viviparous Toothcarp *Xiphophorus helleri*

Recently it was stated that morphogenetic movements in gastrulation of the viviparous teleost *Fundulus heteroclitus* occur in a normal fashion in cases in which cytokinesis is prevented by treatment with the antimitotic alkaloid colchicine[1] To establish whether a more general significance should possibly be attached to this phenomenon a corresponding investigation was performed with a fish bearing live young, namely the viviparous cyprinodont *Xiphophorus helleri*, the swordtail For this purpose pregnant females in the first phase of gestation were injected intraperitoneally with 0 5 ml of 0 001 *M* colchicine solution During gastrulation in stage 12 (beginning gastrula), 13 (advanced gastrula), 14 (further advanced gastrula) and 15 (closure of blastopore) both treated and control females were killed by decapitation and the ovaries were fixed in Bouin's fluid The blastoderms were sectioned at 5μ and stained with Heidenhain's iron hæmatoxylin The mitotic index, representing the ratio of cells in mitosis to the total number of cells, and expressed as per cent, was determined in each blastoderm on the basis of cell counts of the sections

Table 1 Mitotic Indices of Colchicine-treated and Control Embryos of *Xiphophorus helleri*

Stage 12 = beginning gastrula, 13 = advanced gastrula, 14 = further advanced gastrula, 15 = closure of blastopore

Stage	Mitotic index (per cent) Colchicine treatment	Control
12	6 9	2 2
13	15 3	2 4
14	28 4	3 0
15	35 6	3 7

As in *Fundulus*[1], also in *Xiphophorus* the colchicine treatment did not have any apparent effect on gastrulation, which was similar to that in control blastoderms and consequently morphologically normal The colchicine-treated blastoderms showed typical c-mitoses[2] On the strength of mitotic index determinations for control and colchicine-treated blastoderms it could be established that in the treated blastoderms the number of *c*-mitoses increased, indicating that mitosis was completely blocked (Table 1) Thus, in stage 15, 35 6 per cent of the cells are in mitosis, while the mitotic indices of control blastoderms are relatively low during gastrulation Consequently, also in early morphogenesis of viviparous teleosts cell division can be dismissed as a causative factor and apparently provides only material for morphogenetic movements[3] This result, corresponding very well with the findings of Kessel[1] obtained with *Fundulus* and consequently of more general importance, is an argument for the view that gastrulation is a problem of mass movements and not of growth[4,5]

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Oxidase Activity in the Saliva of a Plant-Bug

In an account[1] of the origins of the salivary materials of a lygæid, *Oncopeltus fasciatus* (Dall.), it was reported that an oxidase other than cytochrome oxidase occurs in the accessory gland of the salivary apparatus, but not in the principal gland. No direct evidence was put forward for the discharge of the oxidase during salivation, but it was stated that the stylet-sheath material shows non-enzymic oxidizing properties that are not apparent in the precursors of the sheath material in the anterior and lateral lobes of the principal gland It was therefore suggested that an oxidase from the accessory gland acts on the precursors as they are discharged However, other evidence put forward suggested that the secretion of the accessory gland is a normal constituent of the watery saliva, which can be discharged independently of the sheath material Evidence for the existence of an oxidase in the watery saliva of another lygæid, *Aphanus sordidus* Fabr (the peanut trash bug or groundnut bug), has now been obtained, together with further evidence for the oxidation of the sheath precursors as they are discharged

The principal and accessory salivary glands of *Aphanus* were dissected into insect Ringer's solution and immediately transferred to Burstone's oxidase reagent[2], with or without inhibitors In the absence of inhibitors, the cells of the entire salivary apparatus coloured within half a minute, in the presence of 0 003 *M* potassium cyanide, there was no reaction, and in the presence of 0 003 *M* sodium azide, the whole of the accessory gland and the principal salivary duct, but no other part of the salivary apparatus, were coloured In view of Burstone's report[3] that cytochrome oxidase is inactivated if tissues are left in physiological saline for 30 min , it was interesting to observe that salivary glands which had been left in Ringer's solution overnight and were then transferred to the oxidase reagent showed the same reaction as fresh glands incubated in the reagent plus azide, that is, only the accessory gland and salivary duct were coloured When the lobes of the principal gland were ruptured in the reagent, either with or without azide, the emerging contents did not become coloured and no coloured precipitate was formed

Watery saliva was collected from the insects by forcibly extending the rostrum and collecting the liquid which exuded from the tip The salivation of bugs in these circumstances was erratic often no saliva could be collected, but sometimes a bug appeared to salivate so freely that the whole of the rostrum and head capsule became bathed in liquid To provide against the possibility that the latter phenomenon was due to bleeding caused by damage to the bug during handling, tests for oxidase activity were made both on the liquid assumed to be watery saliva and on blood withdrawn from the insect by puncturing an abdominal intersegmental membrane with a fine pipette

When the collected saliva was discharged into a drop of reagent on a white tile, a purple-blue precipitate formed, usually within a few seconds, and a blackish-purple border began to form around the drop of reagent within 5 min In contrast, the reagent alone left only a pink residue when it evaporated The insect's blood gave no reaction When the saliva was discharged into the reagent diluted with an equal volume of 0 005 *M* potassium cyanide, a slow, weak reaction occurred, and there was no reaction if the saliva was first mixed with its own volume of cyanide and then discharged into the reagent On the other hand, when the saliva was first mixed with 0 005 *M* sodium azide or with water, a rapid reaction occurred.

Sheath material was collected as the 'collar'[4] left at the surface of feeding punctures on peanuts In the oxidase reagent, the sheath material developed no more than a faint pink colour, and there was thus no evidence for oxidase activity in the material itself. However, experiments with oxidation-reduction indicators showed that the sheath material had a higher oxidation-reduction potential than the contents of any of the lobes of the principal gland A satisfactory indicator was 0 01 per cent thionin blue in veronal-acetate buffer *p*H 7 0 Before use, it was reduced by the dropwise addition of 1 per cent sodium dithionite until the indicator was almost colourless A salivary gland was dissected into a drop of insect Ringer's solution made 0 005 *M* with respect to potassium cyanide, and pieces of fresh sheath material were placed alongside All the lobes of the principal gland were punctured and the whole was enclosed under a coverslip in the reduced thionin The sheath material and the walls of the gland immediately became coloured, whereas the contents of the gland, which were observed both as they emerged and after some had coagulated, remained colourless On the other hand, when the indicator had first been re-oxidized by exposure to air, the contents of all the lobes stained to some extent, although the contents of only the anterior lobe approached the same intensity of staining as the sheath material

These experiments showed that an oxidase other than cytochrome oxidase is present in the accessory salivary gland and in the salivary ducts of *Aphanus*, that a similar oxidase is present in the watery saliva, and that the stylet-sheath material has a higher oxidation-reduction potential than the contents of any lobe of the principal gland The results are consistent with the view that an oxidase acts on the sheath precursors as they are emitted, however, the presence of a similar enzyme in both the accessory gland and the salivary duct is a complicating feature, since either or both parts of the glandular system could provide the oxidase in the watery saliva and an enzyme which oxidizes the sheath precursors Possibly both parts are functionally similar, and the accessory gland should be considered as purely a development of the salivary duct, as suggested by Baptist[5]

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Pre-Harvest Prophylaxis for Infestation Control in Stored Food-Grains

STORED food-grains suffer from considerable losses due to insect infestations in the tropical and sub-tropical regions of the world[1] During post-harvest storage, cross-infestation by insect pests and the multiplication of pests as a result of field-infestation occur in stored grains even within a storage period of a few months in warehouses[2-3] Fumigation with noxious gases such as methyl bromide, carbon disulphide, carbon tetrachloride, hydrocyanic acid, ethylene oxide and others, although of use in the control of most infestations, is not easy for the producer or the warehouse-man to apply[2] Parkin[1] observed that application of such measures unfortunately lags behind the advance of knowledge in all but the most highly developed countries, and even in those is not always so widespread or up-to-date as it could be A new approach has therefore been made to replace the fumigation treatment with a relatively simpler prophylactic spray treatment of the grain under pre-harvest condition, using a chemical of low toxicity for mammals, for the control of pest infestation during post-harvest storage

Canada and the United States have set a tolerance limit of 8 p p m for malathion in stored food products[4] The U K Ministry of Agriculture, Fisheries and Food has permitted the admixture of malathion with raw unprocessed grains for their storage Godavari Bai *et al* [4] observed that more than 50 per cent of malathion from wheat flour was lost in one month through *A*-twill bags. The average concentration of malathion was less than 2 p p m. after two months storage At this concentration, malathion could exert little prophylactic action on pests of stored grain. The possibilities of disinfesting grain under field conditions with malathion spray at its permissible dosage and affording longer protection from cross-infestation by storing it in sacks impregnated with insecticides, were therefore investigated

The panicles of sorghum crop (*Sorghum vulgare*) were sprayed with malathion (Premium grade, American Cyanamid Co) water-dispersible emulsion at various dosages on different plots The grain was harvested, threshed and stored in lindane-impregnated *B*-twill sacklets Grain samples were analysed for malathion by the method of Norris *et al* [6] Sorghum harvested from the control plot was stored in impregnated and unimpregnated sacklets and, for comparison, control grain was fumigated and stored in these bags Experimental grain sacklets were incubated in an infestation room having large populations of *Sitophilus oryza* (Linn) and *Tribolium castaneum* (Hbst) for 150 days

The results of the experiment, shown in Table 1, demonstrated that this simple treatment can replace the fumigation treatments for pest control in stored grain.

Table 1 EFFECT OF PRE-HARVEST SPRAYING OF MALATHION ON THE POST-HARVEST STORAGE OF *Sorghum vulgare*

Treatment	Malathion concentration in p p m after 3 days of spraying	Living adults (No /kgm) after 150 days incubation at 24–28° C and rel hum 48–83 per cent			
		T castaneum		*S oryza*	
		Impregnated*	Control	Impregnated*	Control
Pre-harvest malathion spray	1 9	2	8	0	63
	2 4	1	3	0	21
	7 2	0	2	0	8
	7 7	0	1	0	12
	14 2	0	0	0	0
Control	0 0	3	29	49†	283
Fumigated‡	0 0	0	32	0	308
Post-harvest malathion spray	8 1	0	2	0	9

* Impregnated bag at 75 mgm lindane/sq ft
† As a result of field-infestation
‡ Grain fumigated with 32 mgm /litre methyl bromide for 48 hr

In most cases stored food-grains are fumigated in warehouses only when they show open signs of insect infestation, for untrained warehouse-men, in the tropics and in many other parts of the world, cannot detect incipient infestation On the other hand, field-spraying of crops with a pesticide is much more commonly adopted throughout the world The newer pesticidal substances having differential toxicities[7] to insect pests and mammals, such as malathion, present a unique means of introducing simple pest control treatments for stored food-grains. Spraying of malathion on the crop before harvest at a required level to obtain a concentration below the permissible limit in the threshed grain and storing in an insect-proofed structure or jute sacks can afford satisfactory protection from pest infestation in post-harvest storage Treatment of harvested grain with malathion would require elaborate machinery for spraying the regulated grain-flow on a conveyor, but applications in the field on the crop would be easy. This new approach to pest control problems of stored food-grains probably offers a simpler solution for large-scale application where fumigation with toxic gases cannot be easily applied. Although malathion is rapidly dissipated from the treated grains, the possible residual toxicity to consumers, of traces of the chemical on food-grains, needs further assessment in the present context

We thank Dr. V Subrahmanyan, director, and Dr A Sreenivasan, deputy director, of this Institute, for their interest in the work

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Perennial Nodules on Native Legumes in the British Isles

IT is generally maintained that the normal life span of legume nodules in temperate climates is restricted to one season or growth cycle of the host plant Frost, drought[1] and insect damage[2], or developmental changes in the host such as flowering and fruiting[3], are recognized agencies leading to destruction of nodules

Nodules on certain tropical woody legumes and perennial species of *Lupinus* are classed as exceptional in developing annual or seasonal increments of bacterial tissue Various anatomical features are associated with the prolonged activity of such perennial nodules[4].

In an earlier paper[5] a successful over-wintering of nodules was described as a common event in winter annual, biennial and perennial legumes in Northern Ireland. During four seasons of investigation (1952–56) involving 29 native species, a life-span of 10–20 months was recorded for nodules surviving into a second season No evidence was obtained of nodules persisting into a third season of plant growth.

This communication reports the finding, in February 1961, of what are considered to be truly perennial nodules on the roots of gorse (*Ulex europaeus* L) and broom (*Sarothamnus scoparius* Koch) growing on drained peat bog near Randalstown, Co Antrim The nodules are multibranched structures measuring some 1–1½ in in length and weighing as much as 1 gm They are borne on thin fibrous roots traversing the upper layers of partially decomposed peat. Many show apogeotropic growth forms similar to those to be observed in root nodules of *Alnus* and various cycads.

The following findings support the view that the nodules are several years old and clearly equipped for at least another season of growth and nitrogen fixation :

(1) 3–6 annual growth rings can be counted in the parent roots of the larger nodules This suggests that the nodules are of a similar age, since nodule formation in both gorse and broom occurs at infection sites underlying the piliferous layers of very young roots

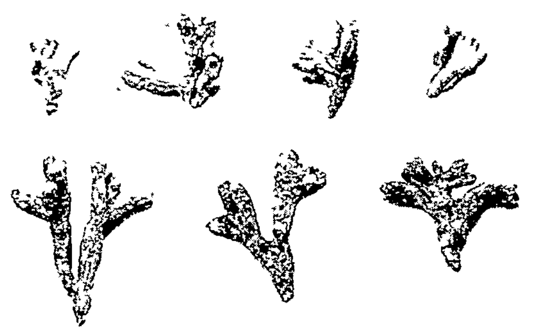

Fig 1 Perennial nodules on native legumes in Britain Upper row from broom, lower row from gorse Material obtained from a drained peat-bog, near Randalstown, Co Antrim, N Ireland (× ½)

(2) A mature nodule may show many constrictions along its length (Fig 1) These constrictions apparently demarcate seasons of growth activity

(3) Sectioning reveals several features reminiscent of those described for perennial nodules by other writers[4,6] Outer layers of the nodule cortex develop heavy suberization in all mature parts of the nodule Vascular tissue persists in healthy condition in older portions of the nodule, although all other inner tissues decay and may even disappear with age Healthy meristems terminate each nodule lobe Behind these occurs a zone of bacterial tissue pigmented with hæmoglobin Adjacent older tissues show an accumulation of green bile-type pigments The nodule cortex in these younger regions contains abundant starch, particularly in lines of cells adjacent to the vascular tissue Paper chromatography of the soluble-nitrogen fraction reveals that aspartate, glutamate, and their respective amides, asparagine and glutamine, accumulate in quantity in the nodule The latter amides and starch are the principal storage products in other parts of the over-wintering plants

It would be interesting to know whether perennial nodules are developed on native or introduced legumes in other parts of the British Isles. Nodules on gorse and broom are clearly equipped anatomically for perennation, yet this event may be achieved in Nature only after a series of mild winters, and possibly only in soil conditions where decay of older parts of the nodule is arrested.

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Cultivation of Goitrogenous and Non-Goitrogenous Cabbage

RESULTS of earlier work on the goitrogenous properties of plants[1-3] supported the assumption that goitrogenous activity of vegetable foodstuffs, particularly that of *Brassica* species, depends to a large extent on the content of sulphur compounds as goitrogenous substances Therefore, in an attempt to supply experimental evidence for the relationship between cabbage goitrogenicity and the intake and rate of utilization of sulphates from nutrient environment, cabbage (*Brassica oleracea*, var. *capitata*, subsp Amager) was cultivated in pure silica sand (SiO_2), in vegetation pots, by means of nutrient solutions in which only the sulphate content was made to vary The results of the first experiment in this series had confirmed the above assumption, namely, that goitrogenous activity of the cabbage increased in relation to the sulphate content in the nutrient solution[4,5] However, efforts to grow cabbage without goitrogenous properties had failed In the second experiment I endeavoured to grow both the goitrogenous and the non-goitrogenous variants of cabbage side by side The same species of cabbage was again grown in vegetation pots with silica sand by means of nutrient solutions The composition of the solutions for variants I and II is given in Table 1

Table 1 COMPOSITION OF NUTRIENT SOLUTIONS

Type of nutrient salt ions	Concentration of nutrient expressed in mgm equivalents/l of nutrient solution Cabbage var I	Cabbage var II
Sulphate	1	25
Nitrate	25	23
Magnesium	8	8
Calcium	15	15
Phosphate	8	8
Potassium	12 66	12 66
Ammonium	0	2
Chloride	8	0
Sodium	1	10

Iron and micro-elements added in equal amounts expressed in mgm /l of nutrient solution for both the cabbage variants

Iron	5 0 mgm	(0 025 gm of ferric citrate)
Manganese	1 0 mgm	(0 00406 gm $MnSO_4$ $4H_2O$)
Copper	0 1 mgm	(0 0003928 gm $CuSO_4$ $5H_2O$)
Zinc	0 1 mgm	(0 0004397 gm $ZnSO_4$ $7H_2O$)
Boron	0 5 mgm	(0 002858 gm H_3BO_3)
Molybdenum	0 02 mgm	(0 000037 gm $(NH_4)_6Mo_7O_{24}$ $4H_2O$)

Beginning with the second half of the growth period, all sulphates were withdrawn from the nutrient solution for variant I by leaving out 1 mgm - equivalent of sulphate ion and sodium ion. The remaining nutrients, such as iron and microelements, continued to be supplied in equal amounts to the nutrient solutions for both the cabbage variants, as seen in Table 1 At the end of the period of growth the content of sulphur compounds was determined in both the variants. Total and inorganic sulphur, expressed in terms of sulphates, was determined gravimetrically, thiocyanates according to Aldridge's method[6], and SH-compounds polarographically according to Zuman[7] The results are summarized in Table 2

It was found that the content of total, inorganic and organic sulphur is several times greater ($P \leqq 0 0005$) in cabbage variant II than in variant I Thiocyanate content of variant II was likewise significantly higher ($P \leqq 0 02$) The values for SH-compounds were significantly higher ($P \leqq 0 001$) in the green leaves of variant II than in those of variant I, while no differences were noted in the cabbage heads In general, the content of sulphur compounds, with the exception of thiocyanate and SH-compounds, was markedly higher in the green leaves than in the cabbage heads

The goitrogenous activity of the cabbage variants was assayed on rats, where the relative thyroid weights, the thyroidal iodine-131 uptake in 24 hr. and the level of protein-bound iodine-131 in serum were followed and a histological evaluation of thyroid preparations was made A strong goitrogenous activity was observed in rats fed with cabbage variant II, while variant I failed to show any activity when the

Table 2. Results of Sulphur Compound Determinations in Cabbage

Type of test (n = 10)*	Variants of cabbage			
	I		II	
	Green leaves	Cabbage heads	Green leaves	Cabbage heads
Desiccated cabbage (per cent)	11.64 ±1.48	8.36 ±0.58	10.58 ±1.76	8.46 ±1.34
Total sulphur in mgm per cent sulphate	46.6† ± 9.6	66.1 ±29.5	427.3 ±208.1	164.0 ±45.6
Inorganic sulphur in mgm per cent sulphate	17.3 ± 8.2	34.1 ±29.1	322.8 ±156.2	89.6 ± 42.5
Organic sulphur in mgm per cent sulphate	29.3 ±10.0	31.9 ±12.9	104.5 ± 66.6	74.4 ±26.7
Thiocyanate, according to Aldridge, in mgm per cent SCN′	1.6 ±0.51	2.14 ±0.46	2.62 ±1.12	2.68 ±0.81
SH-compounds expressed in mgm per cent SH-glutathion	3.82 ±2.12	8.01 ±2.74	9.12 +3.04	9.89 ±1.36

* n, No. of determinations in the experiment.
† Average ± *S.E.*

results were compared with those from a control group of rats, which had received Larsen's diet only. The nutritional value of both the cabbage variants was the same, for I failed to notice any significant differences in the sugar content, vitamin C, total nitrogen and calcium values between the two cabbage variants.

The plant material as defined here, considered from the point of view of cultivation, and the possibility of cultivating goitrogenous and non-goitrogenous variants of cabbage side by side, offer new opportunities for experimental research in this field.

I am grateful to Mr. Š. Kaločai, Mrs. J. Schillerová and Mrs. K. Gschwendtová for their technical assistance, and to Mr. P. Tkáč for translating the text into English.

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Biologically Active Materials in Coal

That materials extractable from coal with polar organic solvents inhibit the growth of certain bacteria and fungi has been known for some years[1–5]. The name 'vitricin' has been applied to the group of biologically active substances found in coal[1]. Mills recently reported work on acetone and methanol extracts of coals of various ranks[5]. He found that bacteriologically active extracts contained acidic, basic, neutral, and phenolic components; attempts to separate active components by extraction of ethereal extracts with aqueous acids, alkalis or buffers met with little success. Testing the crude extracts against *Bacillus subtilis* in nutrient broth demonstrated bacteriostatic activity of the solute at a concentration of 30 µgm./ml. Mills did, however, by steam distillation of an acetone extract of Tupton coal (high-volatile bituminous), obtain an oil fraction which inhibited Gram-positive bacteria and certain moulds.

Research workers at the Federal Bureau of Mines prepared and investigated several methanol and acetone extracts of various coals. In agreement with previous workers[3–5], the most active extracts were obtained from high-volatile sub-bituminous or bituminous coals. Peat and lignites yielded lesser amounts, and anthracite practically no acetone- or methanol-extractable material. Contrary to Mills's observations, the most active material obtained was the 5 per cent aqueous sodium hydroxide-soluble fraction from ether solutions of materials obtained by acetone extraction of coals high in volatile matter. This material, recovered from the hydroxide solutions by precipitation with acid and centrifugation, was active in nutrient broth dilution tests against *Bacillus subtilis* at concentrations of 5–10 µgm./ml.

We recently obtained a large sample of material for more detailed study by treating 200 lb. of a high-volatile sub-bituminous *B* coal (Rock Springs, Wyoming) with 150 gal. of acetone. The work was carried out at room temperature on coal ground to pass a 200-mesh screen. When the solvent was evaporated, a brownish-black, soft tarry material remained. This represented about 1 per cent of the original weight of coal. Previous observations were confirmed with this extract.

A 400-gm. sample of the extract was subjected to steam distillation and 14 ml. (11.6 gm.) of a volatile yellow oil was obtained. The oil was separated into its acidic, phenolic, basic, and neutral components and these were tested in nutrient broth against *Bacillus subtilis*. Only minor activity was observed in the case of the acidic (partial inhibition at 100 µgm./ml.) and phenolic (partial inhibition at 40 µgm./ml.) fractions.

A 50-gm. sample of the residue from the steam distillation was cooled to − 68° C., crushed, and agitated for 5 min. in a Waring blender with 10 per cent (w/v) aqueous sodium hydroxide. The resulting suspension was mixed with a small amount of 'Celite' and then filtered. The filtrate was acidified with 6 *N* hydrochloric acid and extracted with ether continuously for 16 hr. This procedure yielded 6.4 per cent of the original residue. The hydroxide-soluble material inhibited *B. subtilis* at a concentration of 8 µgm./ml. in nutrient broth.

To confirm that phenolic materials were responsible for the observed activity at 8 µgm./ml., the trimethylsilyl ethers ($(CH_3)_3SiOR$) of the phenolic hydroxyls were prepared. A small sample of the hydroxide-soluble material was refluxed with hexamethyldisilazane for 8 hr. After refluxing, residual hexamethyldisilazane was removed by warming in a vacuum. The reacted material was active against *Bacillus subtilis* at a concentration of 40 µgm./ml. The reacted material then was refluxed for 6 hr. in ethyl alcohol (70 per cent v/v) to hydrolyse the trimethylsilyl ethers, and then heated (54° C.) *in vacuo* to remove the alcohol, water, and hexamethyldisiloxane formed during the reaction. The reconstituted material was active against *B. subtilis* at 8 µgm./ml. of nutrient broth and confirmed complete recovery of the originally observed activity.

The original acetone extract and samples obtained at the various concentration stages were analysed by mass spectrometry and were also examined in the ultra-violet. Mass spectrometer analysis of the total acetone extract showed peaks at mass units (in order of decreasing intensity) at 342, 324, 374, 376, 340, 350, and 392. These masses indicate a range of compounds in the six-ring series. Since ultra-violet spectra indicated a high percentage of three-ring systems, the six-ring compounds detected by mass spectrometry probably consist of diphenanthryl, or similar systems Control runs with acetone alone (no coal) demonstrated that polymerized solvent was not responsible for the mass peaks observed

The acetone extracts differed mainly from the parent coal in elemental composition in having a higher hydrogen content Analyses showed (per cent)

Coal C, 79 31, H, 5 41, N, 1 74, S, 0 90, O, 12 65, ash, 1 96

Extract C, 78 87, H, 9 92, N, 0 23, S, 0 21, O, 10 92, ash, 0 02

Acetone might be expected to extract more polar compounds from the coal matrix, enriching the oxygen content of the extract, but apparently this did not occur The oxygen content of the extract was 10 92 per cent, that of the whole coal 12 65 per cent Perhaps materials soluble in acetone serve as solvents for other materials in the coal which are not acetone-soluble On long contact with the coal, the extract then serves as a continuously changing solvent, contrasted to a soxhlet-type extraction, with resultant extraction from the coal of materials of high mass and low oxygen content

Calculation of the amounts of hydroxide-soluble materials present in the original coal indicate a concentration of 150 μgm /gm of coal The material inhibits the test culture at 8 μgm /ml of broth It is possible that such materials play a part in the resistance of coal to biological oxidation, and the limitation of the coal flora[6] to non-susceptible forms

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MICROBIOLOGY

Relationship between Phage Type and Host Plant in *Pseudomonas mors-prunorum* Wormald

Pseudomonas mors-prunorum causes bacterial canker of cultivated *Prunus* spp. in England and is widely distributed on the plum (*P domestica*) and on the cherry (*P avium*) Strains from plum and cherry show some degree of pathogenic adaptation to their original host[1], but have not been distinguished by routine physiological or biochemical tests We now find that we can differentiate between them by phage typing

The bacterial strains used in these experiments were collected over a period of 7 years from plum and cherry plantations within a restricted geographical area of about a half-mile square at East Malling Research Station in Kent Bacteria were isolated from leaf surfaces, rain-water draining down the trees, and lesions on branches or foliage. The typing phages, with the original propagating host species in parenthesis, were as follows *A*1, *A*2, *A*3, *A*7, *A*32 (*P mors-prunorum*) and *S*3 and *S*8 (*P. syringae*) These phages were selected from a group of 57 isolated from soils by a method previously described[2] The phages were purified by repeated selection from single plaques, and stored as high-titre preparations of approximately 10^{10}–10^{11} particles/ml over chloroform at 4° C In these conditions most of the preparations have remained stable for two years

For phage-typing, 10^7 viable cells of the bacterial isolate under test were poured into plates with 12 ml of a medium having the following composition· 0 3 per cent Difco yeast extract, 0 5 per cent Oxoid bacteriological peptone, 2 0 per cent glycerol, 1 0 per cent B.D H Japanese agar When set, the plates were dried with the lids removed for 2 hr at 30° C and then spotted with 0 02 ml of the phage preparation at the routine test dilution, that is, the lowest dilution giving confluent lysis on the original propagating strain Finally, the plates were dried for about another hour and incubated overnight at 25° C The results of tests on plum and cherry strains are summarized in Table 1 and compared with similar tests on *Pseudomonas syringae* isolates obtained from diseased pear blossoms in widely separated localities of south-east England during 1959–60

Table 1 PHAGE-TYPING OF PLUM AND CHERRY STRAINS OF *Pseudomonas mors-prunorum* AND *Pseudomonas syringae* FROM PEAR

No. of bacterial isolates of each phage-type		Typing phages						
		*A*1	*A*2	*A*3	*A*7	*A*32	*S*3	*S*8
(*A*) *P mors-prunorum*								
(1) from cherry								
	14	+	+	+	+	+	–	–
	15	+	+	+	+	(+)	–	–
	2	(+)	+	(+)	+	+	–	–
Total 35	1	+	(+)	+	+	+	–	–
	1	+	(+)	+	(+)	(+)	–	–
	1	+	(+)	(+)	+	(+)	–	–
	1	+	+	+	+	(+)	–	+
(2) from plum								
	6	+	–	*s*	–	–	–	–
	1	+	–	*s*	–	–	–	+
	1	(+)	–	*s*	–	–	–	–
Total 12	1	+	–	*s*	–	–	+	–
	1	+	–	*s*	–	+	–	–
	1	+	–	*s*	–	–	+	+
	1	(+)	–	–	–	–	–	–
(*B*) *P syringae*								
	9	–	–	*s*	–	–	+	+
	1	–	–	*s*	–	–	+	(+)
	1	–	–	*s*	–	–	(+)	(+)
Total 22	2	–	–	–	–	–	+	+
	2	–	–	–	–	–	–	–
	2	+	–	*s*	–	–	+	+
	3	(+)	–	*s*	–	–	+	+
		s	–	*s*	–	–	+	+

+, Confluent lysis, (+), innumerable plaques, *s*, isolated plaques, –, no reaction

Within each species or strain group there were different phage types The plum strains of *P mors-prunorum* differed from the cherry strains in failing to react with phages *A*2 and *A*7, and in giving no reaction or isolated plaques with *A*3 They were also less sensitive to *A*32 They resembled *P syringae* in typing pattern, except that, as a group, they were more sensitive to *A*1 and less sensitive to the *P syringae* phages *S*3 and *S*8 One plum isolate gave a typing pattern identical with one of the *P syringae* isolates

Phage sensitivity does not appear to be correlated with biochemical activities All the *P mors-prunorum* isolates tested, for example, were physiologically homogeneous and quite distinct from *P. syringae* in respect of the characters shown in Table 2

Over the period of 7 years during which the *P. mors-prunorum* strains have been collected there have been no signs of any major change in the phage types associated with cherry or plum in the field Some strains have been in culture for several years

Table 2 BIOCHEMICAL DIFFERENTIATION OF *P mors-prunorum* AND PEAR ISOLATES OF *P syringae*

Species	Æsculin and arbutin hydrolysis	Gelatin liquefaction	Casein hydrolysis	Tyrosinase activity	Growth in nutrient broth + 5 per cent sucrose
P -mors-prunorum					
(*a*) Cherry strains	–	–	–	+	white
(*b*) Plum strains	–	–	–	+	white
P syringae	+ *	+	+	–	yellow

* One isolate failed to hydrolyse æsculin and arbutin

without passage through the host and appear, therefore, to be relatively stable *in vitro* The difference between the plum and cherry strains may merely reflect a different history of phage-infection in the field The cherry strains, for example, may have failed to acquire resistance to *A*2 and *A*7 or related phages because these are absent on cherry or their activity is in some way inhibited Alternatively, the phage types may be a result of selection by the host plants This would imply some form of association between phage sensitivity and specific virulence factors in *P mors-prunorum*

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Serotonin in the Small Intestine of Conventional and Germ-free Chicks

THE function of serotonin (5-hydroxytryptamine) in the tissues of the small intestine is not understood even though it is found in relatively large quantities, particularly in the enterochromaffin cells[1] The most frequently repeated interpretation ascribes control of intestinal motility and vascular permeability to serotonin[2,3] Thus, if the tonus of the gut is dependent on serotonin-levels, germ-free animals, which possess poor tonus of the tract and distended cæca[4], might be expected to have a lower serotonin content in the gut than conventional animals On the contrary, however, this communication provides evidence that intestinal serotonin-levels are actually depressed by the presence of micro-organisms in the lumen of the gut

White Leghorn chicks were raised in a germ-free environment by methods similar to those previously described[5] on a complete starch-casein diet[6] Fertile eggs were obtained from local hatcheries after 19 days of incubation, immersed in 2 per cent mercuric chloride solution for 10 min at 39° C and put into sterile 'Plexiglas' isolators where they hatched Control chicks were treated in the same manner but were reared in a non-sterile isolator After four weeks the chicks were decapitated, the small intestine was excised and washed in cold water All germ-free animals were examined[7] for bacterial contamination when killed The various segments of the intestine were homogenized in 0 1 *N* hydrochloric acid in a Ten Broek apparatus at 5° C, and serotonin was assayed[8] on fresh specimens in a Turner photofluorometer (G K. Turner and Associates, Palo Alto, California)

Table 1 SEROTONIN IN THE SMALL INTESTINE OF GERM-FREE AN CONVENTIONAL (CONTAMINATED) WHITE LEGHORN CHICKS AT FOU WEEKS OF AGE

Treatment	No of chicks	Serotonin (μgm /gm fresh tissu Duodenum		Ileum	
Germ-free	20	9 0 ± 1 0*		11 0 ± 0 7	
Conventional	20	8 3 ± 1 3		6 1 ± 0 8	
Significance of differences by the '*t*' test					
		t	*P*	*t*	*P*
Germ-free vs conventional		0 84	< 0 4	8 8	< 0 01

* Mean ± standard deviation

A significantly higher concentration of serotoni was found in the ileum of germ-free chicks than in control birds (Table 1) This is consistent with the observation[9] that rats and mice given peroral anti biotics produce significant increases of serotonin in the small intestine However, no such difference was apparent in the duodenum (Table 1) There is insufficient evidence to determine whether these results reflect differences between the microflora of the duodenum and ileum or an influence of the intense microbial activity of the cæca[10,11] due to the proximity of the ileum to the latter In any event, the presence of intestinal micro-organisms clearly affects serotonin in the ileum of chicks

Several reports suggest alternative explanations for the microbial inhibition of serotonin A few isolates of *Aerobacter aerogenes* and *Alcaligenes faecalis* appear to be capable of oxidizing serotonin *in vitro*[12] although it is not known if this event occurs in the lumen of the intestine In addition, many species of lactobacilli which are present in large numbers in the chick[13] require pyridoxine or its derivatives[14], pyridoxal phosphate is required as a co-factor in the decarboxylation of 5-hydroxytryptophan to serotonin[15] Thus bacterial removal of the coenzyme may inhibit serotonin synthesis A similar effect might be achieved by the interaction of a bacterial product with the coenzyme such as has been reported for norepinephrine and 3,4-dihydroxyphenylalanine[16]

Further work is required to determine the reason for the difference in intestinal serotonin between germ-free and conventional chicks and the physiological significance of this observation

This work was aided by research grants *E*–2999 and *A*–2660 from the U S Public Health Service

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VIROLOGY

A New Mutant of Dermovaccinia Virus

During the course of serial one-day egg passages of a strain of the chorioallantoic membrane-adapted dermovaccinia virus (*DIE* strain)[1], a new minute pock mutant was observed. This arose at the 13th one-day egg passage, when tiny pocks mixed with larger pocks typical of dermovaccinia were seen. The former increased in proportion during further one-day egg passages, and finally excluded the original virus. The mutant strain (*DIs*) was obtained in a pure form by two cycles of clone purification. These were initiated by a single pock produced on one chorioallantoic membrane by the inoculation of limiting dilutions of the fifteenth one-day egg passage. Some biological characteristics of this mutant were investigated and are described in this communication.

(1) *Serological specificity.* The strain *DIs* was neutralized specifically by antivaccinia rabbit serum, but no neutralization was observed by normal rabbit serum or by antiherpes simplex rabbit serum. When inoculated on to the chorioallantoic membrane of 12-day eggs, it produced vaccinia–common complement-fixing antigen and hæmagglutinin. These were separable from infectious particles by differential centrifugation. As the size of pock of the mutant resembles that produced by a strain of pigeon pox, comparative chessboard complement-fixation tests were carried out. The results summarized in Table 1 indicated no cross-reaction between the strain *DIs* and anti-pigeon pox–pigeon serum, though a slight cross was observed between the pigeon pox chorioallantoic membrane antigen and antivaccinia rabbit serum. This could be eliminated by ether treatment of the antigen.

(2) *Host range.* The strain *DIs* grows well in one-day eggs, on the chorioallantoic membrane of 12-day eggs, in the yolk sac of 7-day eggs and in the amniotic sac of 11-day eggs. No significant growth was observed, however, when it was inoculated into the allantoic cavity of 11-day eggs. When a large amount of the virus was inoculated intradermally into adult albino rabbits, it produced a slight reddening and flat swelling on the first few days, which then waned. The reaction was nearly the same as that observed with variola virus inoculated via the same route. No lesion could be seen following inoculation on to the scarified skin of rabbits. The mutant produced no swelling when inoculated into the rabbit testicle, no keratitis when inoculated on to the scarified cornea of rabbits. New-born, weanling and adult mice showed no clinical signs, when inoculated intracerebrally, intraperitoneally or subcutaneously. Infection of the scarified plantar skin of weanling and adult mice from an ectromelia-free colony failed to give rise to any local symptoms. No clinical symptoms were observed in newly hatched chicks inoculated via the intracerebral, intraperitoneal or subcutaneous routes. Roosters and guinea pigs produced specific antibody without any sign of clinical symptoms after several weekly injections administered via the subcutaneous or the intravenous route.

(3) *Physical properties.* The mutant showed the same resistance to ether as did the parent strain. The infectivity of the mutant was as stable as that of the original strain at low temperatures (−70° C and 4° C). Electron microscopy of the elementary bodies of the strain *DIs*, partially purified by fluorocarbon treatment, indicated that the shape and size of the elementary bodies were approximately the same as

Table 1. Complement Fixation Test of the Strain *DIs* and the Strain Nakano (Pigeon Pox) against Anti-Pigeon Pox Pigeon Serum

Antigen		Anti-pigeon pox pigeon serum							No serum
Code	Dilution	4	8	16	32	64	128	256	—
Nakano	6.3	4	4	4	4	4	tr	0	0
	12.5	4	4	4	4	tr	0	0	0
	25	4	4	4	tr	0	0	0	0
	50	4	4	tr	0	0	0	0	0
	100	1	tr	0	0	0	0	0	0
	200	0	0	0	0	0	0	0	0
DIs	6.3	0	0	0	0	0	0	0	0
	12.5	0	0	0	0	0	0	0	0
	25	0	0	0	0	0	0	0	0
No antigen		0	0	0	0	0			

Table 2. Complement Fixation Test of the Strain *DIs* and the Strain Nakano (Pigeon Pox) against Anti-Vaccinia Rabbit Serum

Antigen		Anti-vaccinia rabbit serum							Normal rabbit serum	No serum
Code	Dilution	8	16	32	64	128	256	512	8	—
DIs crude	20	4	4	4	4	4	2	0	2	0
	40	4	4	4	4	3	tr	0	0	0
	80	4	4	4	4	tr	0	0	0	0
	160	4	4	3	tr	0	0	0	0	0
	320	4	1	0	0	0	0	0	0	0
	640	tr	0	0	0	0	0	0	0	0
	1,280	0	0	0	0	0	0	0	0	0
DIs ether-treated	10	4	4	4	4	3	0	0	0	0
	20	4	4	4	1	1	0	0	0	0
	40	4	4	2	0	0	0	0	0	0
	80	4	2	tr	0	0	0	0	0	0
	160	2	0	0	0	0	0	0	0	0
	320	0	0	0	0	0	0	0	0	0
Nakano crude	10	4	4	3	tr	0			4	0
	20	4	4	3	tr	0			3	0
	40	4	3	0	0	0			tr	0
	80	2	tr	0	0	0			tr	0
	160	tr	0	0	0	0			tr	0
	320	0	0	0	0	0			0	0
Nakano ether-treated	2.5	2	tr	0	0	0			tr	0
	5	tr	0	0	0	0			0	0
	10	tr	0	0	0	0			0	0
	20	0	0	0	0	0			0	0
No antigen		0	0	0	0	0			0	0

those of the parent stram The mutant was as labile to heat as the parent strain, showing a marked loss of infectivity after being heated at 55° C for 30 min

(4) *Cytopathogenicity in tissue culture·* A moderate cytopathic effect was observed in primary monkey kidney monolayers culture infected with the mutant No cytopathic effect was observed when HeLa cells and *FL* cells were exposed to the mutant, while typical cytopathic effects accompanied by luxurious growth of virus were shown by the parent strain A cytotoxic effect was observable with *L* cells, when infected with a large amount of either the mutant or parent strains

(5) *Production of hæmagglutinin·* On the chorio-allantoic membrane of embryonated hen's eggs, the strain *DIs* multiplies with concomitant production of hæmagglutinin and complement-fixing antigens However, the ratio of pock-forming units to hæmagglutinin units appeared to be slightly lower (4 6 in $\log_{10}$ units) than that observed in the case of the parent strain (5 05) (ref 2) In HeLa cells in which no visible cytopathic effect could be obtained, a considerable amount of hæmagglutinin was nevertheless produced This was characterized by a low ratio of pock-forming units to hæmagglutinin units (3 0 at the twenty-fourth hour of infection, as compared with 4 46 with the parent strain[3])

These results indicate that the strain *DIs*, a minute pock-size mutant of dermovaccinia, is characterized by a reduced, or absent, virulence.

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GENETICS

Segregation-ratio Advantage of Abnormal Hæmoglobins

As a consequence of extensive investigations on abnormal hæmoglobins in Jamaica[1-4], in many instances where this was feasible family studies were undertaken The impression was gained (as suggested in ref 3) that some unusual inheritance patterns energed and for this reason a statistical analysis of the available pedigrees was undertaken Only two of the most significant deviations from the expecte Mendelian ratios will be presented here

Since only limited material was available fron investigations in Jamaica, a search through th literature was undertaken In four publications relevant family studies were reported which hav been included in the analysis It should be pointe out that any deviation from expected ratios fron the Jamaican material was always present too in th same direction in these family studies In the com bined material approximately half the individual are derived from my own investigations, the othe half from ref 5

The results which will be discussed here come from families where one parent has the genotype *AS* and the other the genotype *AC*, these results are presented in Table 1

The most unusual finding is the very uneven distribution of *A* and *C* genes in the offspring of families where the mother has the genotype *AC*—twenty-four *C* against seven *A* with a probability of 0 002 When the father has the genotype *AC* there is no significant difference in the number of the *A* and *C* genes in his children, with, however, a very strong preference for the daughters (as compared with the sons) to carry a *C* gene ($P = 0\ 01$ for sex distribution) A further observation in accord with this is that of the nine children whom I examined from mothers with the genotype *SC*, eight inherited the *C* gene and only one the *S* gene ($P = 0\ 05$)

The finding that deviations from Mendelian expectation do occur is not new Apart from plants and *Drosophila* this is known to happen in *Sciara*, a mosquito[6], in the mouse in relation with the *T* locus (tail formation)[7], and has recently been described in two instances in man Stevenson and Jennings[8] explain the preponderance of affected sons of affected fathers in the dominant condition of ectrodactyly by germinal selection Shaw *et al* [9] describe the marked deficiency of the autosomal dominant condition of aniridia in the offspring of aniridic parents (100 affected for 164 normal children)

The findings reported here might therefore represent the third instance of non-Mendelian segregation ratios found in man and could be explained on the basis of gametic selection or differential gametic survival

It is suggested that the maternal or paternal gamete containing an *X* chromosome and the chromosome carrying the hæmoglobin *C* gene has a considerably better chance to occur or to result in a viable fœtus than the gamete containing the *X* chromosome and the hæmoglobin *A* gene The gamete carrying the *Y* chromosome would not have such a selective preference

Table 1 DISTRIBUTION OF GENOTYPES IN THE OFFSPRING OF MARRIAGES WHERE ONE PARENT HAS THE GENOTYPE *AC* AND THE OTHER THE GENOTYPE *AS*

<table>
<tr><th colspan="5">Father *AC* (10 families)</th><th colspan="3"></th><th colspan="5">Mother *AC* (8 families)</th></tr>
<tr><th>Genotype</th><th colspan="2">Sons</th><th colspan="2">Daughters</th><th colspan="3">Total *A* or *C* genes with exclusion of index case</th><th colspan="2">Daughters</th><th colspan="2">Sons</th><th>Genotype</th></tr>
<tr><td>*AA*</td><td>10</td><td colspan="2">*A* genes from father</td><td>1</td><td rowspan="2">15</td><td rowspan="2">*A*</td><td rowspan="2">7</td><td>1</td><td colspan="2">*A* genes from mother</td><td>1</td><td>*AA*</td></tr>
<tr><td>*AS*</td><td>3</td><td>13</td><td>2</td><td>1</td><td>4</td><td>5</td><td>2</td><td>1</td><td>*AS*</td></tr>
<tr><td>*AC*</td><td>6</td><td colspan="2">*C* genes from father</td><td>4</td><td rowspan="2">18</td><td rowspan="2">*C*</td><td rowspan="2">24</td><td>10</td><td colspan="2">*C* genes from mother</td><td>3</td><td>*AC*</td></tr>
<tr><td>*SC*</td><td>5</td><td>11</td><td>17</td><td>13</td><td>10</td><td>20</td><td>11</td><td>8</td><td>*SC*</td></tr>
</table>

Another possible explanation could be post-zygotic election, although nothing is known concerning xcessive abortions in the families reported here

If one accepts for the time being these findings as pproximately correct and representative, arguments an be presented to explain the high frequencies of he C gene in a small area of Africa For this purpose will postulate that the S gene has a neutral value (in view of the other family investigations which are not eported here, this is probably not true) and that herefore the effect of the C gene would be identical in AC–AS and AC–AA matings Starting with 0 001 per cent AC in a population, one can calculate, using the distribution of A and C genes presented in Table 1, that approximately thirty-two generations (about 800 yr) will be required to bring the frequency to 10 per cent This is a very short period of time and agrees well, therefore, with Neel's suggestion that the C gene must be the effect of a recent mutation, since it has not yet had time to spread beyond the Gold Coast

One might also expect that the number of AC heterozygotes is not evenly distributed among the sexes, the male to female ratio should be approximately 2 3 Only little proof for such a statement is available. For work on incidence of abnormal hæmoglobins in Jamaica[1], results from pregnant women were used, possibly leading to an unusually high figure Schneider[10] finds among 505 Negro blood-donors fifteen males out of sixteen with the genotype AC, but her blood-donors are predominantly male Smith and Conley[11] comment on the fact that of the fourteen cases of AC which they discovered, thirteen were females Garlick and Barnicot[12] find thirteen girls against six boys with the genotype AC in a group of 303 Nigerian schoolchildren with otherwise equal sex distribution

The calculations which I have used here are based on a very small body of material, and although the deviations from Mendelian ratios appear to be statistically significant, more extensive material will be required to confirm the views presented It seems possible, however, that besides balanced polymorphism (as in the case of hæmoglobin S (ref 13)) and a greater potential fertility of unions in which one parent carries a single abnormal hæmoglobin gene[14], still other forces may exist to explain the unusually high frequencies of abnormal hæmoglobins in certain populations

L N Went

Institute of Neurological Sciences,
University of Leyden

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SOIL SCIENCE

Method of measuring Soil Hydraulic Conductivity *in situ*

Soil permeability or hydraulic conductivity is of great importance in the engineering design of foundations, dams, sub-surface drainage systems, irrigation systems, etc In spite of its importance, soil hydraulic conductivity remains one of the most difficult of soil properties to assess Laboratory methods have limitations due to the size of the samples Considerable progress has been made in the development of methods for measuring soil hydraulic conductivity in the field when a water table is present However, water tables are not always present in areas requiring seasonal drainage

In order to overcome some of these difficulties, a new field method of measuring the soil hydraulic conductivity is proposed In short, the method consists of measuring the rate of flow of water out of a cavity beneath a pipe The method bears some resemblance to the piezometer method described by Luthin and Kirkham[1] for the measurement of soil hydraulic conductivity beneath the water table

When water infiltrates into soil from a cavity as shown in Fig 1, for some time after the process of infiltration has started the wetting front forms a sphere around the cavity During this initial phase, the driving force can be considered due to the pressure gradient that exists between the cavity and a locus of points behind the wetting front The force of gravity has negligible effect on the pattern of movement during this initial pressure phase. The duration of the pressure phase depends on the pressure in the cavity and the pressure behind the wetting front as well as the soil properties Measurements during this initial phase should offer a method of determining the hydraulic conductivity of various soil layers or soil horizons The following analysis is based on flow due to pressure forces alone

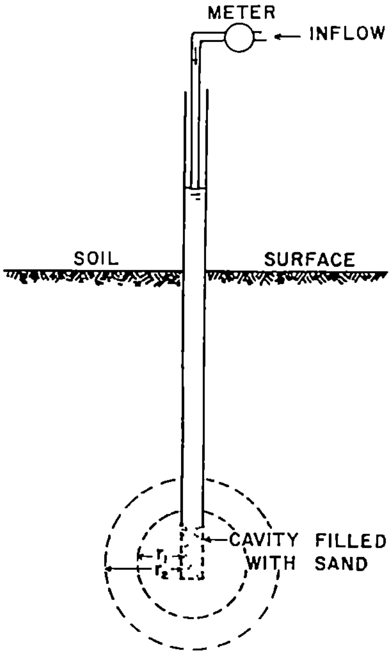

Fig. 1. Diagram of proposed method of measuring the soil hydraulic conductivity *in situ*

The assumptions which are made with regard to the flow régime are (1), The wetting front is sharply defined and the water pressure is known at some point behind the wetting front, and (2) the soil moisture content behind the wetting front does not change appreciably with time and is close to saturation

If Q is the volume flow per unit time out of the cavity, then as the wetting front moves radially outward·

$$Q = S4\pi r^2 \mathrm{d}r/\mathrm{d}t \qquad (1)$$

where S is the change in moisture content expressed as the volume of water change per unit volume of soil The $4\pi r^2$ term represents the circumferential area of the wetting front and this is multiplied by $\mathrm{d}r$ to give the volume of soil An alternative expression for the flow can be obtained by a consideration of the pressure distribution around a spherical source. The equation for the flow per unit time is given by:

$$Q = [k4\pi(p_w - p_c)]/(1/r_w - 1/r_c) \qquad (2)$$

Setting these two equations equal and solving for the hydraulic conductivity k, we have, after introducing the boundary conditions

$$k = [1/(t_2 - t_1)][(1/3)S(r_2{}^3 - r_1{}^3)/r_w(p_w - p_c)] - 1/2[S(r_2{}^2 - r_1{}^2)/(p_w - p_c)] \qquad (3)$$

where p_c is the pressure head behind the wetting front, p_w is the average pressure head in the cavity, r_w is the equivalent radius of the cavity and $t_2 - t_1$ is the time required for the wetting front to travel from r_1 to r_2

The above equation is used to calculate the hydraulic conductivity following the procedure outlined below Two measurements are needed the rate of flow from the cavity and the time for the wetting front to move from r_1 to r_2 as shown in Fig 1 The change in moisture content, S, is calculated by $S(r_2 - r_1) = Q(t_2 - t_1)/(4\pi r^2)$, where r is taken as the average of r_1 and r_2 The progress of the wetting front from r_1 to r_2 is measured electrically.

A laboratory experiment was performed to test the validity of the assumptions used in the development of the theory The experiment was performed in a sector tank filled with a Columbia fine sandy loam soil Pressure measurements were made with tensiometers located at various points in the tank The results of the tank measurement indicate that the assumptions are reasonable and the values of hydraulic conductivity obtained in the tank indicate that the method can be used to get an approximate value of hydraulic conductivity in the field

The method is at present being developed in the field and as additional information is obtained a further report will be made

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[1] Luthin, J N, and Kirkham, D, *Soil Science*, **68**, 349 (1949)

Nature of Sulphate Retention by Acid Soils

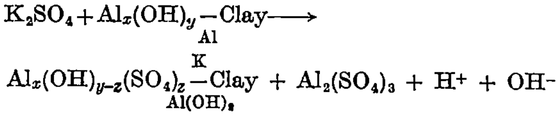
Certain soils containing oxides which occur in tropical and subtropical regions are known to retain an appreciable amount of sulphate when equilibrated with dilute solutions of sulphate salts[1,4] In our earlier work we found that the cation and anion are held in about equivalent amounts, with an increase in retention occurring as the pH was lowered or the sal concentration was increased So far as we are aware no satisfactory explanation for all the phenomena observed has yet been given

Recently we have found that the amount of sulphate which is held by an acid red soil increases with time of equilibration up to eight weeks (the longest period studied) Sulphate was added as K_2SO_4 and the amount retained was measured in two ways In the first, the K_2SO_4 was labelled with sulphur-35 and its disappearance from solution was recorded In the second, the cations in solution were measured and their sum assumed to be equal to the sulphate in solution The first method has the disadvantage that isotopic exchange with native soil sulphate occurs and makes the results higher than the true values The second suffers from lack of precision Taken together, however, they show about the same trend in the retention of sulphate with time As can be seen from Table 1, the greatest increase in sulphate held occurred during the first 24 hr

Table 1 EFFECT OF TIME ON THE RETENTION OF SULPHATE FROM 0 01 N K_2SO_4 BY CECIL SOIL

Equilibration time	Sulphate retained (m equiv /100 gm) By sulphur-35	By cation sum
1 hr	1 50	0 42
24 hr	2 20	1 74
1 wk	2 40	2 04
2 wk	3 24	1 92
4 wk	3 60	2 02
8 wk	—	2 38

It has been found[3] that the hydrolysis of exchangeable aluminium and the formation of hydroxy-aluminium compounds proceeds at a greater rate in soil systems to which a salt has been added In acid red soils the sulphate can exchange with some of the hydroxyl ions on the hydroxy-aluminium (or iron) polymers The overall reaction is described as follows



$$K_2SO_4 + Al_x(OH)_y\underset{Al}{-}Clay \longrightarrow$$

$$Al_x(OH)_{y-z}(SO_4)_z\overset{K}{\underset{Al(OH)_2}{-}}Clay + Al_2(SO_4)_3 + H^+ + OH^-$$

The replacement of hydroxyl groups from the hydroxy-aluminium groups results in the neutralization of hydrogen ions from the hydrolysis of exchangeable aluminium Similar reactions also occur with salts of chloride and nitrate

A reaction of this general form explains the increase in sulphate retention with increasing concentration and decreasing pH since under both these conditions hydroxyl ions would be replaced more easily The increase in retention of sulphate with time is explained by the hydrolysis of exchangeable aluminium to a dihydroxy-aluminium species[2] and the subsequent neutralization of the hydrogen ions thus formed by replaced hydroxyl ions This reaction provides sites on which both potassium and sulphate ions can be held

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk * are open to the public*)

Monday, October 30

University of London (at the Postgraduate Medical School of ndon, Ducane Road, London, W 12), at 4 p m —Dr R F Mahler lycogen Storage Diseases" *

University of London (in the Physiology Theatre, University llege, Gower Street, London, W C 1), at 5 p m —Prof J N David-n "The Biosynthesis of Polynucleotides" * (Last of two lectures)

University of London (at King's College, Strand, London, W C 2), 5 30 p m —Dr F A E Pirani and Dr G F Elliott "Ballistic issiles and Nuclear Weapons—Missile Guidance and Control" * hird of seven lectures in War Studies Further lectures on November 13, 20, 27)

Institution of Mechanical Engineers, Thermodynamics and luid Mechanics Group (at 1 Birdcage Walk, Westminster, London, W 1), at 6 p m —Discussion Meeting on "The Extent to Which ata can be Made Available for Engineering Design"

Society for Visiting Scientists (at 5 Old Burlington Street, ondon, W 1), at 7 30 p m —Discussion Meeting on "Information heory" Chairman Dr A M Uttley Speakers Prof E C Cherry, rof D M Mackay and Dr H Kalmus

Monday, October 30—Friday, November 3

Institute of Welding, Royal Institution of Naval Architects, Institution of Engineers and Shipbuilders in Scotland, orth-East Coast Institution of Engineers and Shipbuilders, nd the Institute of Marine Engineers (at the Institute of Marine ngineers, 76 Mark Lane, London, E C 3)—Symposium on "Welding n Shipbuilding"

Tuesday, October 31

Institute of Petroleum (at the Royal Institution, 21 Albemarle Street, London, W 1), at 4 p m —Sir Stephen Gibson, C B E "The Birth of a Science" (Sixth Cadman Memorial Lecture)

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Prof R G Harrison "Functional Aspects of the Vascularization of Tissues" * (Fifth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

Institution of Mechanical Engineers, Steam Plant Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "The Varieties of Feed Pump Drives"

Tuesday, October 31—Wednesday, November 1

Plastics Institute (at the Institution of Electrical Engineers, Savoy Place, London, W C 2)—Conference on "Engineering with Plastics"

Tuesday, October 31—Friday, November 3

Second Effluent and Water Treatment Exhibition and Convention (at the Seymour Hall, Seymour Place, London, W 1)

Wednesday, November 1

Geological Society of London (at Burlington House, Piccadilly, London W 1), at 5 p m —Dr D Flinn "On Folding during 3-Dimensional Progressive Deformation", Dr P E Brown "The Tectonic and Metamorphic History of the Pre-Cambrian Rocks of the Mbeya Region, South-west Tanganyika"

Institute of Petroleum (at 61 New Cavendish Street, London, W 1), at 5 30 p m —Mr D Scott and Mr D Jamieson "Molybdenum Disulphide as a Lubricant Additive in Rolling Contact—a Study of Compatibility with other Additives"

Royal Meteorological Society (at 49 Cromwell Road, London, S W 7), at 5 30 p m —Dr F H Ludlam "Giant Hail"

British Institution of Radio Engineers (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Prof M R Gavin "The Teaching of the Theory of Transistors and other Semiconductor Devices"

Institution of Electrical Engineers, and the Royal Aeronautical Society, London Joint Group (at Savoy Place, London, W C 2), at 6 p m —Mr H J H Sketch "Generation of Power in Satellites"

Society of Chemical Industry, Industrial Water and Effluents Group (at the Royal Institution, Albemarle Street, London, W 1), at 6 45 p m —Dr B A Southgate "The Industrial Water and Effluents Group" (Inaugural Meeting)

Society for Analytical Chemistry (at the Chemical Society, Burlington House, Piccadilly, London, W 1), at 7 p m —Scientific Papers

Thursday, November 2

Royal Society (at Burlington House, Piccadilly, London, W 1), at 10 30 a m —Discussion on "Mechanisms of Virus Infections" opened by Prof Wilson Smith, F R S

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1 15 p m —Dr J L Watling "Probability" *

Chemical Society (at Queen Mary College (University of London), Mile End Road, London, E 1), at 2 p m —Symposium on "Electron-spin Resonance".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Prof J J Pritchard "Bone Healing" * (Sixth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

University College, London, and the Feldberg Foundation for German-English Scientific Exchange for Experimental Medical Research (in the Physiology Theatre, University College, Gower Street, London, W C 1), at 5 30 p m —Prof Herbert Hensel (University of Marburg) "The Electrophysiology of Thermoreceptors" * (Feldberg Foundation Lecture)

Institution of Electrical Engineers (at Savoy Place, London, W C 2), at 6 p m —Mr T Somerville "Acoustics and the Electrical Engineer"

Institute of Refrigeration (joint meeting with the Refrigeration Servicemen's Association, at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Borough Polytechnic, Borough Road, London, S E 1), at 7 p m —Mr J E Kumler "Modern Designs in Thermostatic Control for Refrigeration, including Air-Conditioning, Automatic Defrosting and Heat Pump Applications"

Royal Institute of Chemistry (joint meeting with the Brunel College of Technology Chemical Society, at the Brunel College of Technology, Woodlands Road, Acton, London, W 3), at 7 p m —Dr J Lewis "Modern Developments in Co-ordination Chemistry".

Friday, November 3

Mineralogical Society, Clay Minerals Group (at the Geological Society, Burlington House, Piccadilly, London, W 1), at 11 a m. —Autumn meeting

Institute of Physics and Physical Society, Colour Group (joint meeting with the London Section of the Society of Dyers and Colourists, at Burlington House, Piccadilly, London, W 1), at 6 p m —Mr K MacLaren "A Daylight Illuminant"

Institution of Electrical Engineers, Medical Electronics Discussion Group (at Savoy Place, London, W C 2), at 6 p m —Discussion on "Information Theory in Relation to Biology" opened by Prof D M Mackay

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S W 1), at 6 30 p m —Prof B Lythgoe "The Wittig Reaction and its Application in Organic Synthesis"

Royal Institution (at 21 Albemarle Street, London W 1), at 9 p m —Dr S Brenner "The Handing on of Genetic Information in Living Matter"

Saturday, November 4

Association of Clinical Biochemists, Southern Region (at Queen Mary's Hospital, Roehampton, London, S W 15), at 2 30 p m. —Symposium on "Special Applications of Electrophoresis"

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 3 30 p m —Mr Frank W Lane "Kingdom of the Octopus" *

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Assistant in Biochemistry in St Salvator's College—The Secretary, The University, College Gate, St Andrews (November 4)

Lecturer, Grade I (preferably with experience in the biochemistry of proteins or nucleic acids) in Biochemistry—The Registrar, The University, Leicester (November 4)

Lecturer (with a good degree in zoology, and preferably research experience) in Zoology—The Bursar, College of Advanced Technology, Gosta Green, Birmingham 4 (November 4)

Lecturers (honours graduates in engineering or in science), to teach the subject of applied thermodynamics to students of engineering—The Secretary of University Court, The University, Glasgow (November 7)

Assistant Lecturer or Lecturer (preferably with a special interest in Africa) in Geography—The Secretary, University College, Gower Street, London, W C 1 (November 10)

Assistant in Genetics—The Secretary of University Court, The University, Glasgow (November 10)

Lecturer (veterinary graduate with a special interest in the field of reproduction) in the Department of Animal Husbandry at the Veterinary Field Station, Langford, near Bristol—The Registrar and Secretary, The University, Bristol (November 11)

Superintendent (with administrative experience, and preferably some scientific experience) of the University Biochemical Laboratory—Prof F G Young, Department of Biochemistry, The University, Tennis Court Road, Cambridge (November 16)

Lecturer in the Electronic Computation Laboratory of the Applied Mathematics Department—The Registrar, The University, Sheffield (November 18)

Lecturer or Assistant Lecturer (with a good honours degree or equivalent in engineering, physics or mathematics and some research experience) in Vibration Theory—The Secretary and Registrar, The University, Southampton (November 18)

Botanist (with a degree, preferably majoring in botany, non-graduates with a knowledge of taxonomy and herbarium experience may be considered) in the Botanic Garden Department, Adelaide, South Australia, for general herbarium, duties, determination of plants, and the supervision of botanical assistants—The Agent-General and Trade Commissioner for South Australia, South Australia House, 50 Strand, London, W C 2 (November 20)

Wykeham Professor of Physics—The Registrar, University Registry, Oxford (November 23)

POSTDOCTORAL RESEARCH FELLOW IN ORGANIC CHEMISTRY at the University of Melbourne—The Registrar, University of Melbourne, Parkville, N 2, Victoria, Australia (November 25)

LECTURER (with postgraduate qualifications and research interests in experimental or social psychology) **IN PSYCHOLOGY** at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, November 27)

CHAIR OF PATHOLOGY in the Medical School, University College of Rhodesia and Nyasaland, Salisbury—The Registrar, University of Birmingham Edgbaston, Birmingham 15 (November 30)

CHAIR OF PHYSIOLOGY in the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (November 30)

LECTURER (with a degree in electrical engineering from a British university, or equivalent, preferably with first- or second-class honours, corporate member of the Institution of Electrical Engineers and preferably with experience in teaching at university level and/or of research work) **IN ELECTRICAL ENGINEERING** at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Hong Kong and London, November 30)

PROFESSOR OF EXPERIMENTAL PHYSICS—The Registrar, The University, Nottingham (November 30)

REGIUS CHAIR OF SURGERY in the University of Aberdeen—The Private Secretary, St Andrews House Edinburgh 1 (November 30)

SENIOR LECTURER/LECTURER IN BOTANY, a **SENIOR LECTURER/LECTURER** (preferably with qualifications in inorganic chemistry) **IN CHEMISTRY**, and a **SENIOR LECTURER/LECTURER IN MATHEMATICS** at the University of Khartoum—The Registrar University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (November 30)

LECTURER (preferably with experience in teaching, research and in the design and construction of hydraulic works and apparatus) **IN CIVIL ENGINEERING** (Hydraulics) at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall London, S W 1 (December 1)

BURNETT-FLETCHER CHAIR OF HISTORY AND ARCHAEOLOGY—The Secretary, The University, Aberdeen (December 9)

RESEARCH ASSOCIATE (with a Ph D in chemistry or physics) **IN SPECTROSCOPY**, for research in the field of chemical aspects of electron paramagnetic resonance spectroscopy—Industrial Engineering Services Research Council of Alberta, 87th Avenue and 114th Street, Edmonton, Alberta, Canada (December 15)

SENIOR LECTURER (with an honours or higher degree in science or engineering, and teaching and/or research experience in the field of physical metallurgy) **IN PHYSICAL METALLURGY** at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (December 15)

VISITING PROFESSOR OF OCEANOGRAPHY at the University of Cape Town, South Africa—The Secretary Association of Universities of the British Commonwealth (Branch Office), Marlborough House Pall Mall, London, S W 1), and The Registrar, University of Cape Town Private Bag, Rondebosch, Cape Town, South Africa (December 31)

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree in biology (including zoology), F I M L T or equivalent qualifications, and preferably some experience in histological techniques) **IN THE PHYSIOLOGY DEPARTMENT**, to assist with histological investigations related to the endocrine research programme of the Department—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield Reading, quoting Ref 61/22

ASSISTANT LECTURER (Grade B) (with a degree or equivalent qualifications, teaching experience, able to teach to higher national certificate standard, and preferably some industrial experience) **IN CHEMISTRY AND METALLURGY**—The Clerk to the Governors, South-East Essex Technical College, Longbridge Road, Dagenham, Essex

ASSISTANT PROFESSOR (with two or three years postdoctoral experience in a field allied with virology, and prepared to participate in a research programme on the virology of tumours) **ON CANCER RESEARCH**—Dr R Bather, Department of Cancer Research, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

FISHERIES OFFICER (male, qualified biologist, with an honours degree in natural science, preferably zoology, and experience in research work) in Tanganyika, to investigate the ecology of inland waters in relation to fish production—Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S W 1, quoting BCD 63/8/012

GRADUATE (with a degree in a biological science and an interest in cytogenetics) **IN THE BIOPHYSICS SECTION**—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berks

LECTURER or **SENIOR LECTURER IN CHEMISTRY** in the Training Department—The Warden, University of London Goldsmiths' College, New Cross, London, S E 14

RESEARCH FELLOW IN ORGANIC CHEMISTRY—Prof R C Cookson, Chemistry Department, The University, Southampton

RESEARCH FELLOW or **ASSISTANT** (with a good honours degree in mathematics or engineering and preferably experience in programming for a digital computer) **IN THE DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS** for work in the field of hypersonic gas dynamics—The Secretary and Registrar, The University, Southampton

SECRETARY (with administrative experience in a responsible capacity in university of equivalent posts) of the College and Clerk to the Governors—The Rector, Imperial College of Science and Technology, South Kensington, London, S W 7

STATISTICIAN (graduate) **AT THE RADIOBIOLOGICAL UNIT** of the Medical Research Council, for work which includes a wide variety of physical and biological problems—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berks

TECHNICIAN or **SENIOR TECHNICIAN** (with general biochemical laboratory experience and a knowledge of electronics), for duties involving assistance with research and occasional classwork and maintenance of spectrophotometers, high speed centrifuges, etc—The Secretary, University College, Gover Street, London, W C 1 quoting "Biochem /1"

TECHNICIAN (Scientific Assistant grade) (preferably with experience of tissue culture methods), for work on respiratory diseases of animals—The Administrative Secretary, School of Veterinary Medicine Madingley Road, Cambridge

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletins of Marine Ecology (formerly *Hull Bulletins of Marine Ecology*) Edited by Sir Alister Hardy, Dr C E Lucas and R S Glover No 43, Vol 5 The General Hydrography of the Clyde Sea Area, Scotland Part 1 Description of the Area , Drift Bottle and Surface Salinity Data By Dr H Barnes and the late E F W Goodley Pp 112–150 11*s* No 44, and 45, Vol 5 The Multi-Depth Plankton Indicator By R S Glover A Modification of the Small Hardy Plankton Sampler for Simultaneous High Speed Plankton Hauls By David Miller Pp 151–172 6*s* 6*d* (Edinburgh Scottish Marine Biological Association, 1961) [258

University of Bristol The Annual Report of the Agricultural and Horticultural Research Station, (The National Fruit and Cider Institute), Long Ashton, Bristol, 1960 Pp 171+6 plates (Long Ashton, Bristol Agricultural and Horticultural Research Station, 1961) 15*s* [258

Agricultural Research Station Soil Survey Research Board Soil Survey of Great Britain, Report No 13, 1960 Pp iv+57 (London H M Stationery Office, 1961) 5*s* net [258

Empire Cotton Growing Corporation Progress Reports from Experiment Stations, Season 1959–60 Tanganyika Territory—Coastal Provinces Pp 12 2*s* 6*d* Survey of Reports Pp 7 2*s* 6*d* (London Empire Cotton Growing Corporation, 1961) [258

Ministry of Agriculture, Fisheries and Food. At the Farmer's Service a Handy Reference to Various Services available to Farmers in England and Wales Pp 81 (London Ministry of Agriculture, Fisheries and Food, 1961)) [258

Other Countries

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Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO , LTD
ST MARTIN'S STREET, LONDON, W C 2
Telephone Number Whitehall 8831 Telegrams Phusis Lesquare London

Advertisements only should be addressed to
T G Scott & Son, Ltd , 1 Clement's Inn, London, W C 2
Telephone Number Holborn 4743

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A NATIONAL YOUTH SCIENCE PROGRAMME

RECOGNIZING the great strides being taken by science to-day, the fact that, according to Sir Bernard Lovell, of all the scientists who ever lived 90 per cent are with us to-day, and, above all, universal acknowledgment of the fact that all and sundry are now in some way or another responding to the impact of science on themselves and on society (whether conscious of it or not), one must admit that there is need for everybody to learn something about what is behind this great and rather sudden social upheaval

Science yields power it renders physical power to industry and travel, further means of interpretation in the various arts, more facilities for enjoying one's leisure, but it is to be hoped that it will never succeed in giving power to the few who might use it for controlling the many Yet that risk seems very real to-day, due mainly to the widespread lack of understanding of the true aims and objectives of science As Mr Dean Rusk recently said ' Power is not a matter of arms alone Strength comes from education, fertile acres, humming workshops and the satisfaction and pride of peoples". Strength assuredly comes with education, and in this science must play a much greater part from the earliest years of a child's life This has also been emphasized recently by Lord Hailsham, the Minister for Science (see p 393 of this issue)

For many years one referred to pure science and applied science, this eventually being altered to fundamental and applied science Gradually it has become recognized that there is no hard and fast line of demarcation between the two—that one inspires the other, and that fundamental science is not entirely basic to applied science and vice versa To-day, one hears of science and technology, perhaps the day is not far off when the implied distinction between these two will disappear Accepting, perhaps with some reservation, Murray Schoonraad's statement that "to-day's science is to-morrow's technology" (*Nature*, **191**, 848, 1961), it would seem that there is no real distinction between science and technology, but that over a period of time one merges into the other and that the length of this period is quickly becoming shorter Science should eventually, therefore, be recognized for what it really is, namely, the search for the truth (using its own peculiar means), the exposition of the truth and its application wherever possible to man's needs and comforts

The 'balance of power' periodically changes even within the ranks of the scientists themselves, such changes being inspired by popular appeal or curiosity or sudden recognition of profitable application For example, in the Victorian era, Darwin and his supporters stirred the world with the conception of the origin of species As these ideas appeared to strike at the very roots of the religious faiths and beliefs of millions of people at that time, one can easily imagine how science (in this case, biology) was brought to the foreground in public debate, discussion and sometimes heated argument as exemplified by the Huxley–Wilberforce contretemps at Oxford and, much later the growth of so-called Fundamentalism in the United States which, starting as a denominational interest, eventually became a national concern when, in 1925, a teacher in Dayton, Tennessee, was tried and convicted for teaching the principles of evolution in a State school Thus, for a time, science made a spectacular appearance before a world-wide audience

One can think of other scientific spectacles, those of to-day obviously being feats in space science But such spectacles can sometimes do more harm than good to science itself, for it would be undesirable to have a scientific *élite* in any community, such as is now developing in the U S S R Science is actually proceeding on a very broad front, and many of its facets, highly important though they are, can never be spectacular So it can be only a very small majority of people who recognize this general advancement, and, to-day, no one person can be expected to understand all of it. Even now it would be a very small percentage of those who have not read biology to a fairly advanced level in school who could give the names of the scientists who discovered penicillin or insulin So clumsy is the way in which science is presented to the public that it is unlikely that Fleming's name will ever become such a household word as that of Gagarin Macromolecular biology is now making great strides in the field of scientific endeavour, but it will be some time before the non-scientist will be able to understand and appreciate this

This does not imply that all thinking people are not aware of the need for a much wider and deeper appreciation of science and its impact on human society The main problem is how to set about it It seems better to acknowledge the fact that among adults only those who wish to know something about this 'Age of Science' will take the trouble to learn This minority is fairly well catered for already In Britain, the British Association for the Advancement of Science, the Workers' Educational Association, the extra-mural departments of the universities, many local authorities, the British Broadcasting Corporation and television authorities are doing excellent work to enlighten those who wish to learn

This is by no means enough, for it is not only the mature grounds which need attention but also the newer, uncultivated yet potential fertile soil—men and women of to-morrow, who are recognizable as soon as they are able to speak

Governments, local authorities, industry, etc , are bringing pressure to bear in an all-out attempt to increase, widen and improve the standards of science teaching In some cases, no doubt, the results are proving disappointing and mediocre, this being due to many conditioning factors, the chief of which is the dearth of science teachers, unsuitability of some of these, lack of space and equipment, finance, and so forth, but above all the time-factor in school terms and hours.

Science teaching seems to have become too rigid, especially in its attitude towards the applications of science. This may be due to a schism in thinking of science as a cultural discipline in contradistinction to science as a practical need. (The examination systems may be largely responsible for this.) Heuristic methods of teaching, as practised and advocated by H. E. Armstrong earlier in this century, served a useful purpose at the time in that they pointed the way to an effective means of teaching the scientific method of experimentation. But such methods aim largely at training potential scientists, and though they inspire some children who have no intention of becoming professional scientists they might well repel others. They are, therefore, not entirely satisfactory if we are to capture the imagination of the majority of the very young in the appreciation of science. The practical application of *A* to *B* may not be so interesting as the application of *A* to self, for what touches the heart of the layman is the effect scientific progress is having on himself—that is, the practical applications of science to man, his health, his job, his creature comforts and, above all, his future. The laboratory is not the only room where science can be taught: it is not necessary to dissect a rabbit in order to learn about the bones of the mammalian skeleton, neither is it necessary to use earthworms with the ancillary apparatus to understand about respiration in animals. Many observations and experiments can be carried out on the pupil's own body, thus becoming more interesting. None of this need undermine the ultimate aim of treating science for what it is, namely, a cultural, and therefore not solely a technical, discipline.

Where, therefore, are the other and earliest grounds for successful scientific seeding? When does a child reach the critical point when he might become inspired with things scientific? The first lies within the ægis of the primary school (many of which have no laboratory), and the second depends on how successful is the seeding in the first. It was with the view of considering how the initial seeding could best be accomplished that the British Association for the Advancement of Science convened a conference, during September 29–30, jointly with the Association of Teachers in Colleges and Departments of Education, on "The Approaches of Science in the Primary School".

At this conference, Mr. N. Isaacs said that the "approaches" in this case are through a disciplined way of thinking, a way encouraged by a definite method of exploration of the physical, biological and psychological worlds. The natural learning processes of the child are akin to the development of scientific thinking—exploration, collecting and piecing together experiences, building up these into a connected scheme of knowledge and foresight. Such processes can be observed in the developing child, and become most marked when the child, in its third or fourth year, has sufficiently mastered language to discover the way of question-forms. The method of seeding should be one of accentuating and directing the natural learning process. This would, it is hoped, be achieved by creating in the primary schools specific environments aimed at developing the individual's interest by setting the scene, catching his innate curiosity and spurring him on to observe and bring forward the right kind of question and hence an attitude to exploration. So, by the time he enters the secondary school, the child is competent to know his world, capable of planning and ready for things scientific. Given an interest in science he will need nothing but opportunity to develop it. However, unless the child is deeply and naturally instilled with the human implications of his knowledge in the primary school, his approach to science teaching in the secondary school will not be satisfactory.

Much more can be done also outside school hours. A great deal has already been done in the United States in this highly important field of extra-mural endeavour. By 1940 some seven hundred 'science clubs' had been founded there. The movement was somewhat retarded by the Second World War, but the project gained momentum during the post-war years, and now there are about 25,000 science clubs of which approximately 500 are outside the United States. Through what is termed 'Talent Searches' individual boys and girls showing special aptitude are encouraged, and it is now claimed that 95 per cent of those winning honours in the Science Talent Search proceed to the universities. According to Science Service's *Growth of the Science Youth Program* (October 10, 1959), honours were awarded to about 10 per cent of the entries and about 50 per cent of these eventually gained doctorates. Yet it is to be hoped that those who were not so selected are now progressing through life with a better understanding of science than they would otherwise have had. If so then Science Service (Washington) is doing excellent work. This Service also inaugurated 'Science Fairs', first on a local and regional basis. By 1950 these had become so numerous that the National Science Fair was instituted. In 1958 this Fair became international through the entry of finalists from Japan's National Fair.

This project could well be followed elsewhere, as indeed it is in Britain, though two other methods of somewhat similar pattern might be considered. First, a central organization might be set up to encourage and direct the attention of science teachers to possible extra-mural activities aimed at young children and teenagers in most branches of science—astronomy, the physical sciences, archæology, natural history, engineering, agriculture, horticulture and so forth. Such an organization might invoke the aid and advice of national and local scientific societies. Thus it should be possible to encourage school teachers to become more flexible in their teaching by organizing extra lectures and exhibitions, and above all, by making available opportunities for practical work during term-time at specific centres.

These activities might, though not necessarily, be divided into age-groups, but should on no account be confined to those pupils who are aspiring to the science sixth form

It can be taken for granted that, once established, such a scientific centre would be able to rely on much practical assistance from local industry, though that would depend considerably on the personality and tact of those running the centre In any event, the time seems ripe for academic and industrial research centres to open up their laboratories, workshops and even factories more often than they do for organized visits by school children Museums play their part, but this is not enough Of course, a great deal is already being done, but much more is needed, for this will help to inspire interest among the many who may never become professional participants in future years, and it must be recognized that these are the laymen of the future so far as science is concerned

Certain sections of industry actually do arrange campaigns, and send their representatives to schools and universities, but their object is clearly to catch the future scientists, and in any event they, and others, might do still better by 'selling' themselves on their own premises rather than in the school lecture theatre

A second method of approach takes into account the fact that it is asking a great deal of any school teacher to take on still further responsibility, especially out of school hours, terms or grounds Yet much could be done, as in the Boy Scouts and Girl Guides movements, that is, not only outside school hours but also away from the daily school teacher with all due respect to him, a change of senior personnel might prove stimulating

The British Association is doing much towards educating youth During the recent annual meeting at Norwich there were three young people's lectures, a schools exhibition, the President's tea party for student exhibitioners and the annual session devoted to "Science in the Sixth Form" organized by Section X This is good and points the way to further endeavour with still younger children in view Nevertheless it must be recognized that such events are confined to a few days each year, to the comparatively small number of large towns and their catchment areas and to a fairly advanced age-group With its lecture services, young people's programmes, international youth science conferences, area committees, science fairs, etc , the British Association has made increasing and comprehensive contributions to the development of more widespread scientific education But it would be a greater step forward if such a body as the British Association made a survey of the needs and potentialities of the whole country, for there is much to be found—the entire country is not lying fallow There are numerous local scientific societies, many of them, it is true, confining themselves to natural history and archæology, but there they are, and some have junior sections Then what always seems to be overlooked is the fact that there are many individual enthusiasts and sometimes small groups of enthusiasts who are interested in a very practical way in most branches of science It is often stated that the days of the 'amateur' scientist are gone, but we believe this is far from the truth It was Will Hay who made very important contributions to astronomy using his 6-in Cooke refractor mounted in his home-made observatory, and by profession he was a stage and screen actor We believe there are many of his type, in various scientific disciplines, cultivating their interests to-day

Most of these local societies, clubs, and individual enthusiasts appear to depend mainly on themselves Yet it is they who might be prepared to form the nuclei for still further local endeavour with the young as their main objective They might well take into consideration the lines of approach suggested by Sir Eric Ashby in his British Association Granada Lecture in 1960 He proposed that an understanding of science could be imparted by emphasizing the importance of technique in science, for the ordinary man is a craftsman and he appreciates craftsmanship. This might well be extended to the children and youth of to-day

Such local individual and collective movements might be loosely unified under a sponsoring body such as the British Association, which is not only well equipped with the necessary contacts but also has the nucleus of the necessary staff Such a sponsoring body, set up on a national scale but using and inspiring local men and women, could offer to the boys and girls of Great Britain the encouragement in scientific activity and appreciation that the National Playing Fields Association, with the invaluable patronage of the Duke of Edinburgh, is giving them in sport and recreation

ELECTRODYNAMICS AND MECHANICS

Electrodynamics of Continuous Media

By L D Landau and E M Lifshitz Translated from the Russian by J B Sykes and J S Bell (Course of Theoretical Physics, Vol 8) Pp x+417 (London and New York Pergamon Press, 1960) 84s net

Mechanics

By L D Landau and E M Lifshitz Translated from the Russian by J. B. Sykes and J S Bell (Course of Theoretical Physics, Vol 1) Pp vii+165 (London and New York· Pergamon Press, 1960) 40s net

THE titles of the volumes in this series cover a vast range of topics and there seems to be little in physics on which the authors are not very well informed The volume on electrodynamics conveys a sense of mastery of the subject-matter on the part of the authors which is truly astonishing I myself have read only one other volume of the series and cannot lay claim to any specialized knowledge of any of the topics dealt with These remarks, therefore, cannot criticize any details of the physics discussed in this volume

The broad lines adopted by the authors become clear very quickly Maxwell's equations are assumed to hold on a microscopical scale and all macroscopical results are derived by a process of taking averages. Forces and energy relations are obtained by a systematic use of the concepts of thermodynamics—there is no place here for the transport of current filaments from infinity and similar 'elementary' devices. The ambiguities of sign sometimes associated with energy expressions are firmly resolved and all such quantities put into their proper perspective as thermodynamical quantities. But such matters are not the chief concern of this volume.

With the help of straightforward ideas of symmetry, linear dependence (and contour integration) the basic ideas of electromagnetism are developed to apply to a long list of topics. These include electrostriction, piezoelectricity, ferroelectrics, the Hall effect, contact potential, electrocapillarity, thermoelectricity, gyromagnetism, ferromagnetism, superconductivity, eddy currents, the skin effect, magnetohydrodynamics, dispersion, wave-guides, double refraction, etc. This list is by no means complete. The standard of explanation is admirable, being sufficient to give a moderately informed reader a grasp of the principles concerned, both physical and theoretical, without elaborate detail. The approximations which must be made to make a theoretical treatment feasible are explained and the theory developed starting from the field equations in most cases. It is the physical insight which accompanies and guides this development that conveys the sense of mastery mentioned here.

To-day the division of the subject-matter of physics into heat, light, sound and properties of matter is being superseded; this book will accelerate this change. In fact, one of the impressive features of the book is the way in which diverse phenomena, listed above, are shown to be related through the dependence of ε (permittivity), μ (permeability), or σ (conductivity) on the frequency of the radiation. Phenomena, which in elementary physics are explained by a closed and mathematically elegant theory, here appear related as a result of expanding ε, say, in terms of the frequency. The general effect is to demonstrate the full power and generality of electrodynamic theory in a way seldom achieved in a textbook.

The translators are to be congratulated on the effectiveness of their translation; the language is direct and could well be original English text. There are surprisingly few typographical errors, about a dozen were noticed, most of these being the omission of a letter or a symbol. Throughout the volume there are references from time to time to modern work, mostly in Soviet journals, a large proportion being available in translation. In short, *Electrodynamics of Continuous Media* is a book which can be thoroughly recommended to every physicist.

In the history of science it has always required a great intellectual effort to break free from established modes of thought and to establish a new outlook, exemplified in the change from the Ptolemaic to the Copernican view of the universe. To-day we are in the midst of a similar change with the advent of modern physics, and the volume on mechanics will contribute to the establishment of a new outlook.

Although the subject-matter is, of course, Newtonian mechanics, the basic outlook is different from the traditional Newtonian outlook. From the outset Hamilton's Least Action Principle is taken as the fundamental law governing all motion; an inertial frame is one for which space and time are isotropic and homogeneous, and the basic transformation is the Galilean transformation between two inertial frames. Considerations of symmetry lead to the law of inertia, and the equivalence of two inertial frames leads to the introduction of a coefficient identified as the mass of a particle. A system of particles which interact with one another but with no other body requires an addition, $-U$, to the sum of the Lagrangian functions of the particles to give the Lagrangian of the whole system. This function U, depending on the co-ordinates only (non-dissipative systems only ?) is the potential energy. There is no indication of how to find the form of U in any given case. The use of the equations of motion now introduces the concept of a force and we arrive at Newton's Second Law of Motion. Throughout this part one feels that without the familiarity gained from the traditional outlook and the associated intuitive appreciation of mass, force, etc., it would be difficult to appreciate the proper significance of many of the results. In some of the succeeding chapters these intuitive ideas are still necessary to 'feel what is going on'—the escape from traditional views is not complete.

On one point of the dynamics of a rigid body the treatment is not quite satisfactory. On p. 109 it is said that the equation for the rate of change of angular momentum "May be regarded as Lagrange's equation . . . for the 'rotational co-ordinates' ". Apart from the inverted commas it is not pointed out that there are no such co-ordinates, that a component of angular velocity is not, in general, the rate of change of any one angle, as is shown afterwards in equation (35.1). This topic should, strictly, be treated as a non-holonomic constraint according to the methods of a subsequent section. Nevertheless, this is a familiar point and the reader already knows the results. It does not affect anything else in the book.

When the reader has adjusted himself to the new outlook then the rest of the book, the greater part, is a vindication of the effort of making this adjustment. The scope and power of the methods used are made abundantly evident in fields of importance to physicists; little time is spent on artificial classical problems. The theory of collisions between particles is developed in a form in which physicists need it; this is followed by chapters on small oscillations, the motion of a rigid body, and finally Hamilton's equations, Poisson brackets, Liouville's theorem, Hamilton–Jacobi equation, etc. This last chapter lays the foundations for the transition to quantum mechanics.

In undergraduate courses on dynamics the treatment of small oscillations often leaves the impression that the theory is self-contained without room for development. Here it is clear that the familiar theory is one stage in the approximations necessary for the discussion of such motions. The development to anharmonic and non-linear oscillations follows naturally. This is typical of the way in which the reader is shown that classical mechanics is still capable of development. The illustrative problems are very carefully chosen and the accompanying solutions are admirable for conciseness, the salient points only being given.

It is a remarkable achievement to write a text on advanced analytical dynamics in the compass of 165 pages and to produce such a stimulating account. The translation is excellent and errors of typography are very few and far between.

B. H. CHIRGWIN

A STUDY OF DIABETES

Diabetes in an English Community

A Study of Its Increase and Natural History By Joan B Walker and David Kerridge Pp 46. (Leicester The University Press, 1961) 7*s* 6*d*

A VALUABLE survey of the incidence and natural history of diabetes in a rural parish (Ibstock in Leicestershire) has been made by Dr Joan Walker of Leicester Royal Infirmary and David Kerridge of the University of Sheffield

The inquiry was initiated in 1955 by the British Diabetic Association in an attempt to arrive at a numerical evaluation of the problem of diabetes in Britain The figures available from the General Register Office vary from two to four diabetics per thousand of the population, but these figures refer to known diabetics It was well known from the United States and Canada that, in previous surveys, as many unknown diabetics were found as those already diagnosed

Many of the methods used by other investigators depended on the study of clinics, schools, factories, prisons and other such institutions, which are special groups with age and sex selection, while detection drives, although not restricted to special age or occupation groups, depend on voluntary initiative and tend to have low response-rates, and therefore allow too much room for bias Throughout the year 1956, these ways of determining the incidence of diabetes in Britain were discussed It was essential to find a method which would be practical, easily applied elsewhere, and be, at the same time, accurate. It was agreed that no census of known diabetics attending clinics could produce the information required As it was most important for the purposes of the investigation that follow up should be possible, it was finally decided to investigate a compact stable community

Because the diabetic clinic at Leicester Royal Infirmary served the City and whole County of Leicestershire, and because some of the Leicestershire villages had exceptionally static populations, it was decided to investigate a village community with the view of its becoming part of any larger national or international survey which might be undertaken later

The objectives of the inquiry were as follows· to establish the number of diabetics in a natural area where the population is static, to discover the undiagnosed cases of diabetes, to investigate the possible hereditary factors; to investigate the possible ætiological factors of physique, diet habits, stress. occupation, and parity in women, to follow up the cases of glycosuria discovered by the investigation, if possible, for as long as twenty years, and to watch for new cases developing in the area, to repeat the whole survey at a future date and to repeat it also in another area of England for comparison or contrast.

Every person living in the village who was over the age of five years was asked to supply a specimen of urine and to answer a questionnaire Altogether 2,071 males and 2,034 females were tested making an 81 per cent response of the population over the age of five years Details of thirty-three previously diagnosed cases were collected from general practitioners' and clinic records So far as could be determined the 19 per cent of non-co-operators were not different in age or other environmental factors from the rest, and in calculating rates, it was assumed that they were a random sample of the whole population

The results show that there are about thirteen diabetics per thousand of the population, half of whom are asymptomatic The relationship of diabetes to various social factors was examined. Plans for a follow-up study of the population are also described in the booklet

ANALYTICAL INSTRUMENTATION

Physical Methods in Chemical Analysis

Vol. 1 Edited by Walter G Berl Second Edition Pp xiv+686 (New York Academic Press Inc , London Academic Press Inc (London), Ltd , 1960) 19 dollars

ADVANCES and refinements in analytical instrumentation must, of necessity, call for periodic reviews of those publications which form such an essential part of that ever-expanding physicochemical noosphere from which the modern scientist draws both inspiration and knowledge This present revision (Volume 1) of a well-known authoritative treatise on instrumental methods in chemical analysis belongs to this category

The more elementary student may find the advanced and detailed form of presentation somewhat bewildering, but as his knowledge increases and he joins the ranks of the experts, he will undoubtedly appreciate the lucidity with which the various specialist contributors have described those practical and theoretical problems which are concomitant with the complex field of contemporaneous instrumentation This is, however, a book which has been written by experts for experts, and, at the academic stage, it will undoubtedly be of the greatest assistance to honours graduates and research workers Most modern instrumental methods are described with much attention to practical laboratory detail Nearly a quarter of the 670-page work is devoted to X-ray diffraction techniques as applied to powders, metals and polymeric fibres Spectrophotometry, emission spectrography and infra-red spectroscopy are given comprehensive treatment in chapters of about 80 pages each The diagrammatic illustrations are clear and well annotated and each chapter concludes with an extensive bibliographical reference section

Later sections deal with Raman spectra, measurements of refractive index, mass spectrometry, and electron microscopy and diffraction The latter technique is particularly valuable for those crystallographic investigations on surfaces where the affected layer is too thin to be detected by X-rays The section on electron microscopy gives some extremely interesting reproductions of electron micrographs of films of various materials

The applications and design of simple and tandem mass spectrometers are described in some detail. Reference is made to the usages of such instruments in the analysis of chemical mixtures, the leak-testing of evacuated vessels, measurements of appearance potentials and related thermal quantities, reaction kinetic studies, etc.

This new edition undoubtedly provides up-to-date information on progress in the development and application of physical instrumentation and will be eagerly acquired by all chemists having an interest in the theory and practice of these specialized analytical techniques D T LEWIS

Materials for Nuclear Engineers

Edited by Dr A B McIntosh and T J Heal Pp ix+373 (London Temple Press, Ltd, 1960) 75*s* net

WRITTEN by specialists on the United Kingdom Atomic Energy Authority staff, this handbook on nuclear materials should prove invaluable as a reference volume not only to nuclear engineers but also to many scientists and technicians working in the nuclear power field The materials covered are uranium, plutonium and thorium metal fuels, together with their more useful ceramic compounds magnesium, beryllium and zirconium canning materials and graphite as the most important solid moderator The occurrence, extraction, fabrication, physical and mechanical properties, compatibility in various reactor environments and behaviour under irradiation are discussed for each material The data are presented in suitable tabular and diagrammatic form and have been selected as the best available from published information to mid-1959

It is regrettable that space could not be found to include stainless steel canning materials, ferritic steels which are of current interest for pressure vessels, and beryllia, which is the most promising alternative solid moderator to graphite, particularly for high-temperature gas-cooled reactors Equilibrium diagrams are given for sixteen plutonium alloy systems and it would be valuable to have diagrams also included for the technologically important alloys of uranium and thorium

As this is a rapidly expanding field, new or revised data, especially on the irradiation behaviour of fuels, are continually forthcoming, and it is to be hoped that suitably revised editions will be produced in good time In particular, the nuclear engineer will need to be kept up to date on the progress of ceramic and cermet fuels which are becoming of increased importance

Neuro-Psychopharmacology

Proceedings of the First International Congress of Neuro-Psychopharmacology, Rome, September 1958 Edited by P B Bradley, P Deniker and C Radouco-Thomas Pp xv+727 (Amsterdam Elsevier Publishing Company, London D Van Nostrand Company, Ltd, 1959) 135*s*

THOUGH neuropsychopharmacology might be shortened as a word, the book deserves no shortening, for the contributions are uniformly of a high standard, and the book reflects credit on all associated with it Its encyclopædic range and its great quantity of recorded facts make it an essential reference book to every worker in the subject The editorial work has been so well done that the great quantity of material is everywhere clear and orderly Two indexes are provided, and it is well printed and bound The discussions given are apparently second thoughts, for they are terse and meaningful, much better than the obscure muddle of the truly verbatim record

Comment on such a wealth of topics is difficult, but I noticed one fact that seems remarkable The better understanding of higher mental functions, and their mechanistic basis, that has come in the past decade through cybernetics has shown the outstanding importance of 'memory', both short- and long-term, in every aspect of the higher cerebral functions Yet our knowledge of the physical basis of memory in the brain is to-day almost nothing Our knowledge of brain function is grossly out of balance of what happens from one milli-second to the next a great deal is known, but how a present event in the brain will influence its action in an hour's time, or in a day, or a year, is almost totally unknown Yet it is the action that persists over a day or a year that is the really significant one for our everyday life This lack of balance in our present-day science is shown in the book, for of the 152 contributors, only one specifically considers the effects of a drug on memory But the same paper also shows that research in this direction is by no means forbiddingly difficult

W Ross Ashby

Life in the Sea

Text by Prof Gosta Jagersten Photography by Lennart Nilsson Pp 184 (London G T Foulis and Co, Ltd, 1961) 50*s*

THIS is a book of photographs, a few in colour but mainly black-and-white, of marine invertebrates and a few fishes, taken by Lennart Nilsson The text, and doubtless much scientific guidance, is supplied by Prof Gosta Jagersten, of the Zoological Institute, Uppsala, and director of the University of Uppsala Biological Station, Klubban, Fiskebackskil, on the Gullmar Fjord, where most of the photographs, apart from a few at Trondhjem, were taken

It is much the best book of its kind I have seen Apart from the high technical skill, especially of many of the photomicrographs, notably of protozoa, diatoms and a wide assortment of planktonic organisms, there is a remarkable quality of 'aliveness' about almost all the photographs, scarcely one is a set piece Technical skill and artistic selection are blended with results which will come as a revelation of beauty as well as of biological information to professional zoologists no less than to amateurs and to school children Perhaps the most striking pictures are those showing young pipe fishes emerging from the egg pouch of the male parent, the series (some in colour) showing conflict between an octopus (*Eledone*) and a lobster and another between two bristle worms, reared up with bared jaws Of greatest scientific interest are the pictures of *Protodrilus* with Prof Jagersten's description of the habitat of wave-washed sand and the effect of this on metamorphosis of the larvæ Altogether a book for everyone and fully worth its price

C M Yonge

Water Gardening

By Frances Perry Third, revised edition Pp xviii+338+105 illustrations (London Country Life, Ltd, 1961) 30*s* net

THE content of this book ranges far beyond the scope of the title, and the bulk of it consists of lists, somewhat arbitrarily defined, of plants which all supposedly have some relation to an aquatic environment But the author's net has been cast much too widely, and the rather bewildering catalogue of species and forms could have been with advantage reduced to a judicious selection of plants which are readily obtainable, known to be of some horticultural merit and likely to be amenable to cultivation Instead, it seems that the literature, not always the most up-to-date or authoritative, has been combed and many of the plants listed have little, apart from botanical interest, to commend them in the aquatic garden or its surroundings The reader will find much of interest and profit in those chapters dealing with design, construction and planting of water gardens.

G Taylor

SCIENCE AND GOVERNMENT IN A FREE SOCIETY*

By The Right Hon VISCOUNT HAILSHAM, P.C
Lord President of the Council and Minister for Science

IT is now a commonplace to say that in some respects a nation is highly regarded in proportion as its science and technology command respect I do not, I think, have to prove this proposition But I would wish to begin by qualifying it

It is easy, of course, to demonstrate that the wealth of nations in peace, and their force and power of resistance in war, have largely been a function of their industrial and scientific strength This is why all nations, slave and free, developed and undeveloped, do their best to achieve a high level of scientific and technological investment within their own borders With most statesmen it is a simple question of the survival of their national societies, and the growth of their national wealth, that they should do so

These considerations are obvious, what is not so obvious, and may, therefore, be in more need of argument, is that, at least in its higher reaches, science is not intrinsically a matter that should—or indeed can for long—be pursued solely for the sake of the wealth or the power that its pursuit may bring

All genuine science has its origin in the questioning nature of the free human spirit, in its creative genius, in its power of insight It is not something which can be dictated to either by theologians or ideologues—or, in the long run, by rulers It may be that they can destroy it by repression, or even starve it for lack of material facilities But they cannot by their own act make it live It is doubtful how long they can even cause it to grow by supplying the means of growth It is certain that they cannot, simply by supplying the means, dictate the nature or scope of the discoveries that can be made Far less, indeed, because it is in the nature of scientific research to discover something which is intrinsically new If anyone were able to predict, or pre-empt, the answers to scientific problems, they would not be genuine scientific problems, for the essence of the matter would already be known in advance The discoveries of a Newton, an Einstein, a Darwin, a Planck, or a Rutherford are genuinely not known in advance, and, when they are made, their practical applications are not immediately perceived even by the inventors This is something which lies deep at the root of the subject, and which statesmen and their people would do well to ponder more deeply than they do at present

I say all this without for one instant losing sight of the spectacular achievements of science under the patronage of government, and without doubting for an instant that these achievements will be multiplied in our time—nor that a great deal of my own time as Minister must be spent precisely in seeking to multiply them in my own country Nevertheless there is a sense in which I continue to regard these wonderful achievements as something of a paradox and I am tempted to speculate whether, in the end the influence of a so obviously materially motivated interest by government in science will not obscure the very insights on which creative science is essentially based In the U S S R , and even perhaps in the United States, to look no nearer home, I see some signs that this may ultimately be so, if it does not prove to be the case it will be because the creative human genius in science as in the arts, may prove too strong for the material motivation of the Governments which seek to direct its course

However this may be, I begin by insisting that pure science is intrinsically as much a branch of culture as history, philosophy or poetry, and that the relationship between Government and pure science is essentially the same as that between an enlightened Government and the artist—that is, the relationship of an enlightened patron and not that of an employer My point is that at its most vital and critical growing point, that of fundamental discovery, the Rutherfords and the Darwins have to be treated with not less respect than the Shakespeares and the Miltons and the Goethes Historically, science is born of two highly respectable parents, the belief in the uniformity of Nature which was the natural child of monotheism in religion, and the freedom of expression and thought which was the product of the Renaissance I would claim that if Government sought to interfere with the scientist in the absolute freedom of his explorations, and the integrity and independence of his speculation, it would ultimately destroy its real source of vigour, or alternatively frustrate its purpose by undermining the scientist's confidence and his will to co-operate

But to this thesis there is a clear antithesis The constant temptation on the part of Government to do this thing is matched by a constant need on the part of the scientist, especially in some ways the purest of the pure, to seek Government help on an ever-increasing scale In most fields the day of the gifted amateur in science is rapidly closing Some of the research tools of the modern scientist are very expensive The education of the modern scientist is not only protracted and complicated, but almost as expensive as the tools he employs He is increasingly dependent on the patronage, in some form of Government

But in exercising its patronage the Government is not, and cannot be disinterested The immense advantages which enure to a State that makes use of the discoveries of science—and trains its citizens to make them—are such that all States make some sort of an attempt to do so, and compete eagerly with one another in the attempt Who will deny that, in the process, both the State and science have to some extent been corrupted ? Three-quarters of British Government money spent on science come directly from the defence budget The proportions in the United States and the U S S R are probably greater, indeed, it is questionable whether the basic organization of scientific endeavour in the U S S R makes any serious attempt to give civil science as such an independent life and existence at all In Britain the creation of a Minister for Science whose terms of reference excluded defence was something of an act of faith, and has come in for some criticism. It is probably fair to admit that a price had to be paid for this. Nevertheless, I am still of the opinion that, in the long run, the marriage between science and defence is corrupting, and will at best turn science from a liberating to a destructive force, and at worst ultimately dry up the wells of inventiveness in the scientist himself. There is a point, indeed, at which technology itself will perhaps corrupt and destroy the scientific inventiveness on which it was based.

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* Substance of an address to the Arbeitsgemeinschaft fur Forschung des Landes Nordrhein-Westfalen, Dusseldorf, on September 27

I therefore earnestly hope that at least a proportion of scientists in all countries will retain their freedom and integrity. It may be that the mere necessity to train all scientists in the genuine techniques of free speculation and invention will cause them to break their shackles. Certainly I am glad myself that my own position of Minister for Science is organized in such a way that neither defence nor even the short-term requirements of technology have been allowed to claim complete mastery over scientific endeavour.

But I would think that the strongest guarantee of a correct balance lies in a healthy relationship between Government and universities—not simply as regards the faculties of natural science, but generally in relation to their whole cultural purpose. There may be many means of making adequate government support available without infringing academic freedom. We are naturally proud in Britain of our own system, which allows government funds to be administered, in effect, by the universities themselves. I am not myself saying that this is the only possible way of achieving the desired object. But it remains, I think, at the basis of any healthy relationship of Government and science.

Indeed, whatever the temptation to militarize or commercialize science, there is a practical consideration which tends in the other direction. No country in the world has ever successfully set up a Department of Science, in the sense of a Ministry directly controlling the pace, the scope and the methods of scientific research. This is due to two considerations. The first is that the strategic planning of science cannot be undertaken without full participation of the scientists themselves—and by these I mean, not just a staff of administrators with scientific degrees, but also men and women who actually carry on scientific work, whether in universities, government research stations, or industry. This is as true of the socialist U.S.S.R. as it is of the capitalist United States, and it is not less true of Britain. Until a short while ago, Russian science was run by the Academy, and though the recent change by which it is now subordinated to a co-ordinating committee is almost certainly a move towards tighter control by the State, it is not a means of taking scientific decisions out of the hands of scientists, or reducing science to a mere branch of government administration.

There is also a theoretical argument which tends in the same direction. There is a sense in which there is no such thing as science, but only sciences. Another way of stating this is to say that science is in fact an all-embracing term, and that scientific researches into particular fields are functions of those fields and not of a comprehensive entity called science. From one point of view, medical research bears a much closer relation to the climate, population, health, diseases and economic activities of a nation than to their nuclear physics. In terms of science, as distinct from economic policy, it would be meaningless for a Finance Ministry official to try to block a grant for medical research on the ground that the money was needed for a synchrotron. It is true that both projects must take their stand in the queue for the general investment programme. But they are related to other items in the programme more closely than to one another.

What is wanted, therefore, is not a single scientific general staff forming part of a single department of science, but a series of scientific general staffs or Research Councils directing scientific research in the general fields to be occupied, independent of day-to-day bread-and-butter work in those fields, but in close contact with them. At the same time there must be, of course, some body to perform the more generalized function of supervision, looking for gaps or signs of imbalance, and fitting the parts into the general economic, cultural and social pattern of the entire community. This is the pattern of British organization. So, taking medical research again as an example, we have a Medical Research Council, independent alike of the Department of Scientific and Industrial Research and of the Ministry of Health but under a Minister for Science, and of equivalent status to other Research Councils serving other scientific fields, commanding the respect and enjoying the co-operation of the scientists, but serving the public interest through its subordination to a Minister responsible for science, who, in turn, is advised by a general council on scientific policy.

The question arises, however, whether such an organization does not give rise to an ivory tower mentality on one hand, and lack of scientific-mindedness on the other. Critics have undoubtedly claimed that this is so, and it is sometimes said that on one hand British industry is too little conscious of the need for technological progress, and on the other hand, that the Research Councils are too strongly academic and too pre-occupied with fundamental research. I agree that the organization which I have been attempting to outline is not in any way a substitute for adequate technical or qualified staff at the executive and administrative level in industry or Government, indeed, I would go further, and say that it will only work at full efficiency on the assumption that such staff are available and active at every level, and in close contact with the Research Councils. This however, does not diminish the case for the Research Councils, which is based on the need for detachment and independent supervision, and not on the supposition that they are substitutes for an appreciation of scientific and technical factors in those who are responsible for management in Government or industry.

This leads me to another generalization about the nature of my subject. Science is, or at least it should be, all-pervasive, and will not achieve its best results until this is the case. You can never make industry research-minded, whether privately or publicly owned, simply by providing research in government institutions or universities, and, if British experience is any guide, you will not even achieve it only by encouraging co-operative research by associations of individual firms—valuable though such associations be. There is no substitute, at any level, for science 'on the ground'—that is, within the individual industrial or commercial firm, within the individual teaching institution, within the individual government departments and nationalized corporations, and even within the home circle. This may seem impossibly ambitious But I consider it to be an obvious truth, whether you regard science as primarily a branch of culture or as something intrinsically practical in application If the institution of a Ministry of, or for, Science were to obscure the essential truth that science and the scientific approach must be all-pervasive at every level, and cannot be imposed from outside, it would be nothing less than a national disaster

Thus the duty of organizing science in a free society, like all other important duties, begins with the individual and covers the entire society Government is a financing, co-ordinating, participating, training, function Over a large field it is not, and cannot be, directory and executive There are functions in which the main part ought to be played by industrialists, educationists, teachers and scientists themselves.

I now wish to discuss the conditions in which this duty can be carried out, and I begin, as anyone must begin, with the child The clue to the correct organization of science is an adequate pattern of education I would go so far as to say that if we could get this right, we should, in the long run, get everything right If we get it wrong, we shall get nothing right, even in the short run

By far the most important desideratum is the creation of a good general school education You cannot make scientists of the first class out of an uneducated populace. The qualities which go to make a first-class scientist show themselves comparatively late in a child's career In most disciplines a sound knowledge of mathematics is a condition of entry into the profession, in most of the others, such as medicine, a wide general culture is almost indispensable Moreover, from every wider point of view, any attempt—perhaps in order to obtain rapid results, perhaps for short-sighted financial reasons, or possibly even for less-creditable reasons of political or ideological obscurantism—to generate a race of scientists interested only in their own specialities, to the exclusion of general culture or even of other scientific disciplines, would, I hope, be bound to fail. If it were not to fail, and if it were taken to anywhere near its logical conclusion, it would certainly be the most disastrous enterprise on which a developed human society could possibly embark Such a race, narrow, blinkered, unbalanced, humourless, frustrated and—over a large range of human experience—inarticulate, would be one of the most evil that the human imagination could devise, probably a race of slaves designed to serve a race of still more uncultured masters, and, if not slaves, illiterate but technologically proficient tyrants to destroy or enslave the society which brought them into being

To this first proposition, however, I would add an immediate footnote In this sound general education of which I have spoken, science should form an integral part from the first From the time that a child can read, and count up to ten, an element of scientific knowledge and thought should be injected into the curriculum of what he learns, even if it is only the most generalized statements about the universe, the microcosm of the atom, the organization of life and evolution, and the nature of chemical compounds The reason why science is regarded by so many otherwise cultured people as a hidden mystery is because so little of it is injected into general reading and conversation before the age of ten [see also p 387 of this issue] We should not learn our culture —our history, our religion, our ethics, our physics, our mathematics—in a group of watertight vertically divided compartments, or in a series of horizontally divided instalments, but in a wide, integrated, smoothly flowing stream

But behind the general educational pattern lies the need for a sound specialist and vocational training available from the age of fifteen onwards for those who need to know the essential manual skills or crafts, and from sixteen or seventeen onwards for those who seek technician status, or the higher technological or scientific degrees I am not qualified to lay down a detailed or technical prescription for this But I would say generally that there should be more than one road to the top, and that a society which does not make further education, of at least three years, universally available for those who possess the entrance qualification for it, on the basis that it will be availed of by at least 25 per cent of the appropriate age-groups in the population, is seriously under-estimating its requirement. If you put the figure at 40 per cent I would not seriously quarrel, but would say that this remains to be proved

At the spearhead of the scientific movement is the army of research workers, the true frontiersmen of scientific advance, few in numbers, highly specialized in qualification, but on whose efforts depend the success or failure of all How will these work ? Who will direct their work ? How will it be financed and organized ? What is to be their relationship to Government ? These questions are really at the heart of the whole matter, everything else is, in comparison, only on the fringe

In the early days of science, these questions presented no difficulty Science was independent of Government and even, to a large extent, of the formal courses of the university But this is no longer the case The men of whom I now speak take about thirty years to train—five in the nursery, ten in the school, three or four in the university as undergraduates, four or more learning research methods, and perhaps another five working under some kind of supervision Their research apparatus—originally made of glass and brass in an attic or a garage or outhouse—may now cost millions of pounds, like the proton synchrontron at CERN, or the fast breeder reactor at Dounreay, and the supporting structure of constructional engineering, electronics, computers, served by technicians and craftsmen, employs whole industries and forms the basis of life in whole townships and communities Of those who start off on this road, many fall by the wayside On the genius of a small proportion out of the very few who survive the race depends the success or failure of all

Here my first proposition is that, at the highest level, it is ruinous to separate research and teaching It follows that the most advanced scientific work should be located in or near the universities, where this is possible, or directed by university personnel The contrary course—the creation of separate government-sponsored institutes—is attractive and is sometimes rewarded with spectacular results Moreover, considerations of national security may sometimes dictate it But in the end it can be a primrose path, as everyone from the U S.S R westwards who has tried it knows You may gather together all the most promising intellects in a subject, build them a town, equip them with all the most lavish facilities, and the thing will go off like a rocket—at first But after five years—after ten, after fifteen—what then ?

Unless active steps are taken to redress it, there is a danger that intellects will be staler, new blood will not be forthcoming, the stimulus of youth will be absent From a proportion of those engaged, the research potential may have disappeared, even the scientific questions will have changed Instead of a research institute you would then have a monastery which had lost its faith—isolated stereotyped, remorselessly carrying on activities with a diminishing margin of useful results

There have been, of course, remarkable examples of research institutes where the dangers I have mentioned have been avoided, it would seem, indefinitely In Great Britain I would cite the National Institute of Medical Research and the National Physical Laboratory, and in Germany the Institutes of the Max Planck Society But if the dangers have been avoided, it is precisely because they have been recognized as dangers As a general proposition I adhere to the view that there must be the constant stimulus of teaching, or research will tend to die There must be the constant interpenetration of the mature intellect with the adolescent, or it may petrify. There must be a constant outlet for research workers who are ready to pass into teaching or administration, or research may even degenerate into a racket Therefore, where possible, locate your best research in university or teaching institutions If you cannot, you must do the next best thing and arrange for constant interchange of personnel, and regular exchange of ideas between them.

This does not mean that there is no place for government research institutions, or institutions of research collectively organized by or for the benefit of private industry On the contrary, as will be seen, my thesis is that there is room for more and more of these My point is only this: that however much these bodies proliferate, they should where possible locate their best research in or near universities, and that where they cannot do so they should promote the closest possible links with teaching institutions of all sorts

To begin, therefore, with the Government Most governments—directly or indirectly—build the roads, manage the telephones, dispose of the sewage, construct and maintain the harbours, many manage the railways, generate the electricity and gas All are concerned with standards of measurement, heat, light, health and air pollution Most are concerned with the nutrition and health of their citizens, and few can divorce themselves from the efficiency of agriculture All these activities throw up problems of research and development, many of which cannot conveniently be carried on in conjunction with teaching Some activities are intrinsically public in character. Others can efficiently be financed only by heavy drafts of public money It will be a long time before agricultural research is paid for entirely by farmers. Nor can the pharmaceutical industry, the medical profession, or private generosity, finance medical research on an adequate scale Even in the sphere of industrial research there are many general questions, like those connected with fatigue and creep in metals, which lend themselves to government research financed by Government, because they are not so closely connected with any particular private industry as to attract sufficient private finance

Here I would describe in greater detail a British institution which we owe in part to the genius and foresight of the first Lord Haldane, and which has on the whole won the confidence of the scientific world and created the means of providing government finance without sacrificing either academic independence or scientific integrity This is the institution of the Research Councils, of which there are at present four in Britain, the Department of Scientific and Industrial Research, the Medical Research Council, the Agricultural Research Council, and the Nature Conservancy.

The thinking behind the creation of these Councils is to separate the activity of research from the executive business of government and economic activity—keeping medical research separate from the Ministry of Health, for example—and to concentrate it under a number of Councils, composed mainly of working scientists and industrialists with real executive responsibility for the research programmes under their control, with separate budgets negotiated with the Finance Department, and invested with the responsibility, within their several spheres, for financing the training of research workers no less than carrying forward the business of research All operate stations or institutes of their own All finance research in the universities, and one, the Department of Scientific and Industrial Research, co-operates with particular industries in financing joint programmes of research carried out by organizations known as Research Associations, managed primarily by the industries concerned on a co-operative basis, but attracting also government grants through the Research Council

It would, of course, be a logical and attractive arrangement if one could go on to report that the fundamental or basic research in Britain were now carried out in teaching institutions, and that the applied research was all done in government or industrially based research institutions.

Experience, however, shows that, although this may in theory and in practice approximate to the ideal arrangement, no absolute division of function on these lines is either desirable or possible Apart from the obvious fact that any line drawn between basic and applied research is necessarily arbitrary and imprecise, experience shows that the human problems involved cannot be solved satisfactorily on these lines. However closely applied or related to concrete industrial difficulties the work of a research institution may be in intention, in practice it will not retain the services of high-grade scientific personnel if they are to be completely debarred from undertaking fundamental research

At the other end of the scale, no matter how academic may be the work in a university, it is impossible to exclude applied or practical applications or development Contact with the intellectual frontier is a requisite for the mental health and happiness of one, contact with real life an unavoidable necessity for the other Thus no division is absolute, and nothing will substitute for the management of these highly sensitive, highly qualified human beings by suitably qualified and sympathetic scientific bodies assembled for the purpose A close association between basic and applied science, and a constant interchange of personal contacts between industrial and governmental research, and between both and the teaching institutions, are probably a better prescription than a more perfectly formalized pattern of organization

How far is it possible for national Governments and administrations to guide and fertilize science without warping its character and distorting its scope ? I have already pointed out that the gifted amateur, and even the rich private or industrial patron, whether individual or corporate, cannot possibly cover the sweep of modern science and technology, or even the training of the modern scientist , the needs have reached the national scale

But the nation State also has its limitations, even financially, and more still in its terms of reference It is surely no coincidence that, in one field after another, science not just on a national but also on an international scale is beginning to become an actuality. The International Geophysical Year is only one example—in which even East and West co-operated Europe has recently begun to plan to enter co-operatively into space research Britain and the United States are co-operating in the launching of the first British satellite experiments Western European countries co-operate, under the auspices of the European Nuclear Energy Agency, in the *Dragon* High Temperature Reactor project at Winfrith Heath, and in the Halden project in Norway I have already spoken of the internationally owned proton synchrotron at CERN There are projects on foot for both European and Atlantic universities Much of the aid to under-developed countries will be both co-operative and technical What is the future and what are the prospects for enterprises of this kind ?

I would say that a very considerable scope exists for international scientific effort In the long run, I am quite certain that the really ambitious space research, if it is to continue indefinitely, will have to be financed in this way I cannot myself conceive that either medical or agricultural problems would fail to gain by international treatment Many of the longer-term projects in engineering, for example, in the realm of thermonuclear fusion or the direct generation of electrical energy from heat, may well come into the same category.

At the same time I would emphasize that international co-operation is no substitute for national excellence Our experience has been that co-operation is only possible between nations which each have something comparable to offer to one another It is neither practicable nor desirable to create international scientific organizations to make up for a basic want of scientific organization or effort in the individual member countries The new organizations, when they come into being, will be additions to and not substitutions for what is attempted nationally They will be no excuse for the individual member States to neglect either national education or research on a national scale—although duplication of effort can be avoided, and a scale effect obtained by jointly undertaking projects which are in excess of the capital resources available to any one

Hitherto it has been possible for the nation State to stimulate the growth of scientific effort in a variety of different ways—by supporting scientists in the universities or research institutions, and financing their machines and apparatus, and by developing machines capable of practical application in various directions where the State itself is the ultimate user of the machine

But this has meant, in societies both free and servile, predominantly the development of engines of war, even where these have ensuing or concurrent civilian application Where would the aeroplane, the radio, radar, jet propulsion, or, more recently, space research have been but for the constant urge of governments to prevail in two world wars—or should I say in three ? It is useless for governments to protest that their Gagarins and their Sheppards—or their Titovs—have also achieved technical success in realms of peaceful science and technology. Only those nations which have had reason to develop military rockets have actually reached for the stars, and a principal effect of their space flights has been to emphasize the prestige of their military technologies

Is there no means nowadays of governments developing or helping to develop new devices which are just as revolutionary but without the stimulus of war or the fear of it ? The steam engine in its origin, and the internal combustion engine in its inception, were primarily civilian devices What must we do now to stimulate development in a purely civilian sense ?

I must begin by emphasizing that, as between research and development, it is development which costs the money Of the two hundred and fifty million pounds spent on research and development by government departments in Britain, about fifty million goes on research and the rest, I estimate, on development, and of this, of course, a proportion that I would not care to guess at is spent on projects which never ultimately reach fruition

Secondly, I point out that the role of the would-be financier of development is radically different according to whether the financier is, or is not, the ultimate user of the hardware to be produced One reason why so much successful development has been inspired by defence has not been the over-riding importance of the conception of war in the mentality of modern government, it has been the simple circumstance that the defence forces as the ultimate users of the hardware have been able to formulate their staff requirements, undertake their feasibility studies, and finally proceed to development contracts with the makers, without the discipline of the necessity to make a commercial project, but with all the enthusiasm of a potential user whose life may depend on the excellence of the product

It is not easy, in a free society, to reproduce all these conditions in civilian life—even where, as is by no means usually the case, the Government is the ultimate consumer In civil aircraft, in railway engineering, in road construction, radio engineering, electricity generation, perhaps in machine tools and computers, and above all in the peaceful uses of atomic energy, some of the conditions exist, and only in the latter case have the possibilities been at all adequately grasped—with results which have as yet not been fully seen Admittedly there are formidable difficulties—the formulation of the requirement, the provision of government finance for a project which may ultimately enure at least in part to private profit, the placing of a contract selectively (which could give ground for scandal and abuse); but we shall not advance farther until we get our feet wet It may be that here, too, there is scope for the pooling of resources internationally

Of one thing I am quite certain The preservation of a technological lead cannot long be maintained by means of commercial secrecy Commercial, like military, security always breaks down in the end, and what advantage exists in priority is obtained by industrial experience in production rather than by the maintenance of technical secrets

A nation remains in the forefront technically, not by patenting its devices but by keeping ahead in invention and development, and this is a complex achievement which lies only in part within the province or power of government to promote Let us remember that science is an achievement of the human spirit, and resolve that the European peoples will foster it for its own sake rather than for the wealth and power that it will bring The respect for truth, the freedom of intellect, the moral discipline that it engenders, are worth more than these Get them for their own sake, and my conviction is that what remains will be added unto us

SYSTEMATIC MEASUREMENT OF FALL-OUT IN CZECHOSLOVAKIA UP TO 1961

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SYSTEMATIC measurements of fall-out in Norway[1] showed that monthly average concentration of fall-out materials in precipitation after high-yield test explosions reaches a peak value in about six months, and then decreases with a half-life of about one month Our systematic measurements of fall-out in precipitation in Hradec Králové, Czechoslovakia, during the past four to five years provided evidence that the activity of radioactive fission products from fall-out during November 1956–December 1959 showed a trend similar to that in Norway[2] (Fig 1) The peak of the activity in April 1959 has about the same value, 3,960 $\mu\mu$c /l precipitation The minimal value during November–December 1959 was about 20 $\mu\mu$c /l

Detection of French Nuclear Tests of 1960

In Fig 1 are shown the results of our measurements also during January 1960–March 1961 The increase in activity of rain-water in March 1960 after the first French nuclear test in the Sahara Desert on February 13, 1960, was very pronounced but transitory Activity of fission products reached its highest level in the collection of rain-water of March 1, namely, 25 9 mμc /l In the collections of March 4–12 the activity was about 5 mμc /l The concentration of radioactive fission products from fall-out in the air at ground-level in Hradec Králové during March 1960, studied by measurements made on dust collected by a membrane filter assembly, showed no pronounced increase in activity (The concentration was about 1 $\mu\mu$c / m^3) It is interesting to note the measurements of Patterson and Lockhart on the eightieth meridian network of air-filter stations[3] The activity of fission products reached its highest level at Miraflores, Panama, in the collection of February 26–29, and this station received the first indication of fission products in the 2-day collection of February 24–26 It is therefore probable that the radioactive cloud from the first French nuclear test reached our station in Hradec Králové at the beginning of its second revolution around the globe, its velocity of propagation being about 100 km /hr The attempt to explain the relatively high level of the activity of rain-water and the simultaneously low level of the activity of surface air sampled on March 1 in Hradec Králové is made in the last paragraph A sharp increase in the level of activity occurring some time during February 28–March 1, 1960, in Surrey, England, and evidence which suggests that this was due to the first nuclear weapon test in the Sahara, was described by W Anderson *et al* [4]

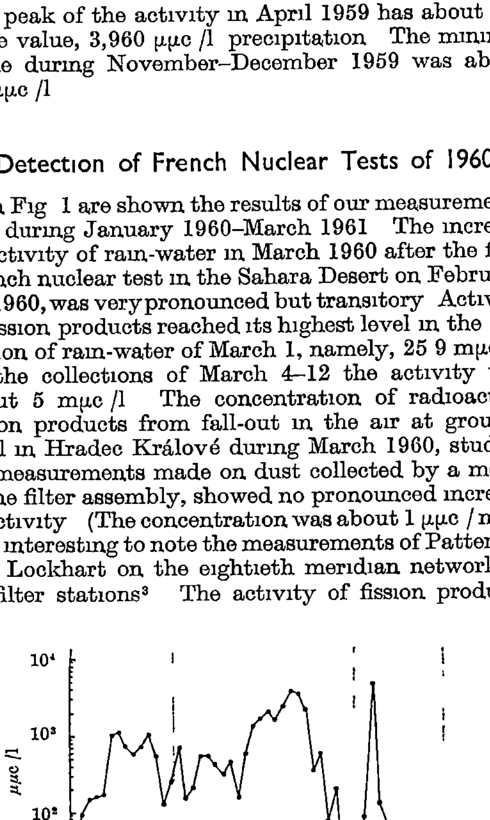

Fig 1 Monthly average concentrations of materials from fall-out in precipitation

Fig 2 shows the decay of three representative samples plotted on a log activity versus log time-scale with February 13 as the origin Fig 2 is similar to the figure published by Y Feige *et al* of the Israel Fall-out Monitoring Service[5], where the main impact of the first French nuclear explosion was already on February 17 Our Fig 2 demonstrates that the exponent n in the formula of the decay $A = kt^{-n}$ is 1 2–1 4 The long-lived background becomes evident after the elapse of 120–160 days, according to the level of the activity of fresh samples We followed the decay during 400 days

After the second French nuclear test, on April 1, 1960, we picked up radioactive materials in rain-water on April 9 The initial activity was relatively low, about 0 4 mμc /l, and average long-lived background activity had to be subtracted after 30 days

Patterson and Lockhart[3] relate the second rise of the activity during early April to the February test, since debris from the French test of April 1 would not be expected to arrive so soon, and debris from the much smaller second test could be hidden by residual activity from the first test If we relate at our sampling station the second rise during early April to the first French test, the exponent n in the formula $A = kt^{-n}$ would be 3–4, which is unlikely The first 'half-life' of our sample collected on April 9 was 9–10 days, which is probably due to the fresh activity

In Rumania, Mageru *et al* observed the transitory increase in activity during February 23–25 and March 16–20, 1960, in the air-streaming from the Mediterranean[6]

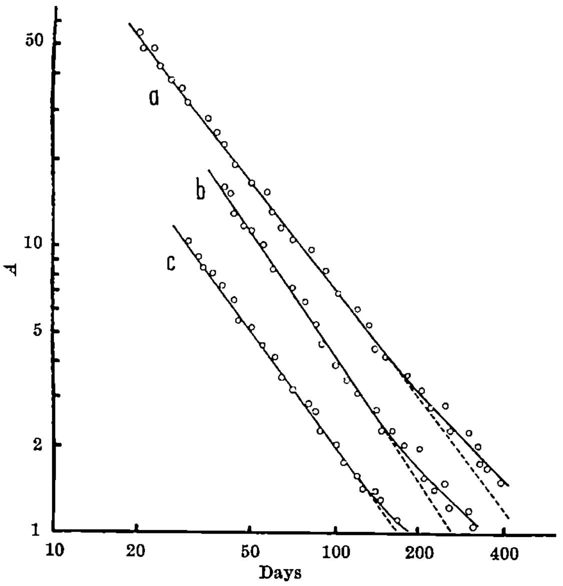

Fig 2 The decay of three representative samples in the collection of March 1–8, 1960, after the first French nuclear test on February 13, 1960

Autoradiograms of radioactive dust particles from the second French test were obtained by V Havlovic in Pilsen, Czechoslovakia[7]

After the third French test on December 27, 1960, we did not observe any marked increase in activity

Collection of Fall-out and Typical Decay Curves

In addition to the collection of rain-water samples, the fall-out was collected every second or third day by the pot method, which is efficient[8]. The collecting dishes, made of polyvinylchloride, have high borders and a collecting area of 1 m ² A thin layer of distilled water is maintained on the bottom. For control purposes the fall-out is also collected once a month After annealing at a temperature of about 600° C., the sample is again moistened and uniformly applied to an aluminium dish, 3 cm ² in area After drying, and three days after collection, the activity is measured with a Geiger-counter for beta-rays The number of pulses is recorded with automatic equipment of our own construction on a paper tape[2] The samples are also changed automatically. Strontium-90 + yttrium-90, obtained from the Dosimetric Department of the Institute of Nuclear Research in Prague, was used as a standard

Fig 3 shows the activity of fall-out in the period when nuclear tests ceased during November 1, 1958–February 29, 1960, and also during March 1, 1960–March 31, 1961 Activities are in mc /km ²/ month, and plotted as logarithm of activity versus time. In Fig 3 are used activities collected monthly, that is, at the end of each month With this value we compared the total of daily activities for the month considering the radioactive decay The maximum in March 1960 is not so pronounced as in Fig 1, because the fresh activity in March 1960 decayed more quickly than relatively older activities at the beginning of 1959 In the first quarter of 1961 the monthly average activity of fall-out was 0 4 mc./km ².

Fig 4 shows the decrease with time of activity of rain-water collected on May 4, 1959 (sample *a*), and on March 1, 1961 (sample *b*) Approximate date of nuclear test was determined graphically. The values of :

$$1 \cdot {}^{1\,2}\sqrt{A} \text{ and } 1 \,.\, {}^{1\,6}\sqrt{A}$$

are plotted on the ordinate axis, thus producing curves a', a'' and b'. (The curve b'' is not plotted) In the period of the first 150 days they are straight lines, which intersect the time-axis in July, and October 1958 and in February 1960 For the first French test one may plot the values of $1 \; {}^{1.2}\sqrt{A}$ to obtain the right date of the test For the sample collected on May 4, 1959, which was one of our last relatively strong active rain-water samples in the spring of 1959, it is impossible to determine by using the rather insensitive graphical method which date of the nuclear test is correct[9] But a control graphical determination makes some date in October 1958 more probable It consists in the fact that for the relation $A = kt^{-n}$ there is a linear dependence between the time difference (τ) of the dates corresponding to the activities, the ratio of which was chosen constant, for example, $A_n \,.\, A_{n+1} = 2$, and the time (T) corresponding to the first activity Relation $A = kt^{-n}$ may be written in the form $A = a\ (T - T_0)^{-n}$, where T is the date of measurement, T_0 the date of the nuclear test, and then transformed in the linear dependence $\tau = c\ (T - T_0)$, where the constant c is dependent on the exponent n, $c = {}^{n}\sqrt{2} - 1$ Thus, $n = 1\ 2$ appears more probable (curve a')

In fresh samples collected during March 1–14 two groups of beta-rays were roughly determined by absorption measurements with aluminium foils 0 7 MeV and 1 5 – 2 1 MeV In agreement with Akpinar[10], it was shown that the energy of beta-rays

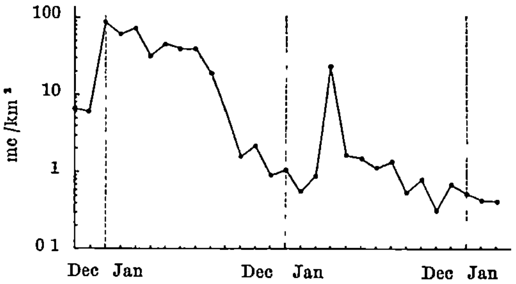

Fig 3 Concentrations of fall-out materials in mc /km ²/month

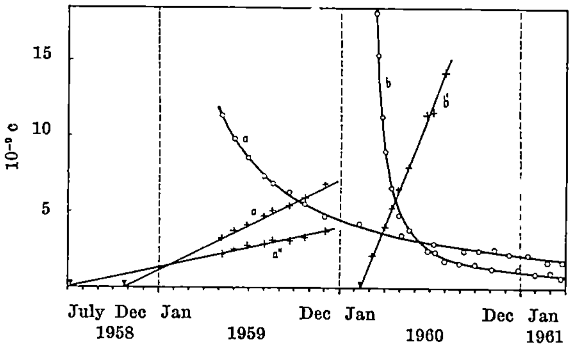

Fig 4 Typical curves of decrease in activity with time of two samples of rain-water collected on May 4, 1959 (*a*), and on March 1, 1960 (*b*) Approximate dates of nuclear tests were determined graphically from the initial part of the curves, where the background is negligible

varies with the age of the sample, but due to relatively large masses of samples we were unable to use this method for rapidly determining the age Old samples collected in spring 1959 and studied by absorption measurements showed the group of beta-rays about 3 MeV

The energy of the gamma-rays of samples in the collection during spring and summer 1959 was roughly determined from absorption measurements with copper and lead plates, with a scintillation counter The result is 0 2 MeV and 0 8 MeV This is in rough agreement with gamma-scintillation spectra of the fall-out[4,11], and with the main gamma-emitting nuclides to be expected in a 6–18 month-old mixture of fission products[12]

The seasonal variation of the first half-life[13] of our samples corresponds roughly to the calendar of nuclear test explosions, as was more exactly determined by D H Peirson *et al* [14] on the basis of the ratio of zirconium-95/cæsium-137, which is a useful index of age or origin of the mixture of fission products

Radiostrontium determinations in the fall-out were made using well-established methods[15] (Separation was carried out by the Chemical Institute of the Medical Faculty of the Charles University in Hradec Králové) The average concentration of strontium-90 found by us in rain-water collected during the first two quarters of 1959 was about 10 μμc /l , in the last quarter about 1 μμc /l , and in the first quarter of 1960 about the same This is in agreement with the measurements of W Anderson *et al* [12], who proved a marked reduction of the average quarterly concentrations of strontium-90 in rain-water collected during 1959

Correlation with Meteorological Data

The reality of the maximum of man-made radioactivity in rain-water measured on the territory of Č S S R at the beginning of March 1960 is confirmed by comparison with the meteorological situation A picture of the upper air flow at the 500-mb level has shown that the contaminated air mass after the first Sahara test reached our country in 17 days During this time the trajectory of the air mass could be traced over northern Egypt, Iran, northern India, Vietnam, southern China, the Pacific, the southern parts of the United States (February 26), the Atlantic Ocean and northern France It is characterized by the very rapid progress, especially in the part above the Atlantic Ocean, where the velocities in the jet-stream surpassed 100 km /hr The trajectory explains the measured maxima of the radioactivity in air samples in Israel on February 17, a sharp increase of the activity in Surrey, England, on February 29 and on March 1 in Germany On an average, however, following the trajectory, we find the maxima of the air activity above the territory of Israel on February 15 or 16 From this we arrive at the hypothesis that a radioactive cloud settled relatively slowly and that the air trajectory in the lower levels was probably not the same as in the 500-mb level

The abnormally high level of artificial radioactivity of atmospheric precipitation on March 1 does not correspond in Hradec Králové to the maximum, which is not expressed so well in the histogram of air activities We explain this fact by the process during which radioactive particles were bound in clouds and washed out below the clouds by the falling precipitation elements In this case a decisive part is played by the evaporation of rain-drops As the intensity of evaporation increases with decreasing dimension of drops, we could expect, at 11 hr on March 1, when the cold front with smaller intensity of precipitation and smaller dimensions of rain-drops passed over our country, a higher radioactivity of rain-water, although the measured values of air activity was relatively low

We are indebted to Prof F Běhounek, member of the Czechoslovak Academy of Sciences, for valuable advice and encouragement during this work.

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RADIOACTIVE FALL-OUT AND THE TESTING OF NUCLEAR WEAPONS

STATEMENT BY THE MEDICAL RESEARCH COUNCIL

IN December 1960 the Medical Research Council published its second report "The Hazards to Man of Nuclear and Allied Radiations" (Cmnd 1225), which included an assessment of the levels of exposure to radiation which had resulted from test explosions up to that time The resumption of nuclear weapon testing in the atmosphere by the U S S R has, however, resulted in the further release of radioactive fission products and, at the request of the Government, the Council has issued a statement (October 24) which attempts to put in perspective some of the more important considerations that result from the new situation A review of this subject would not have been possible without help from many people, and the Council would like to record in particular its indebtedness to the Agricultural Research Council and the United Kingdom Atomic Energy Authority, members of the staff of which have provided valuable advice and assistance

Mechanism of deposition of fall-out Work on the deposition of fall-out from weapons detonated during 1958 and in previous years has shown that the rate at which fission products pass from the stratosphere to the lower atmosphere and thence to the Earth's surface varies greatly according to the size of the explosion and the height, latitude and time of year at which it occurs Deposition from large explosions has been found to be most rapid when debris is released into the lower stratosphere in high latitudes during the autumn

Types and sources of radiation exposure from fall-out The significance of tests involving substantial and rapid fall-out is that, soon after the tests, considerable quantities of short-lived radioisotopes, which will not have been retained in the stratosphere sufficiently long to decay to negligible levels of radioactivity, are likely to be deposited on the Earth's surface These short-lived components of fall-out contain both materials, such as zirconium-95, which are not absorbed into the body but contribute to the external radiation received by the whole body, and materials which are absorbed into the body such as iodine-131, which becomes concentrated in the thyroid gland If present in sufficient amounts, these short-lived materials may, in the short time during which they retain their radioactivity, give doses comparable with those delivered over much longer periods by the longer-lived materials produced in the same explosions

The chief longer-lived components of fall-out which require consideration are cæsium-137, carbon-14 and strontium-90 Cæsium-137 and carbon-14 are of importance because they give rise to general irradiation of the body, including the gonads The dose-rate from carbon-14 is very much lower than that from cæsium-137 but, as it will continue for very long periods, the total dose over hundreds of generations may be similar Strontium-90, both because of its long half-life and because it becomes concentrated in bone, causing prolonged irradiation of bone and bone marrow, is still regarded as the most important single indicator of the possible total hazard to the individual

Assessment of the hazard from fall-out prior to the present tests. The Council's second report on "The Hazards to Man of Nuclear and Allied Radiations" (Cmnd 1225), which assessed the possible extent of the hazard caused by test explosions up to the end of 1958, provides some background for considering the possible consequences of recent tests

With regard to the genetic effect from all test explosions which had taken place up to the end of 1958, the Council concluded that the mutations which might be caused were likely to be few in relation to those produced by natural radiation the latter are themselves believed to form one-tenth or less of the mutations newly arising in any generation

As to effects on individuals, among the most important components in fall-out are iodine-131 and strontium-90 From the amounts of iodine-131 observed in human thyroid glands, the Council concluded that the average thyroid dose delivered by this radioisotope as a result of nuclear explosions which had taken place prior to its 1960 report was unlikely to have exceeded one-quarter of the radiation received from natural sources during any year

Investigations of strontium-90 in human diet in the United Kingdom were commenced in 1955 and were considerably expanded in 1958 The contamination of milk receives particular attention because dairy produce contains most of the strontium-90 which the population ingests Samples of milk drawn from depots handling more than 40 per cent of total production are examined Other foods are also sampled, so that the average strontium-90 content of diet throughout Great Britain can be assessed

Between 1958 and 1959, the ratio of strontium-90 to calcium in average diet in the United Kingdom increased by 50 per cent, but the recent Agricultural Research Council report (*ARCRL* 5) shows that in 1960 it decreased to little above the 1958-level This decrease was due to the reduced rate of fall-out consequent on the cessation of testing and reflected the fact that, when the radioactive fall-out was relatively high, the contamination of diet was due primarily to the direct entrapment of the recent deposit on growing vegetation Strontium-90, however, also enters plants from the soil. This is a slower but continuing process so that, even if fall-out ceased, diet would continue to be contaminated, though at a reduced level, for a considerable period

The results of assays of human bone are now available for the five years 1956–60 In each year the highest concentration of strontium-90 was found in children about one year old In this age-group there was a progressive but slow rise each year until 1958, a doubling of the 1958 value during 1959, but a fall in 1960 which has continued in 1961 The average value in these infants of 4 strontium units (that is, 4 $\mu\mu c$ per gm of calcium) at the peak in 1959 was about a sixteenth of the value (67 strontium units) regarded by the Council as the maximum permissible average value in the whole population

Children born in the year 1955 have been exposed to strontium-90 from their diet during their whole life-time Although in 1956 the average value in their bones was 1 strontium unit, the value in 1960 was only fractionally greater (1 4 units) Older children have formed only part of their bone structure from contaminated food, and the bones of those born between 1945 and 1950 showed only 1 strontium unit in 1960

Estimation of additional exposure due to recent atmospheric tests The recent tests by the U S S R are reported to have included explosions both in the kiloton- and the megaton-range and to have occurred in Central Siberia and the Arctic As would have been expected from experience in 1958, the radioactivity reaching the ground in recent weeks as a result of the present series of explosions has come mainly from short-lived components which are deposited within a few weeks of the explosion The deposition of longer-lived components, such as strontium-90 and cæsium-137, is likely to be greatest next spring

Since iodine-131 is the main short-lived isotope absorbed into the body, extensive measurements are being made of levels of iodine-131 contamination following the present series of tests Early results of the survey, which is being carried out by the Agricultural Research Council, are given in the appendix

It is estimated that the average radiation exposure to the thyroid from this isotope in the fall-out already measured in the United Kingdom from the present tests is likely to be less than the figure of one-quarter of the radiation received from natural sources during any year However, the value for infants in their first year of life may now approximate to that received annually from exposure to natural sources

Because iodine-131 is taken into the body predominantly through milk, the Medical Research Council has had under review the maximum sustained level in milk which might be regarded as allowable. It has based its recommendations on similar criteria to those used in setting the dose-levels permissible in the general population from strontium-90 It has concluded that an acceptable dose would not be exceeded in any age-group of the population unless an average concentration of iodine-131 in milk of 130 μμc per litre was exceeded over a period of one year, or higher concentrations for correspondingly shorter times The level has been set to provide a margin of safety for the section of the population in the group in which the dose-rate would be highest, namely, infants up to the age of 1 year, considerably lower radiation doses would be received by older children and adults consuming the same milk Allowance has also been made for the likely variation in the concentration of radioiodine in local milk supplies The dose referred to will not be reached unless present levels of iodine-131 continue for several months as a result of further weapon tests or are substantially increased A marked decrease may be expected within a few weeks of the cessation of tests

As the highest fall-out rate of some of the longer-lived radioactive substances, such as strontium-90, may be expected in the late spring of 1962, it is not yet possible to predict the total additional radiation dose to which the population has been committed by the recent tests However, as the levels of radioactivity measured in air have been similar to those observed at an equivalent time after explosions in the autumn of 1958, there is at present no reason for believing that tests from which fall-out has so far been detected will involve an additional radiation exposure from external radiation or from strontium-90 which differs greatly from what has been experienced hitherto It may be concluded that strontium-90 from tests so far carried out will not give rise to levels in bone approaching those which the Council laid down in its report of December 1960 as the maximum permissible in the general population

Consideration has also been given to the inhalation of radioactive materials, including plutonium It has been concluded that the extent of radiation exposure from such sources is unlikely to be comparable in importance with that from the other sources mentioned

The Council is informed that it is not expected that underground tests, such as the United States has been carrying out, will contribute to world-wide contamination

Possible consequences of the explosion of a fifty-megaton bomb It appears likely that the explosion of a fifty-megaton bomb during the present series of tests would cause the total energy released from this series to correspond approximately with that of explosions occurring in the two years 1957–58 (according to the Ministry of Defence statement of May 5, 1959) If the bomb is exploded in the atmosphere, the resulting fall-out will depend on the extent to which the energy is due to fusion rather than fission, and on the altitude and site of the explosion

The explosion of such a bomb in the atmosphere in northern latitudes during the autumn could be followed by considerable fall-out of shorter-lived radioisotopes Deposition of radioiodine due to the series might then be substantially greater than that from previous tests, and the contamination of milk in the subsequent weeks might cause the annual mean level to approach that already specified, namely, 130 μμc per litre

Radioisotopes of medium half-lives, such as zirconium-95, might be expected to raise the level of external radiation in the open for some months by an amount comparable with the dose-rate received from natural sources

It is not yet possible to predict with any accuracy the total additional dose of radiation to which the population will be committed by the current series of Russian tests, including the explosion of a fifty-megaton bomb Concentrations of strontium-90 in bone in young children might rise in the course of one year to about twice the maximum level observed hitherto, but the level regarded as the maximum permissible one by the Medical Research Council would not be reached

Arrangements for monitoring and assessing the fall-out situation Regular measurements of radioactivity in rain, drinking-water, food (including milk), air, soil, vegetation and human bone, are continuing according to a country-wide programme under the supervision of the Medical Research Council and the Agricultural Research Council The results of measurement of radioactive fall-out are published at frequent intervals Premature attempts to interpret the significance of individual early measurements directly in terms of human hazard can be extremely misleading Variations in the measurements are to be expected, both from day to day and as between one place and another The significant factors are average levels over long periods of time

The situation with regard to fall-out and the hazards from it is kept under close and continuous review, and the bodies responsible for so doing have undertaken immediately to inform the Government if there should be any major change in their assessments

Appendix: Measurements of Iodine-131 in Milk

When it became apparent that the new series of nuclear explosions might lead to appreciable quantities of iodine-131 in milk, the Agricultural Research Council Radiobiological Laboratory commenced an examination of milk from eight depots or farms located in central and southern England, Wales Scotland and Northern Ireland The samples are analysed frequently, usually daily The mean results so far obtained are shown in Table 1

In the first few days of the week commencing October 15, 1961, the average level fell to below 100 but those results which are now available for the

Table 1 LEVELS OF IODINE-131 IN MILK PRODUCED AT EIGHT SITES IN DIFFERENT PARTS OF THE UNITED KINGDOM

Week commencing	μμc Iodine-131/litre
September 16, 1961	85
September 24, 1961	109
October 1, 1961	138
October 8, 1961	150
Mean for four weeks	120

Table 2 LEVELS OF IODINE-131 IN MILK PRODUCED THROUGHOUT THE UNITED KINGDOM

Mean of values for October 2 and 16, 1961

Zone		μμc Iodine-131/litre
1	Northern England	103
2	North-West England	92
3	East and East Midland	71
4	West Midland	100
5	Wales	153
6	South and South-East England	102
7	Mid-Western England	86
8	Far Western England	105
9	Scotland	190
10	Northern Ireland	165
Country-wide mean weighted for production		113

latter part of that week indicate a return to the levels observed ten days previously

More detailed investigations of the general level in milk throughout the country were made for October 1 and 15 On these dates representative samples were obtained from depots which together handle more than 40 per cent of the total milk produced in each of ten zones into which the whole country has been divided The statistical organization of this elaborate scheme, which is also in operation for strontium-90, has been described in reports by the Laboratory The average results for the two sampling occasions are shown in Table 2.

The means shown in the two tables are in close agreement and the significance of the figures is discussed in the foregoing statement

NEWS and VIEWS

Electrical Engineering at Manchester : Prof. C Adamson

Dr Colin Adamson has been appointed to a newly created (second) chair in electrical engineering in the Faculty of Technology in the University of Manchester. Dr Adamson gained a B Sc (Eng) degree at the University of London in 1947, following work at the Hull and Bradford Technical Colleges He qualified for the degree of M Sc (Eng) in 1952 as a result of research work while working as assistant lecturer in the Bradford College and as research engineer at A Reyrolle and Co , Ltd He was appointed lecturer in electrical engineering at Manchester in 1952, and senior lecturer in 1959 He has been awarded the degree of D Sc this year Dr Adamson has been responsible over the past few years for the development in the Manchester College of Science and Technology of an extensive Power Systems Laboratory, for both undergraduate and postgraduate work, the latter forming a large sub-section of the postgraduate school in electrical engineering His various projects have received substantial industrial support, much of it under contract He is a representative on the British Electrical and Allied Industries Research Association, and has served on committees of the Conférence Internationale des Grands Réseaux Electriques He has recently served, by invitation, as visiting professor at the University of Roorkee, India, under the Colombo Plan, and has lectured at the Universities of Washington and Wisconsin

Municipal Engineering at Manchester : Prof J R. D Francis

Mr John Robert Dark Francis has been appointed professor of municipal engineering in the Faculty of Technology in the University of Manchester, in succession to Prof R J Cornish, who recently accepted a similar appointment in the University of Delhi, India He will take up his post in January 1962 Mr Francis, who is forty-one years of age, gained honours in engineering at the University of London in 1940, following work in the Brighton Technical College After service as lieutenant in the Royal Naval Volunteer Reserve he was appointed to a lectureship in the Department of Civil Engineering at the Imperial College of Science and Technology in 1946 He was appointed reader in fluid mechanics at that College in 1958 Mr Francis has been engaged in research in fluid mechanics and has been concerned in the hydraulic design and model testing of several civil engineering works, including the Owen Falls Dam (Uganda), Tema Harbour (Ghana) and the Baldershead Dam (Durham) He held a fellowship at Woods Hole Oceanographic Research Institution, Massachusetts, during 1952–53, and has recently lectured at the Nigerian College of Technology, Zaria, and the Kumasi College, Ghana

U S National Bureau of Standards : Dr Y Beers

Dr Yardley Beers has been appointed chief of the Millimeter-Wave Research Section, Radio Standards Laboratory, Boulder Laboratories–National Bureau of Standards, U S Department of Commerce He joined the staff of the Bureau in June 1960 as a consultant in atomic frequency and time-interval standards. Dr Beers was a member of the staff of the Radiation Laboratory at the Massachusetts Institute of Technology during 1942–45, a consultant to the Brookhaven National Laboratories with the Atomic Energy Commission, 1947–59, and a teacher of physics in New York University for fifteen years, eventually achieving the status of full professor In 1956, Dr Beers worked as a Fulbright scholar with the Commonwealth Scientific and Industrial Research Organization in Sydney, Australia. During his stay, he was also visiting professor at the University of Sydney His special fields of research interest are nuclear physics, microwave spectroscopy, and the electronics connected with these fields

The Charles F. Kettering Laboratory, Yellow Springs, Ohio

Dr Leo P Vernon has been appointed director of research of the Charles F Kettering Laboratory in Yellow Springs, Ohio He is well known for his work on photosynthesis and has recently returned to his former position as professor of chemistry in Brigham Young University after a year's sabbatical leave, which he spent with Prof Hugo Theorell at the Nobel Institute, Stockholm, Sweden Mr Justin C Crawford has been appointed director of laboratory administration in the Laboratory Mr Crawford will be responsible for all non-scientific activities and business administration A native of Chicago, he has served with the Standard Oil Co of Indiana since 1948, where for the past five years he has been manager of engineering administration

Tristan da Cunha

The island of Tristan da Cunha is the subject of a special exhibition at the Geological Museum, South Kensington The geology is described with the aid of maps and diagrams, and characteristic volcanic rocks collected by the Shackleton–Rowett expedition during the voyage of the *Quest* in 1921–22 are also shown A series of photographs illustrates the rugged topography of the Island, which is related to its volcanic origin A display of projected colour transparencies is a special feature of the exhibit Charts of historical interest, lent by the Admiralty, dating back to the 1827 Admiralty Survey by Cooling, are mounted on a separate screen Although Tristan da

Cunha is a volcanic cone with a crater lake at the summit, there has been no previous record of eruption during historical times The island does, however, lie on the flanks of the Mid Atlantic Ridge, which is a belt of crustal weakness extending from Jan Mayen Island, through Iceland and the Azores, southwards towards the Antarctic The nature and extent of this submarine feature were investigated during the recent International Geophysical Year

Award by the Fleming Memorial Fund

THE Trustees of the Fleming Memorial Fund for Medical Research have made their first grant This is to the University of Oxford and amounts to £48,000 for the Sir William Dunn School of Pathology at Oxford, headed by Sir Howard Florey The grant is in connexion with electron-microscopy work at the School, where the purification of penicillin was achieved and its clinical use made possible Recently, too, cephalosporin *C*, a penicillin-like antibiotic active against some important penicillin-resistant bacteria, was discovered at the Sir William Dunn School of Pathology

The Fleming Memorial Fund for Medical Research was launched in March this year in memory of Sir Alexander Fleming, discoverer of penicillin, to assist basic medical research Its British and international appeal, with a target of £1 million, lasts until March 1962 So far more than £370,000 has been raised

New Thinking in School Chemistry

THE Office for Scientific and Technical Personnel of the Organization for European Economic Co-operation arranged a seminar held at Greystones, Co Wicklow, during March 1960, to deal with the status and development of school chemistry teaching (see *Nature*, 186, 208, 1960), and the report has now been issued with the title "New Thinking in School Chemistry" (Pp iii+215 Paris Organization for European Economic Co-operation—Office for Scientific and Technical Personnel, 1961) The contributions and discussions show that there is considerable difference of opinion on some topics Some favour a dogmatic theoretical treatment beginning with the atom and electron as familiar modern concepts and proceeding to the development of theories of structure and bonding, the teaching being largely centred on the periodic table What one speaker calls 'stick-in-the-mud experiments' will not take a prominent place It is felt that distinction of *s* and *p* orbitals might be too difficult There is sharp disagreement among those favouring this approach as to the meaning of the theories, and teachers using this method will obviously need annual refresher courses An American speaker states that in some schools there the teaching begins with organic chemistry Other speakers, including two from the United States, explain schemes in which models to illustrate atomic linking and molecular structure are made by the pupils Some think that this will take too much time and will be costly Opinions are divided on the value of films Another group of speakers favours an experimental approach and thinks that practical work is desirable Qualitative and quantitative analysis can illustrate general chemistry and inculcate habits of neatness and accuracy Some suggestions for new experiments, including the use of paper chromatography in qualitative analysis, are made Summaries of the discussions, three joint reports, and recommendations for future action close the proceedings

Radiation Botany

THE first number of *Radiation Botany*, a new international journal devoted to plant radiobiology and related subjects, was published in September It is edited by A H Sparrow together with an editorial board drawn from seven countries (*Radiation Botany*, Vol 1, No 1, September 1961 Pp 100 Annual subscription rates (*A*) for libraries, government establishments, research laboratories, etc , 140*s* , 20 dollars, (*B*) for individuals who place their order directly with the publisher and certify that the journal is for their personal use, 70*s* , 10 dollars London and New York Pergamon Press, 1961) This first issue of a hundred pages consists of eleven scientific contributions the subject-matter of which includes, factors affecting responses of plants to radiation, root absorption of fission products derived from underground nuclear explosion, morphological responses to gamma-rays and the influence of radiation on chiasma frequency These and other topics dealt with illustrate the main aim of the journal, which is to be concerned with the effects of ionizing radiations on plants It will, however, also be devoted to the more technical aspects of plant irradiation Each contribution is usefully prefaced by abstracts in English, French and German, and papers are acceptable in any of these languages.

The journal is attractively printed in a style in keeping with the modern nature of the subject The choice of type, paper and general layout makes *Radiation Botany* a very readable journal Table layout is particularly good, and the line illustrations and plates are of a high standard Though all workers in the field of radiobiology will welcome this new outlet for their writings, one may hope that genetical and physiological journals will continue to receive contributions from those who have used radiations to elucidate problems primarily concerned with those sciences

Lanehead Field Centre

THE increasing scientific interest being shown in the 'marginal land' of the British Isles is reflected in the recent foundation of a new research centre in the Northern Pennines The Department of Geography of the University of Durham, under the guidance of Prof W B Fisher, has recently acquired a village school at Lanehead, near Cowshill, in Upper Weardale, and has speedily converted the building into an active centre for field research. Situated near the watershed of the Rivers Tyne, Wear and Tees, the new centre dominates a virgin area for field and ecological investigations Research projects have already been started on the geomorphology, microclimate, soils and vegetation of the region, and progress is being made in the analysis of the social life of the inhabitants of the Upper Pennine Dales In addition to giving field courses to the undergraduates of the University of Durham, the research workers (mainly postgraduate students) hope to add, in some way, to our knowledge of the social and environmental background for future development in highland Britain Many aspects of the background still perplex the technologist and the economist, it is hoped that Lanehead Field Centre will provide at least some of the answers

Rutgers Center of Alcohol Studies

THE Center of Alcohol Studies, which was instituted at Yale University in 1921, is being transferred to Rutgers, The State University, and will be known as the Rutgers Center of Alcohol Studies The transfer of the Center, internationally known for the study of alcohol in its social, biological, psychological and sociological aspects, is being made possible by a grant from the National Institute of Mental Health It is expected that the transfer of the Center, which at Yale has been identified with the Laboratory of Applied Biodynamics, will be completed by the spring of 1962 At Rutgers the new Center will be a unit within the Graduate School of the University, the dean of which is Dr Marion A Johnson The principal functions of the Center will include (1) Research into various problems involving the use of alcohol, physiological, psychological, sociological and economic (2) Teaching of groups with a special interest in problems of alcohol such as clergymen, public health officers, personnel workers, police, social workers, and nurses This will be done in a summer programme, the Yale counterpart of which has been nationally famous for years (3) Documentation of all forms of study dealing with alcohol (4) Publication of literature dealing with the subject, including the *Quarterly Journal of Studies on Alcohol*

Courses in Nuclear Energy

The European Nuclear Energy Agency of the Organization for Economic Co-operation and Development (recently the Organization for European Economic Co-operation) has published a new edition of its *Catalogue of Courses on Nuclear Energy in O E E C Countries, 1961–1962* (Pp 167 Paris Organization for Economic Co-operation and Development, European Nuclear Energy Agency, 1961) The *Catalogue* is designed to help science and engineering students who wish to acquire a better knowledge of nuclear subjects and to find out what training facilities are available University authorities and others wishing to compare the programmes of technical establishments in different countries will also find much relevant information The new *Catalogue* covers the 1961–62 academic year and gives details of training courses in subjects relating to nuclear energy in some 180 Western European universities, technical high-schools and research centres It contains a list of establishments where courses are given on subjects relating to nuclear energy, syllabuses of the main courses on nuclear physics, chemistry and engineering, with details as to lectures, length of courses, terms of admission and diplomas obtainable, and information concerning the principal items of nuclear physics and engineering equipment available for training in each establishment Copies of the *Catalogue* can be obtained on application to the Organization for Economic Co-operation and Development, European Nuclear Energy Agency, 28 Boulevard Suchet, Paris 16

Museum of Comparative Zoology, Harvard College

The one hundred and first annual report of the Museum of Comparative Zoology at Harvard College for 1959–60 records the celebration of the centenary of the founding of the Museum by Louis Agassiz Sir Julian Huxley gave a public lecture, and this was followed by a reception and a dinner The Museum is an integral part of a major university and still maintains research and teaching, which were the main objectives of Agassiz's life Although no major expeditions were sponsored during the year 1959–60, many trips were arranged by members of the staff for field and museum studies and for the collection of materials A long list of publications by the staff is an indication of the great amount of research undertaken

Cyrillic Transliteration

Scientific Information Notes for August–September (3, No 4, 1961) reports that progress toward agreement on the use of a compromise system for transliterating Russian (Cyrillic) characters into the Latin alphabet was achieved at a meeting of representatives of scientific and engineering societies, editors, publishers, librarians and Government agencies on July 14, 1961, in Washington, D C The meeting was convened by the American Association for the Advancement of Science at the request of the National Science Foundation to discuss the adoption of a single, uniform transliteration system for Russian to English The compromise system, tentatively designated as the "AAAS Transliteration System", combines features of the various systems now in use so that the transliteration of Cyrillic to Latin alphabets may be standardized An important consideration of the proposed transliteration system was that it could be typewritten with the minimum use of diacritical marks Systems considered in effecting the compromise were the systems of the Board of Geographic Names, the British Standards Institution, the Library of Congress–American Library Association and the International Standardization Organization The importance and volume of Soviet scientific and technical literature have resulted in extensive efforts to translate Russian material into English The multiplicity of transliteration systems in use has resulted in increased burdens for members of scientific and technical communities, editors, publishers, librarians and Government agency personnel Much confusion existing in this field could be alleviated or avoided by a reduction of the number of varying transliteration systems now in use The tentative system has been forwarded to the *Z*-39 Subcommittee on Transliteration of the American Standards Association It is expected that an *ad hoc* *Z*-39 subcommittee will be formed to deal with the problem of Russian transliterations.

Learning Theory and the Therapy of Neurosis

Monograph Supplement No 13 of the *British Journal of Psychology* (Cambridge University Press, 1961. Pp. 29 7*s* 6*d* net) is entitled "Learning Theory and the Therapy of Neurosis" The author of this clear and admirably concise monograph, Mr. Ralph Metzner, provides a useful review of the literature on avoidance training and experimental neurosis in animals, and examines its relevance to some aspects of human psycho-pathology He is particularly (perhaps excessively) concerned to analyse some recent methods of psychological treatment (so-called 'conditioning therapy') and to indicate their kinship with the animal training procedures Although a comparative approach to behaviour and its disorders is to be welcomed, it might seem important to bear in mind the differences as well as the resemblances between animals and men. This the author signally fails to do As a result, his work will appear to many as a gross over-simplification of the problem of neurosis and as singularly lacking in awareness of clinical reality None the less, it has value as an unusually fair and thoughtful account of experimental work in its field and as a timely reminder that psychotherapy must eventually achieve a rational basis

The Textile Institute

The following awards have been made by the Textile Institute *The Institute Medal,* to Mr K W L Kenchington, senior development officer, Directorate of Stores and Clothing Development, War Department, Royal Aircraft Establishment, Farnborough, for his contributions to the Institute in the field of clothing science and technology, *Service Medal,* to Mr P H S Bell, head of the Department of Textile Industries, Huddersfield College of Technology, and to Mr J Boyd, of Courtaulds, Ltd , *Companionship,* to Mr C. E Harrison, president of the Federation of British Industries, and to Mr F M Stevenson, chairman and managing director of Stevensons (Dyers), Ltd , and Associated Companies.

Fulbright Travel Grants

Under the provisions of the Fulbright programme, travel grants are available to citizens of the United Kingdom and dependent territories to go to the United States of America for academic or educational purposes, provided that they have adequate financial support in dollars for the period of their visit, and have been accepted by an American institution of higher learning for a minimum of nine months The grants cover the cost of direct travel between a candidate's home and the American institution or university They are available during June 1–August 15, 1962, for which applications must be received before March 16, 1962, and August 16, 1962–April 1, 1963, for which applications must be received before June 1, 1962. Application forms and further information can be obtained from the United States Educational Commission in the United Kingdom, 71 South Audley Street, London, W 1

University News .

Birmingham

The following appointments have been announced *Lectureships*. R G. Bird (civil engineering), Dr. J. S Hough (senior lecturer in charge of the Sub-Department of Malting and Brewing in the Department of Biochemistry), A W Kwiatkowski (mechanical engineering), G Martelli (physics), Dr L Riddiford (senior lecturer in the Department of Physics), Dr K R Rushton (civil engineering), Dr. R B Shearn (chemical engineering), N B W Thompson (physical metallurgy) *Research Fellowships*. T Duthie (Wilmot Breeden Fellow in the Department of Mechanical Engineering), Dr H Kaliszer (mechanical engineering), B K Gazey (electrical engineering), R J Heitzmann (chemistry), Dr A E Pedler and Dr C A Barson (senior Research Fellows in the Department of Chemistry), Dr. B Henderson (physical metallurgy), G R Orgam (industrial metallurgy), B Walker (industrial metallurgy) Dr S Swierszczewski (nuclear physics), J R Turcan (engineering production), C J H Williams (James Watt Research Fellow in the Department of Mechanical Engineering)

Leeds

Mr G L Bibby and Dr A E. D Heylen have been appointed to lectureships in the Department of Electrical Engineering, and Dr M Holmes to a lectureship in the Department of Civil Engineering

Liverpool

The City of Liverpool College of Technology has been admitted to affiliation with the University of Liverpool for a period of three years from October 1, for the purpose of teaching pharmaceutical chemistry for courses leading to the ordinary degree of B Sc and to the degree of B Sc with honours in pharmacology During the period of this affiliation the head and four members of the Department of Pharmacy of the College staff will be recognized as teachers of the University

London

The title of professor of social statistics in the University of London has been conferred on Mr C A Moser in respect of his post at the London School of Economics and Political Science

Manchester

Prof P J Collard, of the University College of Ibadan, has been appointed professor of bacteriology and director of the Department of Bacteriology in succession to Prof H B Maitland Prof Collard will take up his duties in April 1962

College of Aeronautics, Cranfield

Dr R L Apps, of Atomic Power Construction Ltd , has been appointed to the post of lecturer in the Department of Materials, Mr A G. Simms, lecturer at Leicester College of Technology, has been appointed to the post of lecturer in the Department of Production and Industrial Administration, Mr J W Mair has been appointed Research Fellow in the Department of Materials, Dr A Younger, of Richard Thomas and Baldwins, Ltd , has been appointed Research Fellow in the Department of Materials

Announcements

Prof J C Mitcheson, professor of mining at the Royal School of Mines, Imperial College of Science and Technology, and a member of the Geological Survey Board since 1959, has been appointed chairman of the Board in succession to Sir Walter Drummond

Mr B N Hughes, at present editor of *Technology* and special writer to *The Times* on technical and technological education, has been appointed a director of Messrs. Chapman and Hall, to succeed the late Mr Geoffrey Parr as chief editor for scientific and technical books

A symposium on "Low Temperature Carbonization of Non-caking Coals and Briquetting of Coal Fines" is being organized by the Regional Research Laboratory, Hyderabad, India, during November 20–22 Further information can be obtained from the Director, Regional Research Laboratory, Uppal Road, Hyderabad 9, A P , India

The Anatomical Society of Great Britain and Ireland will hold its annual general meeting at St Mary's Hospital Medical College, London, during November 23–24 The meeting will be devoted to papers on "Cytology of Nervous Tissue". Further information can be obtained from the secretary, Prof R J Harrison, Anatomy Department, London Hospital Medical College, Turner Street, London, E 1

The British Insecticide and Fungicide Conference will be held at Brighton during November 7–10, under the auspices of the Association of British Manufacturers of Agricultural Chemicals Further information can be obtained from Mr. W. A Williams, secretary, Association of British Manufacturers of Agricultural Chemicals, Cecil Chambers, 86 Strand, London, W.C.2

NEW SOURCES OF ENERGY

THE United Nations Conference on "New Sources of Energy" was convened for the exchange of experiences and new ideas on recent progress in the applications of solar energy, wind power and geothermal energy, and held in Rome during August 21–31 The term 'new' is not perhaps the best description of these sources of energy which have been used by humanity for many centuries, and is used to emphasize that their development has been largely ignored or regarded as uneconomical in the past but that they should now be included in any survey of power resources These three forms of energy are very different in their characteristics, a geothermal energy source is a gift for any nation fortunate enough to possess such a site, for although there are many problems involved in harnessing this form of energy, its utilization follows well-established procedures Solar and wind energies are ubiquitous, though varying in intensity in different localities and at different times, they are both 'low intensity' sources having a maximum energy flux of the order of 1 kW /m ^{2} The latter, however, is in the form of mechanical energy for which Nature has paid the toll of the Carnot cycle efficiency. while the former is in the form of heat, and mankind must pay the toll to convert this to mechanical or electrical energy Consequently, although the conversion of solar radiation to electrical power on a small scale would be advantageous, the major form of utilization should be as heat at low or high temperatures as required

It is not possible to make detailed comment here on the technical proceedings at a conference attended by some 650 delegates from more than fifty countries and at which some 250 papers were presented, about half being on applications of solar energy All the papers were summarized by *rapporteurs* in sections; these reports were discussed later and a final summary was given at the concluding session for each form of energy under consideration Two visits were arranged during the conference. One was to a demonstration of a solar power plant, 2–5 kW capacity, designed by the National Physical Laboratory of Israel This uses air-inflated plastic mirrors and covers in the form of cylinders about 12 m long which are aligned east and west, and only need moving occasionally to allow for the seasonal change in the position of the Sun Steam is produced at a temperature of 150° C and the heat transferred in a storage vessel to vaporize an organic working fluid which is expanded in an impulse turbine, condensed and returned to the heat exchanger This design has been reduced to the simplest essentials and has an estimated cost of 1,000 dollars per kW installed, and a power cost of 5 cents per kW hr based on 6 per cent interest rate and 15 years life The other visit, of contrasting nature, was to the geothermal station of Larderello This power system, in use before the Second World War, has now been extended to a capacity of 2×10^9 kW hr annually, using steam at an average pressure of 5 atmospheres absolute and a temperature of 200° C The first impression is the unusual sight of gigantic cooling towers rising from surrounding countryside, with a relatively low power-house and a maze of snake-like pipes conveying steam from the distributed bore-holes Use of geothermal steam is complicated by the high gas and mineral content of the steam, so that most of the power stations operate by heat transfer to a pure water circuit from which the steam is passed to the turbines This simplifies the gas removal problem and provides for effective recovery of the minerals, mainly boron compounds Conference papers were presented on the recent developments in the use of geothermal power in New Zealand and Iceland, as well as prospective applications in Japan, South America and other volcanic zones

Wind-power units of small output are well established in agricultural regions The problems involved in larger units of 100–500 kW. which could generate electricity for a grid supply are still under investigation, but the tendency would appear to be towards a direct-drive propeller unit geared up to the generator by mechanical or hydraulic transmission and with automatic voltage control

The papers on solar energy applications covered many fields, such as water and house heating, crop drying, distillation, power production by thermal cycles, refrigeration, air conditioning and cooking The direct conversion of radiation to electricity by thermocouples, photo-voltaic cells and thermionic devices was also discussed, and recent developments in solar furnaces Details of these processes were described more fully in my report on the World Symposium on Applied Solar Energy, Arizona, 1955 (*Nature*, 177, 110, 1956) Since that date there has been development in new designs and improved equipment, especially as regards economical construction, but no really new principles have emerged The number of large-scale installations for water heating and distillation at present existing remains very small, and there is still insufficient detailed information available to select the most satisfactory designs for various purposes. Most of the designs in the fields mentioned above have been developed independently, and are still in the experimental stage Large-scale commercial developments seem limited to water heaters in Israel, and some large-area solar-stills in the United States One of the proposals given prominence at the conference was that a series of experimental stations should be established in various countries where the scaling up from the experimental models to the larger industrial installations could be developed and in this connexion mention should be made of the Brace Experimental Station at Barbados, West Indies, recently established by McGill University The extensive funds available for research on space travel and artificial satellite powering will make possible the development of many schemes for the direct production of power from solar radiation which can be adapted for terrestrial use

Perhaps it will be more profitable in the space available to discuss the economic and social implications of solar energy utilization Much of the discussion at the conference concerned the under-developed nations, which might be described more politely as non-industrial, and how these could be helped by the industrial nations One possible procedure suggested would be for the industrial countries to manufacture and sell equipment to the non-industrial countries, but individuals could not afford to purchase such equipment without a national subsidy in the first instance The preferred alternative would be for the industrial countries to develop very simple designs of equipment which could be made in the country where required and thereby establish village industries The first stage might well be along communal lines in villages, for baths, refrigeration and electricity generation, rather than attempting to accomplish these for individual dwellings The importance of simplicity was emphasized, since in solar appliances the capital cost per unit of energy collected is more significant than the thermal efficiency of conversion The sociological feature is the difficulty of introducing a new method of doing something already accomplished by other means, for example, to replace refuse-burning cookers by solar cookers, but where the application is completely new, as for electricity generation or refrigeration, there is not the resistance of tradition

As regards industrial countries, solar energy can only be integrated with other energy sources, and water heating is the most feasible application The prospects of success are greatest in the Mediterranean countries, decreasing but not necessarily being non-existent in the more temperate countries Much consideration was given to the assessment of potential resources in all the fields of energy considered These data are particularly lacking as regards solar radiation and a much closer network of recording stations is required to assess the availability on a micro-climatic basis

The final conclusions would appear to be

Geothermal power is analogous to hydro-power, and obviously should be developed in the few localities where such sources exist, provided that the power can be transmitted to centres of population Wind-power is an established factor in agricultural areas but development is needed before large wind-power generators can be constructed Utilization of solar energy is the most difficult of all to establish Protagonists must have faith in the possibilities, some of which are not yet proved Similar situations have, however, arisen in other technologies now fully established and justified, for example, space travel, where the initial difficulties have eventually been overcome as unforeseen developments occurred The urgent needs are international co-operation to establish satisfactory designs of equipment and the training in Europe and the United States of personnel from the user nations who can return to promote investigations in their own countries

The general impression is that the development in six years is not as great as would have been expected It is, however, encouraging to find that there are many people of high academic ability in various countries who have enough faith to devote their whole, or at least a considerable proportion, of their time to the attainment of a deeper knowledge of the underlying principles of all these sources of energy and in particular of solar energy devices, in order to raise the existing standard of living in under-developed countries Co-ordinated planning during the next six years should promote a significant increase in applications

Long-term predictions for the future are necessarily uncertain, but there is no doubt that energy demands will grow more rapidly than the world population, which is expected to double by the year 2000, and that all sources of energy should be actively investigated and integrated The conference and discussions arising will undoubtedly promote these objectives, and lead to more successful international co-operation in future developments H HEYWOOD

MICROWAVE MEASUREMENT TECHNIQUES

A CONFERENCE on the general subject of microwave measurements was held at the Institution of Electrical Engineers during September 6–8 A large audience of scientists and engineers from many countries assembled to consider some fifty papers covering all aspects of modern microwave measurement

In his introductory lecture, Prof H E. M Barlow, of University College, London, stated that microwave measurement techniques could be grouped broadly under the following headings measurements using field probes, measurements depending on resonance arrangements, attenuation and phase shift measurements, noise measurements and power measurements, and that the general limits of accuracy of microwave measurements at the present day were of the order of ± 0 1 per cent for impedance measurements, ± 1 per cent for power measurements, and about ± 0 01 db up to 3 db for attentuation measurements

Subsequent sessions of the conference considered most of these groups in detail

Prof Barlow also described two new devices utilizing the Hall effect for microwave power measurement In the first, a thin indium arsenide crystal is rotated continuously in a wave-guide The resulting Hall E M F in the crystal is thus modulated at the rotation frequency, unlike the parasitic thermoelectric and rectified E M F s occurring at the crystal contacts, from which it can therefore be separated by a filter The second device was demonstrated, and comprised an array of Hall crystals in cascade which could be used to measure high-power densities in free space The multiplicity of crystals helps to cancel out the unbalanced rectifier effects occurring at the contacts

A supporting paper by R E Larson of the Boulder Laboratories of the U S National Bureau of Standards outlined the calibration facilities offered by the Electronic Calibration Center of the Bureau. These are ultimately intended to cover the measurement of power, impedance, frequency, attenuation and noise over the full frequency-range 300–40,000 Mc /s

An interesting paper by Prof C. Susskind and P O Vogelhut, under the title "Cavity-perturbation Measurements of the Effect of Microwave Radiation on Proteins", described experiments concerned with the biological effects of microwave radiation on living animals Irradiating power densities of the order of 0 1 W /cm ^{2} produce significant changes of body temperature in mice, and accordingly the 'tolerance' level for human beings is accepted as 0 01 W /cm ^{2} Apart from the heating effect, microwave irradiation causes other biological changes—in particular, it brings about a change in the hydration-rate of proteins The measurement problem involved is one of determining the values of complex permittivities of the order of 60 + 30 j, within times of the order of milliseconds These are found by measurements of the Q and resonant-frequency shift of a resonator through which the sample is passed at a constant rate

J E Pearson and J A Jane of the Radio Research Station (Department of Scientific and Industrial Research) underlined the need for power density monitors to measure power densities of the order of 1–20 mW /cm ^{2} for health hazard purposes Calibration of such meters requires a source of microwave energy of known power density and the authors described such a source utilizing parallel plate transmission line for frequencies between 0 3 and 1 5 Gc /s

Further papers presented on the first day dealt mainly with the measurement of impedance, admittance and reflexion coefficients, although papers dealing with noise measurement and problems of measurement associated with travelling wave tubes were also included The former group of papers was largely concerned with the less-conventional types of measuring equipment reflectometers, rotary standing wave indicators and 3-probe lines, together with an equipment for instantaneous impedance measurement described by P. Hargrave and S J Robinson of Mullard Research Laboratories, which utilizes seven coaxial hybrid rings, capable of giving the polar components of an impedance directly to within ± 10 per cent

On the second day the subject of power measurement was reviewed in an introductory paper by Prof A L Cullen of the University of Sheffield The present position could be broadly summarized by stating that most instruments for the measurement of microwave power are accurate to within about ± 2 per cent, although considerable difficulties still exist in providing reliable apparatus for the measurement of small powers, particularly at the higher frequencies Among hitherto unutilized effects suggested by Prof Cullen which might find application in future power-measuring devices were the use of electrostrictive and magnetostrictive materials to convert electromagnetic field energy into mechanical movement and the use of the saturation-level of maser-type devices, which occurs with increased pump-power, as a reference standard A further similar scheme, using second-order saturation effects in ferrites, was put forward as being possible by Dr P J B Clarricoats of the Queen's University of Belfast Prof Cullen also suggested that it should be possible to make a wattmeter depending on the detection of the change of electromagnetic angular momentum occurring when circularly polarized waves are incident at a conical surface

"A Survey of Microwave Power Measurement Techniques employed at the National Bureau of Standards (U S A)" was presented by G F Engen, who dealt in detail with problems of bolometric measurement errors and mount efficiency.

J A Lane gave a "Comparison of Power Measuring Standards at Microwave Frequencies", indicating that measurements of the efficiency of numerous bolometer mounts conducted in various countries, whether determined by microcalorimeters, film bolometers, impedance measurement or torque vane wattmeters, were all in good agreement at power-levels of the order of 1–2 mW The latter three methods exhibited errors generally of the order of ± 1–2 per cent, while rather better accuracy was possible with the microcalorimeter

An interesting new instrument for the measurement of power was described by Dr E Acs of Budapest The power to be measured is absorbed in a film bolometer element terminating a waveguide and the consequent heating of the surrounding air causes air convection currents in an adjacent channel The convected air stream impinges on a vane, the resulting deflexion of which is dependent on the power dissipated in the film.

Further papers by Prof Barlow and D Woods of the Ministry of Aviation described respectively a suggested method for the measurement of high-frequency power using a thin crystal of indium antimonide placed edge-on to the incident wave (it can be shown that in the absence of reflexions from the crystal edges the Hall voltage is directly proportional to the transmitted power), and a coaxial millivoltmeter/milliwattmeter which uses a silicon diode to measure voltage and power to within 0 5 per cent and 1 5 per cent, respectively, at all frequencies from d c to 1 Gc /s

L. Lewin and G H. B Thompson of Standard Telecommunication Laboratories, Ltd , introducing the afternoon session, read a paper emphasizing the difficulty of "Measurements in Microstrip" The reasons for the present unsatisfactory state of affairs are first, the lack of a simple theory on the basis of which components can be designed and, secondly, no satisfactory insertion type detector yet exists.

Two papers presented by R J Turner of the Post Office Research Station (one in collaboration with D L Lisney) covered equipment used for the measurement of group delay and insertion loss of wave-guide networks in the 3 8–4 2 Mc /s band for use in the testing of components for microwave links used for multi-channel telephony and television transmission Other papers dealt with the measurement of wave-guide attentuation, measurement of dispersion of disk-loaded wave-guide and of propagation coefficients in wave-guides with unknown but symmetrical input and output coupling Among a group of papers devoted to attenuators, a paper on "Resistive Attenuators and their Calibration", by D A J Walliker of Decca Radar, Ltd , dealt specifically with the use of metallized glass vanes and their mounts Such attenuators with micrometer adjustment can yield accuracies of 0 04 db /micrometer division at 40-db level in size 10 guide, with resetting accuracies up to an order of magnitude better than this An attenuator of this type in size 16 guide has retained its calibration within ± 0 03 db over a five-year period of laboratory use Practical calibration systems for S and X band attenuators and their general specifications were also described

The final morning was devoted specifically to millimetre wave techniques, the papers presented falling into two distinct groups, namely, those concerned with H_{01} circular wave-guide transmission and those concerned with other topics The former group included a paper by R Hamer and R J Westcott of the Post Office Radio Research Laboratory on the measurement of attenuation in short lengths of circular guide The Q factor ($\sim 10^6$) of a length of guide under test, when used as a resonator, was measured by comparing the resonator response curve when excited by a swept frequency oscillator with that of a standard reference resonator of lower Q The response curves of the two resonators are matched by adjustment of the display sweep frequency and amplitude, the attenuation constant being deduced from the readings of the attenuators used to achieve this control. The ratio of measured to theoretical attenuation for plain copper guide and copper or aluminium helix guide was of the order of 1·4 at 35 Gc./s. for a 2-in. diameter guide in all cases.

R. F. Skedd of the Standard Telephone Laboratories discussed the generation, and measurement of attenuation, of unwanted modes associated with long-haul wave-guides, while a paper by Dr. A. E. Karbowiak, G. Craven and V. H. Knight of the same establishment described an equipment for the automatic recording of wave-guide attenuation. This apparatus was capable of recording the variation of attentuation with frequency over the whole of the 40–80 Gc./s. band in a space of about ½ hr.—an operation which, without the degree of automation used, would require some thousands of independent sets of measurements. An additional paper by Dr. Karbowiak and Mr. Skedd was concerned with an instrument for the accurate determination of relative surface losses in the millimetric band.

Among the other papers, Dr. J. Brown and Dr. A. P. Anderson of University College, London, dealt with the use of a single crystal as a harmonic generator and as a detector at a wave-length of 4 mm. Such an arrangement with the addition of a simple ferrite modulator has been used to measure the impedance of wave-guide components at 4 mm., as well as to determine the permittivity of liquid dielectrics.

W. L. Birch of the General Electric Co. Hirst Research Centre described a milliwattmeter capable of detecting changes of power ~ 10 μW. for the 70–80 Gc./s. band in which the power to be measured is absorbed in a carbon load, the temperature rise in which is detected by a thermopile with which it is in thermal contact. A further wattmeter for use at millimetric wave-lengths was demonstrated by Dr. H. A. French of A.S.W.E., Portsdown, and Prof. A. L. Cullen. The instrument was of the oscillating torque vane type, was capable of measuring power as low as 100 μW., and, like its predecessors, was also capable of absolute calibration.

The construction of Fabry–Pérot interferometers and their use for the measurement of permittivity of such substances as TiO_2 was then considered in two papers by Dr. W. Culshaw and M. V. Anderson of General Telephone and Electronics Laboratories Inc., California, and by Dr. J. S. Seeley and J. C. Williams of Queen Mary College (University of London).

The final session was concerned with measurements on materials. Prof. Barlow described a method for the measurement of permittivity of high-loss dielectrics in which the specimen under test was located in an evanescent guide coupled to a resonant cavity, thus avoiding the disturbance of the field which normally results when the specimen is located within the cavity. For dielectrics with small losses Prof. G. B. Walker and S. P. Luthra of the University of British Columbia used 'ghost modes' for the determination of complex permittivity. A disk specimen of the dielectric is located in a wave-guide and a resonant mode of the specimen—the 'ghost mode'—is excited, the latter being a mode in which the fields outside the specimen are strongly evanescent and thus contain little stored energy. The limitations of the technique were discussed and a further paper by Prof. Walker and Messrs. J. M. Free and M. E. B. Moffat of the University of Oxford showed how these as well as normal cavity techniques could be used for the investigation of the properties of titanium dioxide and magnesium titanate ceramics at *S*, *X*, *J* and *Q* bands. Dr. Hamer and Mr. Westcott described a method of measuring the complex permittivity of dielectric films while R. B. Nichols and A. E. Brown of Elliott Bros. spoke about an apparatus for grading dielectric cubes according to their exact permittivity. The cubes, used for the fabrication of Luneberg lenses, possess permittivities lying between 1 and 2 which are determined by noting the position at which resonance occurs, as a movable plunger sweeps the cube through a test cavity. Further papers by Dr. Clarricoats and by C. P. Aron of Morganite Research and Development, Ltd., described a simple wave-guide method for the determination of the permittivity of cylindrical dielectric rods and an apparatus for the measurement of the tensor susceptibility of ferrite materials.

G. D. SIMS

PROGRESS IN MICROCHEMICAL METHODS AND TECHNIQUES

THE 1961 International Symposium on Microchemical Techniques, organized by the Metropolitan Microchemical Society of the United States under the sponsorship of the Commission on Microchemical Techniques, Section of Analytical Chemistry, International Union of Pure and Applied Chemistry, was held during August 13–18, at Pennsylvania State University, University Park, Pennsylvania. The excellent accommodation and facilities made possible an informal atmosphere of fellowship and individual contacts. The purpose of the symposium was to provide a forum for the interchange of information and ideas among technologists from all parts of the world concerning new methods and techniques or unique applications of microchemical or microanalytical interest.

The symposium was inter-disciplinary both with respect to the interests of the almost five hundred technologists attending and the technical programme of ninety-five papers and lectures. The symposium was truly international in character with more than twenty countries represented. Travel of many speakers to the United States was supported by a generous research grant from the National Institutes of Health, U.S. Public Health Service.

The technical sessions and plenary lectures were supplemented by a three-day commercial exhibition of equipment, apparatus, specialities and literature, by six panel discussions, film sessions on the history of microchemistry and research advances, a practical demonstration session, a popular lecture, and by various social events.

The plenary lectures were of salient interest. H. Lieb (Austria) delineated the history of microchemical techniques and A. A. Benedetti-Pichler (United States) the development of microchemistry in the United States. D. Van Slyke (United States) surveyed the evolution of micro-gasometric methods of analysis, a field in which he has long pioneered. E Sawicki (United States) considered the present status of micro methods for the sensitive and selective detection and determination of aldehydes and aldehyde-yielding substances, emphasizing his own successful studies R Belcher (England) examined methods developed for the determination of elements and functional groups in organic compounds using sample weights of 30–50 μgm , an area in which he and his co-workers have made notable advances N D Cheronis (United States) discussed the challenges encountered in the characterization of organic compounds at the microgram and especially the submicrogram range It appears that many characterization reactions are so slow at concentrations less than about 10^{-5} molar that operationally they may be considered as not occurring The technical papers covered the entire gamut of microchemical and microanalytical interests, and, in almost all cases, presented novel and previously unpublished research findings

A special session on thermo-microscopic methods due to L Kofler was prefaced by a short talk by A Kofler (Austria), which reviewed the course of her husband's hot-stage experiments A. Lacourt and N Delande (Belgium) reported an extended investigation of the identification of some sixteen amino-acids by the hot-stage observation of eutectic temperatures with twenty-five different components, of which oxalic acid and dicyandiamide appear especially good Of the general papers on microscopy, that of L W. Gamble (United States) was especially noteworthy, it introduced a new approach for the characterization of aliphatic hydrocarbons, namely, microscopic study of their crystalline urea and thiourea adducts E Wiesenberger (Germany) described how the electron microscope could be used somewhat analogously to the light microscope for the detection of substances via the observation of chemical reactions The reactions, largely precipitation of metal ions with suitable reagents or electrical or chemical reduction to metals, are conducted on the special carriers used in electron microscopy within a field of 0 004 mm ^{2}. E. Waldmann (Switzerland) considered the systematic differentiation and identification of crystalline deposits in body tissue by microscopic tests

Inorganic microanalysis was represented by many significant papers. Those of H Flaschka and P O Sawyer (United States) and of A. Ringbom (Finland) emphasized the value of photometric titrations for metal ions present in small amounts or at extremely low concentrations using either ethylenediaminetetraacetate or a chelating chromogenic agent as titrant Sawyer and Flaschka also described an elegant micro- and ultramicro-phototitrator using as the light-sensing element a commercial semi-conducting photodiode responding in such a small angular range that titrations may be performed in open daylight. J Zýka (Czechoslovakia) recommended lead tetraacetate as an oxidizing titrant for the potentiometric titration of various metals and organic compounds in acetic acid or in an aqueous medium containing mineral acid. R. Přibil and F. Vydra (Czechoslovakia) based a simple oxidimetric microtitration of cobalt on the finding that iron(III) oxidizes chromium(II) in the presence of 1,10-phenanthroline or 2,2′-bipyridyl, which also masks many interfering cations by complexation. L Erdey and G Svehla (Hungary) described the chronometric determination of metal ions based on their catalysis of suitable reactions in which the elapsed time is recorded between mixing and the appearance of the Landolt effect, the peroxide–iodide reaction permits the determination of 0 1 μgm of molybdenum within 99 per cent accuracy

Quantitative elemental organic analysis, as a major subject for microchemical research, was considered in many papers In the combustion analysis for carbon, hydrogen and sulphur, C Maresh and co-workers (United States) proposed a gas chromatographic finish. H Malissa and E Pell (Austria) reported on their recent investigations on the complete automation of the micro-determination of carbon, hydrogen and sulphur A M G Macdonald and Y Gawargious (England) gave the results of a comparative study of various high catalytic fillings in the combustion analysis for carbon and hydrogen, cobaltic oxide appeared to be the most satisfactory for general work J A Kuck and co-workers (United States) described an instrumented combustion method for carbon and hydrogen in which carbon dioxide is assessed by infra-red absorptiometry and hydrogen by thermal conductivity

G Ingram (England) introduced a direct ignition in an oxygen-filled chamber at 850° C for the determination of carbon, hydrogen, halogens and sulphur, the combustion products are swept into appropriate absorbing reagents and determined. The method requires only 30 min or less per sample and is applicable to difficult materials, such as organic fluorine or phosphorus compounds W G. Guldner and A L. Beach (United States) based a rapid micro determination of oxygen on pyrolysis of the organic sample in a helium stream in an induction-heated graphite crucible and oxidation of the carbon monoxide, after trapping of unsaturated compounds, to carbon dioxide, which is absorbed in barium hydroxide solution and determined conductometrically J. L. Brownlee, jun (United States), presented an intriguing approach to the determination of oxygen in organic compounds involving a neutron-induced reaction in an atomic reactor, namely $^{6}\mathrm{Li}(n,\alpha)^{3}\mathrm{H}$, $^{16}\mathrm{O}(t,n)^{18}\mathrm{F}$; fluorine-18 is separated and determined radiochemically

Noteworthy papers in organic functional group analysis included that of J E. Zarembo and M. M. Watts (United States) on the determination of the carbodiimide function, of E Sawicki and co-workers (United States) on a highly sensitive spectrophotometric determination of azuelene, and of W Merz (Germany) on the determination of active hydrogen using alkyl-substituted metal aluminium hydrides C A Rush and J I Miller (United States) proposed barium isopropyl methylphosphonate as a rigorous reference standard for carbon, hydrogen, phosphorus, barium and alkoxyl S R Palit and P Ghosh (India) based a determination of acids, bases and salts at micromolar concentrations in benzene on the colour behaviour with rhodamine and certain other dyes previously extracted by benzene under specific conditions M. Bobtelsky (Israel) considered his recent research work with microheterometry in work on the reactions and nature of large organic molecules, including dye lakes and metal–protein complexes

Physical measurements on the micro scale also received attention The use of a dynamic flow technique in place of the usual static drop method was recommended by C. L Ogg and co-workers (United States) for the thermistorized determination of molecular weight W Simon and co-workers

(Switzerland) described refined thermistor circuitry and instrumentation for the routine, precise determination of molecular weight using a sample weight of only 2 5 mgm for a molecular weight of 500 P D. Garn and J E Kessler (United States) demonstrated that thermal analysis is feasible for sample weights less than 1 mgm by the use of appropriately designed sample holders

In the session on nucleonics, G F Morrison and J F Cosgrove (United States) described the application of neutron activation to the sensitive determination of the copper activator in single crystals of electroluminescent zinc sulphide weighing less than 500 μgm. B A Thompson (United States) considered how radiochemical techniques and especially neutron activation could be applied to the determination of the principal components, trace impurities and thickness of metal films a few angstroms in thickness and one square centimetre in area, such films are encountered in the performance and design of electronic devices, including vacuum tubes M T Kelley (United States) reviewed the present status of the analysis of highly radioactive reactor fuels and spent products using liquid samples of 5–700 μl , coulometry, spectrophotometry, flame photometry, emission spectroscopy, titrimetry and radiometry, and density measurements are feasible by remote controlled, automatic or 'slave' manipulated operations A Holasek and co-workers (Austria) explained the difficulties and operational problems encountered in the synthesis of milligram amounts of fatty acids and esters labelled with carbon-14, and considered salient factors involved in the selection of suitable synthetic methods

Chromatographic methods were the subject of many interesting papers E. G Wollish (United States) considered critically the accelerating interest in alumina and silicic acid for thin-layer chromatography in the separation and identification of organic compounds, including drugs. H K Mangold and co-workers (United States) exemplified how thin-layer chromatography acts as a valuable analytical and preparative tool in lipid chemistry L S Ettre and W Averill (United States) demonstrated how new gas chromatographic instrumentation using capillary (Golay) columns and ultra-sensitive detectors permit the direct analysis of trace components in samples of micro-size, and set forth criteria for the selection of methods and instrumentation L Wiesner and W -J Schmidt-Kuster (Germany) described the gas-chromatographic performance of a new integrating counter-detector using a flow-type proportional counter containing a source of ionizing radiation and used with a suitable scaler T S Ma and M Gutterson (United States) recommended gas chromatography in the determination of several organic functional groups S Patton (United States) considered the identification of food flavours using a diversity of techniques including gas, paper and column chromatography, and the study of the dinitrophenylhydrazones of volatile keto-compounds Paper chromatography was applied by B Bobrański and L Syper (Poland) to the identification of drugs containing unsaturated radicals following reaction with mercuric acetate A K. Dey and E J Singh (India) explained the behaviour and application of various complexing agents in the separation of metal ions by the Weisz ring-oven technique

The contemporary position of small-scale methods in the teaching of chemistry at the secondary school and collegiate levels was assessed in a special session with speakers from Northern Ireland (C L Wilson, N N Gilpin), England (J B Headridge, H Holness), and the United States (N D Cheronis, T S Ma and J. A Kuck). A Steyermark (United States) considered the training of chemists in industry in quantitative organic analysis

When the *Proceedings* of this symposium become available as a single volume (Interscience, early 1962), the interested technologist will be able to ascertain the position of microchemistry and microanalysis for 1961 in the spectrum of scientific activity, and to comprehend the research trends that will yield the 'to-morrow' of these disciplines In brief, microchemical and microanalytical research faces continuing challenges with ever smaller samples, ever more minute components, coupled with demands for adequate accuracy, precision and rapidity Improved instrumentation, automation and new techniques and reagents, coupled with re-assessment of present methods and approaches, will energize further advances

A J BARNARD JUN
JOHN Y STEEL

INSECT POLYMORPHISM

A SYMPOSIUM on polymorphism in insects, organized by the Royal Entomological Society of London, was held at the Imperial College of Science and Technology, London, during September 21–22 The president, Prof G C Varley, was in the chair at the first meeting and expressed a hope that special attention would be directed to the agencies promoting and controlling this type of variation

Prof O W Richards, in a general introduction, stated that polymorphism is very widespread among the insects and occurs in all the major orders The metamorphosis of insects, itself a mode of polymorphism, perhaps makes them especially prone to such variation There are two principal types of polymorphism the genetic type, where two or more genotypes co-exist in a state of balance, and the induced type, in which some variable environmental factor such as population-density, food, day-length or temperature leads to the development of two or more kinds of individual in a species

Dr E. B Ford, in an account of the theory of genetic polymorphism, stated that the standard examples are only special cases of a more general phenomenon which is often overlooked when the characters involved are not easily visible This is the tendency for selection to favour the heterozygote over both homozygotes when, after some change in the environment, a rare major gene begins to spread The favourable effects of the gene will tend to become dominant and the unfavourable ones recessive, and the heterozygote will have all the advantages and none of the disadvantages of the homozygotes. Where polymorphism results from the interaction of several genes, the whole complex is controlled by a supergene, the link between the units of which may be strengthened by a chromosome inversion The original effects of the controlling mechanism are always liable to evolutionary change by selection of the remainder of the gene-complex

Dr P M Sheppard then reported on recent work on polymorphic mimetic swallowtail butterflies Knowledge of the way in which mimicry arises and is maintained makes possible a number of predictions as to how the polymorphism should be controlled genetically These predictions can then be compared with the genetics of the polymorphic mimics so far as these have been unravelled The most striking results have been obtained in the African *Papilio dardanus*, in which several different forms of female, some mimetic and some not, correspond to a single type of male Recently, methods have been devised for breeding the butterfly artificially in England and a genetic analysis combined with field data on population statistics show a good agreement with theoretical predictions

Prof T Dobzhansky dealt with the dynamics of chromosomal polymorphism in *Drosophila* In the giant chromosomes of the larval salivary glands one can recognize individuals which as a result of inversions of parts of chromosomes have different gene arrangements Several chromosomal types may occur in one population and their proportion may be constant, vary seasonally, or over longer periods, or geographically Such changes in proportion may directly demonstrate the action of natural selection and allow calculation of the selective advantages of the polymorphs The mere order of the genes may itself be an element in the fitness of a polymorph, but perhaps more often an inversion by suppressing crossing-over preserves an arrangement which gives an advantage to heterozygotes Several of the field phenomena can be reproduced in cage populations. The proportions of the polymorphs are easily modified by changes in the environment or in the genetic background Crosses with stocks from widely separated sources have an unpredictable effect on the proportions of the inversions because of the large number of alternative backgrounds provided

The next four contributions were concerned with familiar examples of non-genetic polymorphism, namely, in Hymenoptera, in termites, in aphids and in locusts Prof C D Michener dealt with the first topic and showed that in only a few bees and in ants is the worker caste sharply different from the queen In most social bees and in wasps the differences are slight and overlapping, occurring mainly in behaviour and physiology, but in several ants the worker caste is itself polymorphic. In the least differentiated castes of some halictine bees, the slight differences arise in the adult stage but the castes of all other Hymenoptera, except perhaps *Melipona*, seem to depend on differences in nutrition, sometimes interacting with temperature At some point in development, however, the caste becomes determined In *Pheidole*, the workers produced from a queen's first batch of eggs are small, even if they were well fed, the type of worker is thus determined in the egg, but perhaps here through the nutrition of the queen which laid them The selective advantages of the castes of advanced insect societies is clear but their evolution is anomalous, since the first step seems to be the reduction of fertility in some individuals However, it appears that economy of labour and better defence of the nest make even the most primitive social organization (one queen and one worker in some halictine bees) more viable than isolated queens.

Dr M Luscher gave an account of social control of polymorphism in termites, one of the most astounding of all forms of social regulation in animals Termites may have up to seven different castes in most of which each sex is equally represented The newly hatched insect is capable of developing into any of the castes by a series of moults Some forms may also have regressive moults leading back to an earlier stage in the series Caste determination mainly acts by directing moults in the required direction, but the workers may also reduce a caste to its correct proportions by selective cannibalism The direction of moults is influenced by active substances or pheromones which are exchanged among members of the colony This is most clearly established in the inhibition of the reproductive caste by members of the same caste already present Luscher has shown by fixing a queen in a screen so that her head is in one colony and her abdomen in another, and then varnishing over parts of her body, that the active substance comes from the anus Complete inhibition, however, requires the presence of a male as well as a female reproductive, so that there seem to be two substances Another one is probably involved in the inhibition of the soldier caste There are also stimulating substances, more males, for example, being produced in a colony with a queen but no king than in one without either Termites are only responsive to such influences for part of the time intervening between each moult The substances probably act through an effect on the ductless glands of the insect There may be as many as eight different pheromones, but none is as yet chemically determined

Dr. A D Lees then described the clonal polymorphism of aphids, especially in *Megoura viciae* Aphids may reproduce viviparously and parthenogenetically or be fertilized and lay eggs They may be winged or apterous and there is more than one kind of wingless female The full development of some of the morphal characters may appear gradually during several successive generations Other morphs (for example, sexual-parthenogenetic) are strict alternatives Controlling mechanisms are almost all environmental, acting through the endocrine system Aphid reproduction is extremely precocious so that an embryo will already have ovulated at its deposition and it is thus necessary to determine which of the three generations is being affected Males can be suppressed by suitable temperatures or in some species photoperiod but are otherwise produced in a characteristic percentage Egg-laying females are produced at photoperiods of less than 15 hr and temperatures of about 15° C Winged forms arise as a result of crowding and this is not an indirect effect acting through the host-plant There is also an 'interval-timer' which does not allow sexual forms to be produced for some time (50–60 days at 15° C) By selecting the first and last members of each generation to start separate lines, it can be shown that the interval is a matter of days and not of generations

Continuous polymorphism in locusts described by Dr. J. S Kennedy is different from most of the other examples in that the morphological differences are relatively slight and intermediates are often commoner than the extremes Yet not only the morphology but also almost every aspect of behaviour and physiology are somehow affected Phase transformation is largely controlled by population-density, which may act directly or on the previous generation. Genetic make-up, temperature and other factors may also have some importance The solitary phase may be regarded as more juvenile than the gregarious one, and recent experiments have shown that the prothoracic gland which normally determines a juvenile morphology persists throughout life in the *solitaria* although disappearing early in the adult *gregaria* Dr Kennedy suggested that *solitaria* is adapted to a 'soft' habitat where food is available without much movement *Gregaria* is suited to a 'hard' habitat which can be exploited only by the continuous movement which is typical of the phase. The polymorphism as a whole allows the species to exploit a much wider habitat than one phase could alone

Dr J H Sang, speaking on the environmental control of mutant expression, dealt with a developmental problem In the course of growth a gene may produce a graded response in an enzyme which nevertheless has a discontinuous effect on adult development Thus the gene 'antennaless' in *Drosophila* determines 0, 1 and 2 antennæ but nearly always perfect structures This is because the action of the gene occurs during a very short but well-defined period, namely, between the seventieth and eightieth hours of larval growth. This is shown by transferring larvæ of various ages to a different culture medium, for the effect of the gene can largely be nullified by suitable food. Such examples suggest how polymorphism might be determined by either a genetic or an environmental switch mechanism, in which influences at the right state of growth may produce large and discontinuous changes in the adult

Prof V B Wigglesworth, in a tentative synthesis, drew analogies between the polymorphism of cells in different parts of the body, the polymorphic aspect of insect metamorphosis and the phenomena which the other speakers had discussed He showed that there seemed to be a complete transition from variation in which genetic determination was almost absolute to that in which external environmental factors decided between several alternatives

The animated discussion which followed the invited contributions did not bring forward many really new points but was chiefly concerned with the relative importance of genetic and non-genetic factors in particular examples Terminology was also discussed, and some speakers were unwilling to speak of polymorphism unless the variations were clearly discontinuous The suggestion, however, was made that the best distinction was between situations in which there is selection for increased variability as opposed to the more normal case where deviations are discouraged O W RICHARDS

MOLECULAR BIOLOGY AND POPULATION STRUCTURE

A SYMPOSIUM on "Molecular Biology and Population Structure" was held at Norwich on the afternoon of September 4 at a joint session of Sections D (Zoology) and K (Botany) of the British Association for the Advancement of Science The aim of the symposium was not so much to present new discoveries as to demonstrate to the audience the interrelations of recent discoveries in these fields, and to discuss some of their implications

The symposium was opened by Dr T. Alderson (British Empire Cancer Campaign), who described what we know of the structure of deoxyribonucleic acid (DNA) and brought out some of the evidence that genetic differences as we observe them are the resultant effects of differences of DNA molecules Dr J A Pateman (University of Cambridge) followed with a discussion of the evidence that gene differences have their effects by influencing the structure of protein molecules, though in discussing, at the end of his review, the recently discovered class of genes that appear to have the capacity of "turning on and off" whole metabolic sequences, he pointed out that there is evidence that these do not operate by producing proteins These new regulator genes may well prove of major importance for our understanding of differentiation Prof H. Gruneberg (University College, London) then made the connexion between enzymes and more conventional characters by describing some beautiful examples, from his work with mice, which showed how the little invisible differences in chemical structure may be magnified during embryological development into the larger more obvious differences with which the study of genetics began

At this point the symposium turned to what at first sight was another topic altogether, when Prof J. M Thoday (University of Cambridge) described how a variety of genes may be maintained in a population if that population were exposed to a variety of demands Dr P M Sheppard (University of Liverpool) then went on to discuss some of the evidence, illustrated from his work on butterflies, that the variety of genetic types in a population are not only directly adapted to the variety of conditions which the members of the population can exploit to promote survival, but must also be adapted to one another The population is an integrated unit, and over the generations the genetic contents of different components of the population are judged not only by their individual success but also by the degree to which they co-operate with one another when brought together in sexual reproduction The chromosomes of a population have not only to have genetic properties that are to be understood in terms of the needs of individuals but also other genetic properties only to be understood in terms of the population as a whole Among other things the chromosomes of different individuals have to be co-adapted to one another if the population is to function successfully over the generations

Dr C. A Clarke (Royal Infirmary, Liverpool) followed Dr Sheppard with a little of the evidence that all these principles apply to man just as much as to *Drosophila*, or butterflies

Prof J M Thoday gave the final paper He pointed out that the two most striking discoveries of modern genetics had been brought together in this symposium

The first discovery concerns the molecular nature of the genetic material It has, of course, long been supposed that genes are molecules of some sort or another, but the precise knowledge of their molecular nature we are now acquiring is one of the most spectacular achievements of biology

The second discovery is of a different kind and was less expected. Both mathematical studies concerning the way genes might be supposed to behave in populations, and the biochemical concept that genetic variation means variation of the enzyme content of individuals, led us to expect that a population should be rather uniform genetically, each individual having much the same genetic make-up as other individuals Further, this is the expectation we seem instinctively to tend towards in our attitude to our own populations, in our concept that the people we know and with whom we have a sense of belonging are essentially like ourselves

It was this belief in the relative genetic uniformity of populations that gave such interest to the pioneer investigation of cases of obviously polymorphic populations which formed evident and striking exceptions to this expectation. Polymorphic populations are populations in which two alternative genes are both frequent, whereas simple theory would lead us to expect one to oust the other so that the population would tend to uniformity One case of polymorphism, sickle-cell anæmia in man, mentioned in a number of the papers, provides an example where population genetics and molecular biology tie up closely together

Study of such polymorphisms not only led to their explanation but also helped us to make the discovery that cases of polymorphism such as Dr Sheppard discussed are far from being exceptional They are only the striking and obvious examples of what is a normal phenomenon For the expectation of genetic uniformity is not in the least borne out by fact in any well-studied population The facts are quite contrary. Populations of sexually reproduced species consist of a wide variety of genetically different individuals and the genetic differences are in fact usually greater than the differences that we can see So great is this genetic variety that we may go so far as to say that no two individuals (apart from identical twins) are genetically alike This applies not only to flies and butterflies but also to man, in fact the evidence that it is true for man is probably more complete than it is for any other organism Each of us is genetically different from all the others. This genetic or biochemical individuality is one of the major facts of life

Along with the discovery of this genetic, or biochemical, individuality has come explanation of it at least in general terms The basic explanations are two

The first invokes the fact that we live, and all our ancestors have lived, in an environment that changes slowly with time It changes as the climate changes, and it changes as other organisms change We, that is the species to which we belong, have therefore inherited from the ancestral populations from which we have descended the means to change as the environment changes, a means that permits change in directions the necessity of which could not be foreseen. Sexual reproduction which permits the generation of a variety of genetically different individuals, and death, which permits the elimination of those individuals less-well adapted or no longer adapted to the environment, provide that means of change Sexual reproduction, however, only permits the generation of a variety of genetically new individuals, it does not ensure it unless there already is a variety of individuals. Hence, in the long term, natural selection in a changing environment must result in the continued survival only of populations that contain and always contained enough genetic variety to be able to produce the new variants that new environments require.

The second explanation is one which has developed more recently It is not an alternative to but supplements the first This explanation invokes the fact that the environment in which a population lives is not uniform in space Different parts of the population meet different environments Hence we must expect that, in different parts of a population, genetically different kinds will tend to be the fitter, so that variation of the environment in space must lead to the selection of genetical variety within the population The results of the laboratory work done to test this view lead to the conclusion that this is a most powerful factor, selection for variety is astonishingly successful

We therefore begin to have an understanding of the function in life of this surprising biochemical variety that has been found in natural populations It is the basis of the means of maintaining the population successfully in conditions that vary with space and change with time, and it is the prerequisite of innovation

Prof Thoday went on to discuss at some length some of the implications of this knowledge as it applied to man, pointing out that, though we know little of the genetics of normal variation in man and need much research on it, we do know enough to be sure that within the range of what are called normal humans there is every sort of variety to be found Each of us is different in molecular constituents that affect all classes of characteristics, whether physical or mental How much vitamin we need to remain healthy, what our normal blood pressure is, what our taste preferences may be, how sociable we are, how quick in the uptake, how liable to fall to temptation, most things will be affected by our innate molecular constitution as well as by our upbringing

This is not to deny the plastic adaptability of *Homo sapiens*. Plasticity of the individual is one of the striking properties of man, and environment in the general sense, which includes other people and upbringing in particular, can have great effects on the characteristics of human individuals Nevertheless, we may be sure that the effect that a particular environment and upbringing will have on one man will differ from its effects on other men Their genetic differences will ensure that this is so

Now this is a consequence of our knowledge to which we shall find it difficult to adjust ourselves, but it has a bearing on almost every important human topic we discuss, because most important problems in some way or other involve the question of the justice of inequality in human society Contemporarily, questions of equality in educational opportunity and the justice of giving the more promising, in so far as we can assess them, better opportunities in grammar schools, provide an excellent example of a topic in which the facts of genetic variety are seldom dispassionately considered But we must, some time, face the question of the relation between such topics and our knowledge that most, if not all, differences between people, and between classes of people, are in part genetic as well as being in the remaining part environmental in causation. Our sense of justice goes against accepting the fact that some people are biologically better endowed than others from the point of view of their capacity to profit from education and their capacity to return to society a profit on the cost of their education But it is a fact nevertheless life is not just in this sense It is time that we realized that all men are of equal value just because all men are different Some are better for some purposes, some for others and their variety, which is the basis of the richness of life, is at their initiation there, a cardinal fact of life, arising from the uniqueness of the molecules that form their basic genetic endowment Society should be organized to profit from that individuality, not suppress it, however inconvenient it may be to bureaucrats and mass-production marketers Further, if we could but accustom ourselves to the fact of human variety we might less often fall into the errors to which the type concept leads in our attitude to the differences between classes and between races The hidden genetic variety within a group is much more striking than are the evident differences that distinguish groups within a species

J M. THODAY

BIOLOGY IN BRITAIN

PROGRESS within any major branch of science is inevitably uneven Thus, there has been in the post-War period very rapid scientific advance in certain aspects of biological science particularly amenable to study by modern chemistry and physics Unaware of the outstanding problems of biological science this has led some authors to advance such judgments as "modern science has all but wiped out the borderline between life and non-life"[1] Such over-enthusiastic assessments in their extreme form amount, as Commoner[2] has pointed out, almost to a declaration that biology as a separate science is no longer needed Perhaps more dangerous is the reaction which now calls for a unifying of biological science by amalgamating existing disciplines in a way which, in practice, effects their contraction or elimination in favour of new, fashionable but frequently very restricted 'subjects' Further, the call for such changes is often prefaced by the contention that established biology teachers are out of touch with modern biology. Dr Wyatt's recent article[3] is tinged with this approach

His statement "the classical biology of pre-War years has changed little" is placed in a context which clearly implies its decreasing relevance to the main stream of biological thought rather than emphasizing that its slower development arises both from the resistance of many of its major problems to rapid solution and a lack of research resources His contention that the American biology graduate is better equipped to teach other subjects (a rather doubtful role), to read the journals and to do and to direct research is too sweeping, despite the excellence and breadth of the biological education offered in some American universities His contention that British students may learn more about modern biology from *The New Scientist*, the Penguin Science Survey and television than they gain from the isolated subject studied at a university at best displays lack of knowledge of many existing degree courses and of the separate and complementary role of important media of general scientific education

Dr Wyatt's solutions for the difficult problems facing many of us who are concerned with training biologists are too easy For example, he finds the course for the diploma of technology in applied biology far, I imagine, beyond the hopes or intentions of its originators, to be more suited for academic biology and research than are the courses (and this presumably means any and all courses) offered at the universities

It should be emphasized that there is ample evidence that, in Britain and other countries, teachers of biology are at present deeply involved in a re-assessment of their teaching programmes For example, in March of this year, at a meeting attended by almost all the professors of botany in British universities, very careful consideration was given to such problems as the sixth-form courses most appropriate for those intending to become biologists (emphasizing the need for basic training in the physical sciences and in mathematics), the rapidly changing character of university courses and the need for improved facilities, particularly for teaching in biochemistry, physiology and genetics, the influence of present Government statements on the recruitment to biology of first-class students and the financing of fundamental biological research Further, new science buildings are being used to bring under one roof, and hence into close contact, groups of related departments, as exemplified by the new Biology Building at Birmingham and the Natural Sciences Building in my own University College Biologists who, in their undergraduate courses and postgraduate work, have specialized in biochemistry, biophysics, microbiology and genetics are increasingly being appointed to existing departments of botany and zoology

Dr Wyatt rightly raises the question of the establishment of departments of biology as against continuing along a pathway of increasing fragmentation This is in line with the recent memorandum of a Royal Society Committee consisting of fifteen of its Fellows, representative of all the major fields of biology Insulated, introspective departments, if entrusted with the teaching of any branch of biology, will inevitably tend to deny to their students the value of the collaborative activity which has so evidently been effective in making many recent advances in our science Nevertheless, there are dangers and difficulties in fully and quickly effecting the proposed re-organization One of the difficulties hampering effective biology teaching is only now being eased by the present rapid expansion of higher education. Only recently in many institutions has it become possible to build up towards a teaching staff in biology whose training and research interests are sufficiently varied to give the desired range of related courses One of the dangers is revealed in Dr Wyatt's approach when, specifically mentioning as staff members, an ecologist, a physiologist, a biochemist, a microbial geneticist, a plant pathologist and perhaps an embryologist, he contends that courses in zoology, botany, biochemistry and microbiology, as well as in chemistry, can be made available by a biology department The danger here, in its extreme form, is that 'molecular biology', instead of being visualized as a major and active field of discovery, is put forward as already synonymous with the whole of biology This danger is, in fact, at present a matter of deep concern to many distinguished American biologists The Royal Society Committee, taking a broader view of the scope of biology, clearly recognized the need for some 6–8 professorial appointments together with a substantial number of non-professorial staff in order to establish a really effective unit for teaching and research in the biological sciences

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If biologists wish to avoid "molecular biology", "in its extreme form", then we must devise courses that will attract potential physical scientists Can we be complacent when biochemists are becoming more divorced from biology and when more of the better students study neither botany nor zoology ? While I argued for experimental biology, at the same time I emphasized the greater need for studying live animals and plants not only in field studies but also in the laboratory. It is not that our established biology lecturers are out of touch, but that our organization, syllabuses and examinations are. Because I believe that biology is relevant, I wish to see more physical scientists study it

The memoranda of the Royal Society and of the professors of botany are both in private circulation; other biologists would be glad to read them

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CONQUEST OF HUNGER

A SYMPOSIUM on food supply and population was an important feature at the meeting of the British Association for the Advancement of Science in 1960 in Cardiff Papers bearing on this theme were delivered by a number of leading authorities, followed by discussions (see *Nature*, 188, 9, 1960) The papers have now been published by the British Association in a booklet called *Hunger Can it be Averted ?* edited by Sir John Russell and Dr Norman C Wright* The booklet, with a preface by Sir John, contains the following articles "The Current Food Supply Situation and Current Trends", by Dr Norman C Wright, "Population Prospects and their Implications", by Prof D V Glass, "Economic Conditions for Greater Output", by Prof W A Lewis, "Land Use and Food Production", by Prof L Dudley Stamp, "Industrial and Technological Needs", by Lord Fleck, "What Limits Food Production ?", by Prof H D Kay, "Some Conclusions", by Prof P M S Blackett There is an appendix, with seventeen figures, entitled "Vast Population Increases Intensify the Battle Against Hunger"

The booklet presents the main facts about food supply and population lucidly and without emotional bias, as might be expected from the high status of the contributors What are these facts ? If world population continues to increase at the present rate it will double itself by A D 2000, and nothing likely to happen within the next few decades will reduce this rate of growth, due largely to 'death control', which will become steadily more effective In fact, the rate of increase will probably accelerate as the expectation of life rises in the developing countries and the saving of child life—the field in which modern medicine and public health have achieved their most striking successes—produces a population structure particularly favourable to high fertility.

At present large numbers of people in the world are under-fed and badly fed, so that existing food supplies are inadequate To feed the much larger population of 1980–2000, much more food than is now being produced will be needed. The quantities required, at given levels of consumption, can be roughly estimated Potentially, they can be made available by distributing surpluses, bringing new land under cultivation and increasing yields For economic and political reasons the contribution of surpluses is unlikely to be important There are wide areas, at present unproductive, which can be brought under cultivation at great effort and cost, but the most promising possibilities lie in increasing yields from land already under cultivation Sufficient technical knowledge already exists to increase yields enormously in most parts of the world, and this knowledge is being extended year by year There is plenty of dramatic evidence showing what can be achieved, for example, by using more fertilizers It is a familiar fact that yields of rice in Japan are 3–4 times greater than in India, a difference due to the large amounts of inorganic fertilizers used in Japan, combined with improved seed varieties, controlled irrigation and efficient cultural practices The crux of the matter is whether solutions can be found to the economic, political, social, educational and administrative problems which prevent technical knowledge from being effectively applied As Prof Blackett puts it "to raise the low level of much of the indigenous agriculture and to bring under cultivation more of the abundant cultivable land requires a massive educational and social advance combined with heavy capital investment in communications, housing, schools, agricultural implements, fertilizers, irrigation and drainage and, as Prof Kay has pointed out, barbed wire". The key lies in the "massive educational and social advance"

Departments of agriculture in many countries are at present under-staffed and often ineffective, and great efforts are needed to establish extension services to bring modern techniques to cultivators Food crops for local consumption usually receive less attention from agricultural departments than cash crops Technical assistance to developing countries in fields concerned with food production should be given on a much greater scale These and numerous other facts with an important bearing on the situation are discussed by the contributors.

While the booklet deals adequately with population trends and prospects, it does not go deeply into the basic issue of population control. It is primarily concerned with practical and inescapable problems of food supply and population which mankind must face in the near future. On these it says nearly all that can be said and much that has been said before. The picture is indeed clear enough. In the preface,

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* *Hunger Can It Be Averted ?* Edited by Sir E John Russell and Dr Norman C. Wright Pp v+80 (London British Association for the Advancement of Science, 1961) 5*s*

Sir John Russell comments "the broad conclusion was that hunger need not be feared for at least the next forty years and this result could be achieved on present knowledge if only it were properly applied

These optimistic conclusions from such an authoritative group come as a great relief after the pessimistic gloom that has long surrounded the subject" The booklet may not produce this impression on all its readers, who should be many

W R AYKROYD

PLANT PHENOLICS GROUP OF NORTH AMERICA

THE recent increase of interest in the biology and chemistry of the phenolic constituents of plants becomes immediately apparent on inspection of the lists of contents of many of the world's biological and chemical publications However, workers in the various fields embraced by an interest in the plant phenolics are widely scattered throughout departments of botany, biology, biochemistry, chemistry, microbiology, pharmacy, pharmacology and forestry, in universities, research institutes, government agencies and in industry There is consequently almost no common meeting place for people with such interests, with the result that there is little discussion and exchange of information between workers of the different disciplines

In 1956, the Plant Phenolics Group was founded in Great Britain as an informal organization with the aims of promoting "the advancement of the knowledge of phenolic and related constituents of plants in respect of their chemistry, function, biosynthesis, effect on plant and animal physiology and pathology, and the application of such knowledge in agriculture and industry". Since its founding the Plant Phenolics Group has been very successful in achieving these aims, holding two or three meetings a year The proceedings of many of these meetings have been published

At the ninth International Botanical Congress in Montreal in 1959, discussions with various biochemists and physiologists interested in plant phenolic substances led to the idea that a sister organization be formed in North America The largest problem with forming such a group on this continent, of course, results from its geographical size, which would prohibit such a group from meeting more frequently than once a year.

An organizing committee, consisting of Dr. T. A Geissman (University of California), Dr V. C Runeckles (Imperial Tobacco Co of Canada) and Dr G H N Towers (McGill University), distributed a letter to interested persons in 1960, the response to which was so enthusiastic as to encourage the committee to make plans for an inaugural meeting This was held at Colorado State University, Fort Collins, during August 31–September 1, and took the form of a symposium on the biochemistry of plant phenolic substances. The meeting was sponsored by the National Science Foundation under the direction of Mr. G Johnson of Colorado State University, and Dr Geissman The papers presented were "Recent Studies on the Structures and Bitterness of the Flavonoid Glycosides of Citrus" (Dr R. M. Horowitz, U S Department of Agriculture, Albany, California), "Biosynthesis of Plant Phenols" (Dr. S A. Brown, National Research Council, Saskatoon), "Naturally Occurring Biflavonyls" (Dr. N. Kawano, University of Nagasaki Japan), "Metabolic Fate of Phenolic Substances in Animals" (Dr A. N Booth, U S Department of Agriculture, Albany), "Role of Plant Phenolics in Disease Resistance and Immunity" (Dr I Uritani, Nagoya University, Japan), "Melanin and its Formation" (Dr. G. A Swan, University of Durham) and "Estrogenic-like Substances in Plants" (Dr E M Bickoff, U S Department of Agriculture, Albany) It is hoped that the proceedings of the symposium will be published

More than sixty delegates attended the symposium, the last session of which was devoted to founding the Plant Phenolics Group of North America The officers elected were: *president*, Dr S H Wender (University of Oklahoma), *vice-president*, Dr L Jurd (U S. Department of Agriculture, Albany), *hon secretary-treasurer*, Dr V C Runeckles

It was agreed at the first meeting that membership of the Plant Phenolics Group of North America be open to all persons interested in the Group's aims, on application to the secretary, Dr. V. C. Runeckles, P O. Box 6500, Montreal, Quebec.

V. C RUNECKLES

RESEARCH WITH TROPICAL PLANTS

THE Tropical Products Institute of the Department of Scientific and Industrial Research is continuing its work on the examination of plants from tropical territories, and a recent report outlines the scope of investigations carried out during 1960*

Any new sources of drugs could be of great economic benefit to the countries to which the plants are indigenous, and the Institute's activities follows representations from overseas governments for technical assistance in this field Already 314 plants have been tested, including specimens from North Borneo as well as samples from Kew Gardens A number of alkaloid-containing plants have been found and are being subjected to further tests The Director of the Institute reports the expansion of economic and long-term research work by the Institute to meet the needs of rapidly developing tropical countries This reflects a trend away from short-term inquiries in order to help newly established laboratories overseas with their research and development problems Even more important is the demand for qualified man-power in the territories themselves, and the Institute has helped by sending visiting scientists to 39 overseas territories and by training research workers from five different tropical countries More than 900 inquiries for advice and assistance were received from 62 countries during the year. While only 10 per cent of these inquiries required laboratory investigation, they took up about three-quarters of the Institute's advisory effort.

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* Department of Scientific and Industrial Research *The Report of the Tropical Products Institute Committee with the Report of the Tropical Products Institute* Pp viii+40 (London H M S O, 1961) 3s net.

At the request of certain Malayan pineapple producers, the Institute is investigating the reason for differences between the flavour of Malayan pineapple juice and Hawaiian and South African juices. This may be due either to the effect of local canning process on the flavouring constituents or to varietal differences inherent in the plants. Many countries have a seasonal surplus of fruits and vegetables, and the advice of the Tropical Products Institute has been sought on methods of making better use of these supplies. Although small-scale canning of surplus fresh fruits and vegetables is not generally recommended as the quality and quantity are unlikely to be sufficient for effective processing, advice was given to Fiji and Nyasaland on how to set up a small-scale canning line; to Kenya on packing pineapple slices in plastic cases; to Singapore on making cashew apple wine; and to Aden and Tanganyika on producing tomato paste or purée.

In many parts of the tropics, surplus coconuts are made into copra by drying the kernel in the open air or in special kilns. Most copra is made by the small peasant farmer and quality control is difficult. As moisture content is a critical factor in the keeping qualities of copra, the Institute is examining the effectiveness of a simple moisture meter, which was originally developed at the Pest Infestation Laboratory for testing grain. A simple portable meter of this kind could easily be used by local farmers.

For some years the United Kingdom has been looking to the Commonwealth for new sources of naval stores (turpentine and rosin). Three hundred gallons of pine oleo-resin from Mauritius were examined at the Institute to see whether it would yield good commercial quality turpentine and rosin. The samples compared favourably with American products, and it is hoped that Mauritius will be able to improve its economy by starting a small naval stores industry based on existing plantations.

Work has also begun on an improved type of solar still and the first model is being tested in Malta. In addition, water-heating equipment using solar energy, developed at the Institute in London, is now being tested under field conditions in Malta. It comprises two fully instrumented flat-plate water heaters for research purposes as well as domestic flat-plate heaters for the roofs of private houses and blocks of flats.

FORMATION OF AIR–WATER FROTHS

By J. RENNIE and W. SMITH

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FOR the absorption of gases in liquids or for distillation on a large scale, effective contact between the two phases is obtained by dispersing gas or vapour at a high rate into a pool of liquid. Froths or foams are formed, giving a good rate of heat or mass transfer across the phase boundary. The generation of these froths is in no way dependent on surface activity: froths can be formed from pure components (Fig. 1), so that the surface tension is uniform in the pool. These froths are not stable: they collapse the instant the gas supply is removed.

One very common and simple device used for the dispersion of gas is the sieve plate. This is a flat plate, drilled with evenly spaced holes, from 1/16 to 3/8 in. in diameter, occupying 5–20 per cent of the total area of the plate. Liquid is generally run across the plate, being maintained in depths of 2–8 in. by weirs, and the gas is dispersed upwards through the holes.

In studying the performance of sieve plates, we have concentrated on froths formed by dispersing air in water. The complicating effects of mass or heat transfer between the phases, and the resulting non-uniform distribution of physical properties in the pool, have thereby been avoided. Preliminary work has been mainly concerned with devising techniques for measuring accurately the surface area and density of the dispersions[1]. It has been found that two kinds of dispersion are obtained, depending on gas-rate; relatively low gas-rates produce a stable, immobile foam of large bubbles, and high gas-rates produce an actively moving froth. It is the froth which is of practical interest, owing to the higher distillation or absorption-rates obtained.

Examination of single short-exposure photographs of froth produced over a range of conditions has shown that the important physical characteristics of the froths, such as density or surface area per unit volume, remain the same whether the plate used has one or many orifices; in particular, the size of bubbles or cells in the mass of the froth is almost uniform, and is independent of the diameter of the orifice.

As may be seen in Fig. 1, before coalescence near the surface of the pool, the bubbles in the froth are all about the same size, and smaller than the bubbles formed at the orifices. Leibson *et al.*[2], who investi-

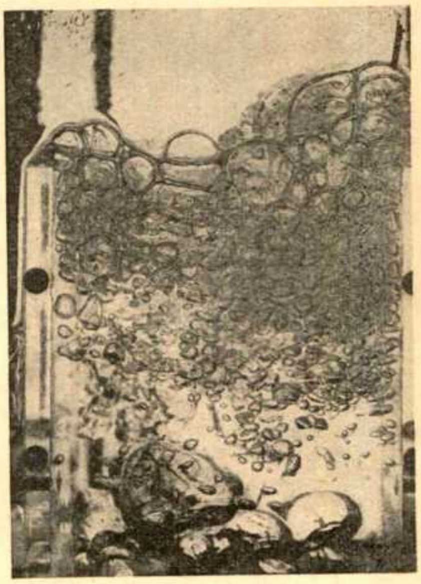
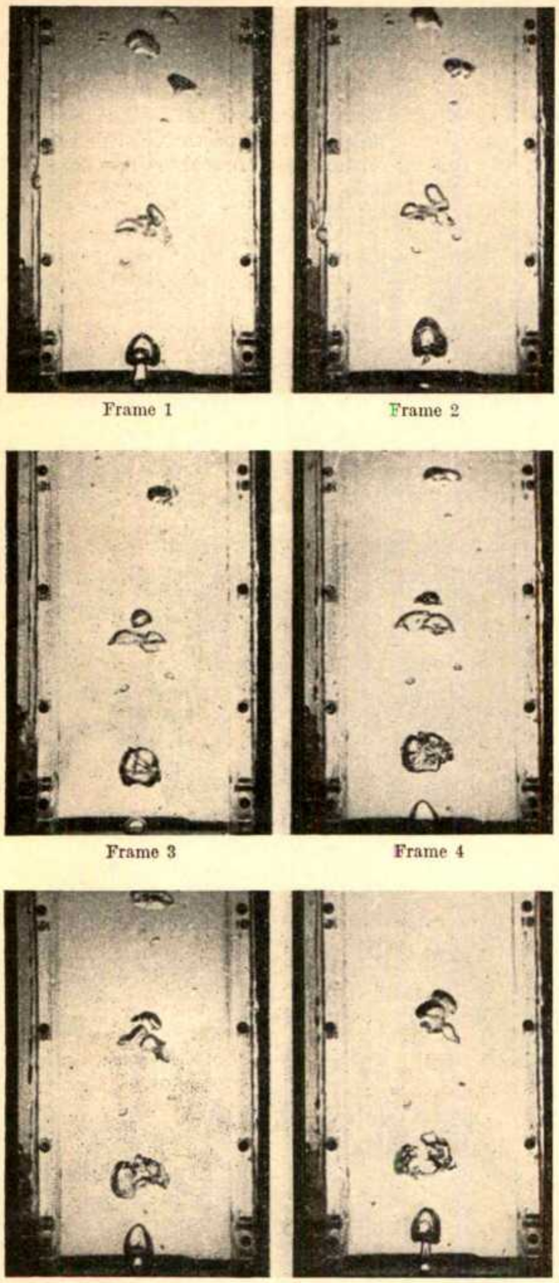


Fig. 1. Froth formed over a multiple-orifice sieve-plate in a shallow pool. Air in water

gated bubble formation at a single orifice, concluded that the small bubbles are produced by sudden shattering of the large bubbles initially formed, and this is indeed what appears to happen when the formation of the froth is followed by a slow-speed ciné camera or by eye. Helsby and Tuson[3], in a study of bubble behaviour at low gas-rates, observed the onset of coalescence between pairs of bubbles near a single orifice. To examine the process in more detail, however, we have taken ciné-photographs of the break-up of bubbles formed by single- and multiple-orifice plates at 64 frames/sec. with synchronized lighting, supplemented by high-speed photographs at 3,000 frames/sec.

Fig. 2 illustrates the behaviour of bubbles before and during disintegration; the sequence of six photographs was taken at intervals of 1/64 sec. These bubbles are formed at a single orifice of 1/8 in. diameter, in a deep pool of liquid. In frame 1 a bubble has been formed and became detached from the orifice. A second bubble is shown penetrating the first bubble, forming a neck of gas connecting it to the orifice. When the 'neck' is detached from the orifice, it may remain attached to the main bubble but separated by a film of liquid, or it may be an integral part of the main bubble. Sometimes no distinct neck is formed, but a second, smaller bubble follows the first in quick succession. In nearly all cases the neck or the second bubble overtakes the main bubble and, being usually of smaller diameter, penetrates it. Frames 2, 3 and 4 show the increasing agitation of the combined bubble as it begins to rise through the liquid. In some photographs filaments and films of liquid from the penetrating bubble can be seen within the penetrated bubble. Frames 5 and 6 show the cells of the combined bubble beginning to move apart and form separate bubbles. The further progress of the break-up can be seen by following the upper group of bubbles. On the rare occasions when a bubble is released without being penetrated by a neck or a second bubble, it remains whole until the bulk of the froth at a higher level is reached.

With slight variations (for example, at very high gas-rates more than two bubbles may interpenetrate) this mechanism of disintegration operates under all conditions which give frothing in the air–water system; the eventual size of bubble or cell of froth is almost constant. The effect of changing the interfacial tension by using other gases and liquids, and the condition where the interfacial tension depends on the life of the bubble, as when mass transfer is taking place, are now being examined.


Frame 1 Frame 2

Frame 3 Frame 4

Frame 5 Frame 6

Fig. 2. Progressive break-up of bubbles. Frames at intervals of 1/64 sec.

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[1] Calderbank, P. H., Evans, F., and Rennie, J., *Proc. Intern. Symp. Distillation* (Inst. Chem. Eng., London, 1960).

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EFFECT OF COPPER ON PRODUCTS OF ORCINOL OXIDATION

By Dr. T. F. SLATER

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THE addition of alkali (final normality approx. 0·01) to a solution of orcinol containing small amounts of copper (approx. 10^{-6} *M*) leads to the rapid production of a red-brown colour. Colours so formed in sodium hydroxide or in ammonium hydroxide are very similar providing the reaction time is short (about 1 hr.). If the alkaline orcinol reaction is allowed to proceed for a much longer time (say, several days) a marked difference between the spectra in sodium hydroxide and ammonium hydroxide becomes apparent due to the formation of the dye orcein in ammoniacal orcinol. Orcein is a

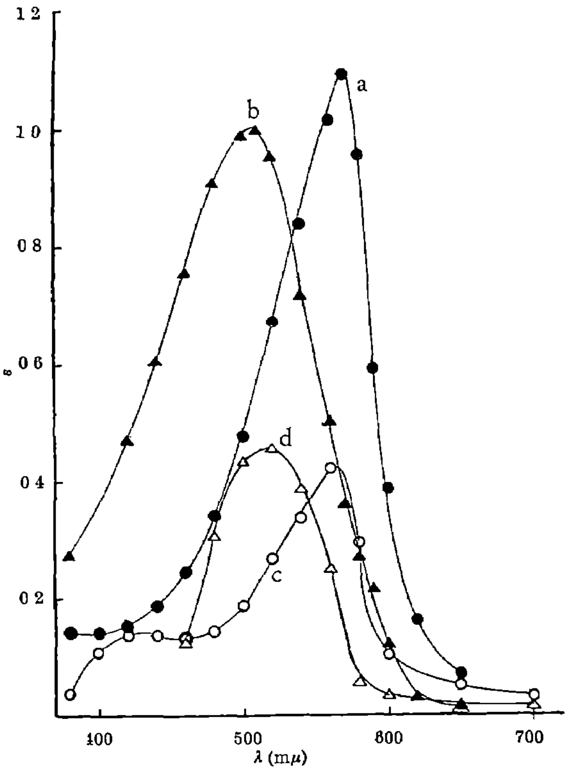

Fig 1 Absorption spectra obtained using a commercial sample of orcein *a*, in 0 1 *N* alkali , *b*, in 0 1 *N* acid , *c*, and *d*, are the alkaline and acid difference spectra for the oxidation of orcinol in the presence and absence of copper ions calculated as follows two flasks containing ammoniacal orcinol were incubated for 4 days at room temperature , one flask, *A*, contained in addition 1 ml 1 per cent sodium diethyldithiocarbamate and the other flask, *B*, contained 2 μgm copper ions After 4 days the absorption spectra of both solutions were determined and the alkaline difference spectra calculated by subtracting spectrum *B from A* Both solutions were then acidified to approx 1 *N* with HCl and the spectra again measured The acid difference spectrum was calculated by subtracting spectrum *B* from *A*

mixture of related products which can be separated by paper chromatography[1] A commercial sample of orcein obtained from British Drug Houses, Ltd , gave absorption maxima in acid and alkali at 510 and 570 mμ respectively (Fig 1)

An unexpected feature of these results was that copper ions inhibited the formation of orcein , this is illustrated in Fig 1 It can be seen that the acid and alkaline peaks formed in the virtual absence of copper ions are identical with the results found with a commercial sample of orcein It would thus appear that the complex reactions leading to the formation of the orcein dyes are inhibited by traces of copper present in the reaction mixture

The reaction between orcinol, alkali and small amount of copper with short times of incubation can be used as a quantitative method for determining low concentrations of copper Lambert[2] has published an assay method for copper based on the catalysis of resorcinol oxidation, but confined himself to assays in pure solutions and did not investigate the applicability of the method to biological samples where trace metal analysis is often required The applicability of the copper catalysis of phenol oxidation as a method for copper analysis in biological samples has therefore been investigated Since this work was started before seeing the report of Lambert[2], it is concerned solely with the oxidation of orcinol Some of the early work on this investigation was done using borate and phosphate buffers but these were later replaced by the more satisfactory pyrophosphate buffer suggested by Lambert[2]

The assay system used was to incubate 2 ml 1 per cent orcinol, 5 ml pyrophosphate buffer (0 1 *M*, *p*H 12), copper solution (0–1 0 μgm) and water to give a final incubation volume of 25 ml at 30° C for 10–30 min with shaking at 120 shakes/min Lambert proposed stopping the reaction by acidification, but this greatly decreases the extinction value and a more convenient procedure was found to be the addition of a strong copper chelator (versene) at the end of the incubation The addition of 1 ml 0 1 *M* versene *p*H 7 4 to the reaction mixture slows the reaction to negligible proportions Colour development increases with *p*H as reported by Lambert for resorcinol oxidation Under these conditions the colour increases linearly with the incubation time and linearly with the amount of copper added (respective limits studied, 1 hr and 2 μgm copper) After correction for blank colour-formation in the absence of added copper, a concentration of 1 8 × 10^{-7} *M* copper in the incubation mixture gave an extinction at 460 mμ of 0 1 (path length, 1 cm) after a 20 min incubation

The effects of metal ions commonly occurring in biological extracts was investigated to see the extent of interference in the copper-catalysed orcinol oxidation The following metal ions had no influence on the reaction when present in large final concentrations (at least 40-fold excess over copper) sodium, potassium, zinc, calcium, magnesium, aluminium and ferrous ions Cobaltous and manganous ions stimulated the orcinol oxidation in a similar manner to copper ions , ferric ions and chromic ions inhibited the reaction Comparative effects of these metal ions are shown in Fig 2 The effects of several of the above metal ions on the oxidation of alkaline orcinol are different from their effects on resorcinol oxidation[2,3] It is apparent from the results in Fig 2 that unless the biological extracts are abnormally rich in manganese or ferric ions compared with copper, the orcinol colour will be largely the result of copper-induced oxidation and, as such, will reflect the copper content of the extracts In general, the manganese content of biological extracts is much less than the copper content[4], but ferric ion inhibition is possible, particularly if appreciable blood is present As was to be expected, the copper-orcinol reaction

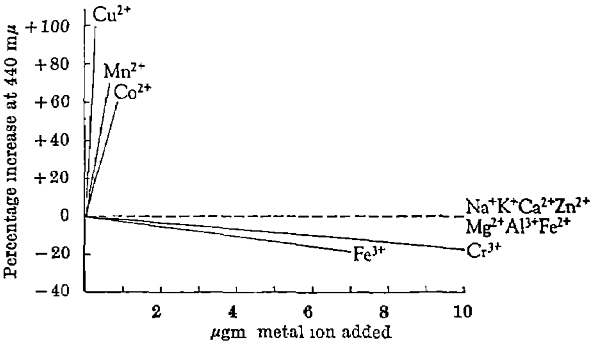

Fig 2 Effect of various metal ions on the alkaline oxidation of orcinol The ordinate expresses the percentage increase in colour (at 440 mμ) produced by the addition of metal ions compared with the colour produced in the absence of added metal, the abscissa is the amount of metal ion added in μgm Other conditions as in the text The results are representative of various incubation times from 10 min to 3 hr but are suitable for comparisons, that is, weight for weight, copper is approx three times as effective as manganese and about six times as effective as cobalt

was inhibited by traces of protein or amino-acids which bind the copper in an unreactive form Reducing and oxidizing reagents (for example, vitamin C and hydrogen peroxide) also interfere Thus if copper estimations by either the copper-orcinol or resorcinol procedures are to be carried out on biological samples, the tissue must be ashed prior to copper estimation With certain high protein/high fat samples (milk, for example) the copper orcinol procedure becomes unsuited for the estimation of low contents of copper due to the high blank values consequent on ashing. It would appear from the foregoing results and from previous work on resorcinol[2] [3] that the catalytic action of copper on phenol oxidation can be used for estimating copper in biological samples providing that the ashing procedure does not require large amounts of digestion material. In other words, the use of the method would seem to lie with samples relatively enriched in copper compared to protein/fat

Much of this work was done during the tenure of a Beit Memorial fellowship held at University College, London I am grateful to Prof. C Rimington and Dr A L Greenbaum for helpful comments.

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PARTICIPATION OF IMIDAZOLE IN INTRAMOLECULAR HYDROGEN BONDING

By Prof. A. LUKTON
Brooklyn College, Brooklyn, New York

IT is generally believed that an understanding of proximity effects of neighbouring groups functioning in concerted action on an enzyme surface will lead to an explanation of the great disparity between the rates of catalysis of enzymes and of their model systems[1] Precise determination of the particular role of groups such as imidazole in enzyme catalysis thus requires that its intramolecular structural relationships be established. In this article, evidence for intramolecular hydrogen bonding involving the imidazole ring is presented

The assignment of the imidazole infra-red absorption bands were made by Otting[2] and include strong bands at 2,600–3,200 cm $^{-1}$ and at 3,475 cm.$^{-1}$. He demonstrated that the strong NH (unbonded) absorption at 3,475 cm $^{-1}$ decreased in intensity but not in frequency with increasing concentration in chloroform solution At the same time the hydrogen bonded NH bands in the region 2,600–3,200 cm $^{-1}$ increased in intensity, indicating an increase in intermolecular hydrogen bonding Recently, Anderson *et al* [3] established the relationship between the absorption intensity change and the length of the linear imidazole oligomers as effected by the imidazole concentration in carbon tetrachloride solution. Such absorption changes should be useful in determining the existence of intramolecular hydrogen bonding in compounds which contain the imidazole ring In such a structure no imidazole absorption should occur in the 2,600–3,200 cm $^{-1}$ region since imidazole participation in intramolecular hydrogen bonding would preclude the possibility of its participation in intermolecular bonding in dilute, non-hydrogen bonding solvents

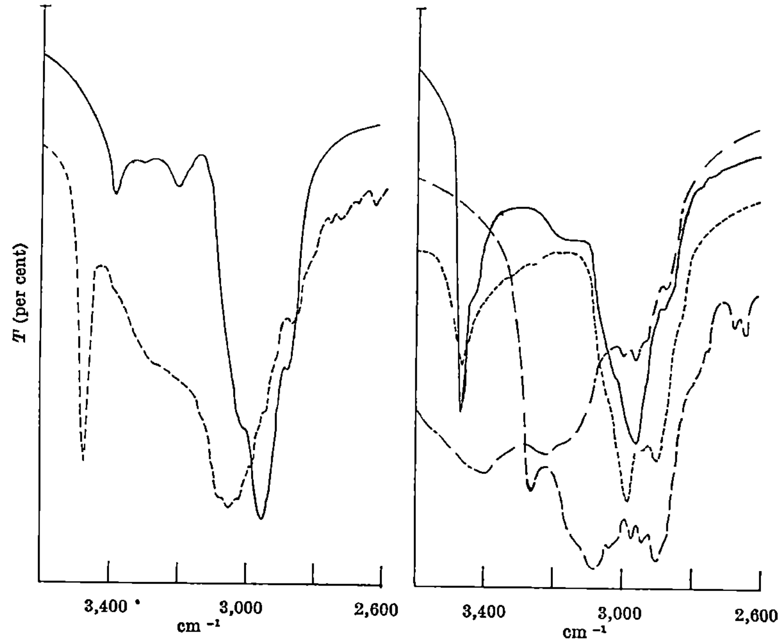
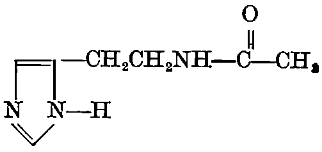
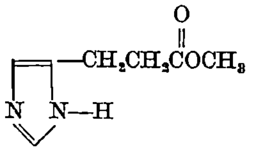


Fig 1 The infra-red absorption spectra of left, 1,4-methylhistamine in $CHCl_3$ (——), imidazole in $CHCl_3$ (– – –), right, acetylhistamine in $CHCl_3$ (– – –), acetylhistamine in KBr (— — —), methyl dihydrourocanate in $CHCl_3$ (——), methyl dihydrourocanate in KBr (— - —)

Acetyl histamine (I) and methyl dihydrourocanate (II) were studied to examine this premise



(I)



(II)

In (I) intramolecular hydrogen bonding could involve the hydrogen linked to the ring pyrrole nitrogen and the carbonyl oxygen of the amide

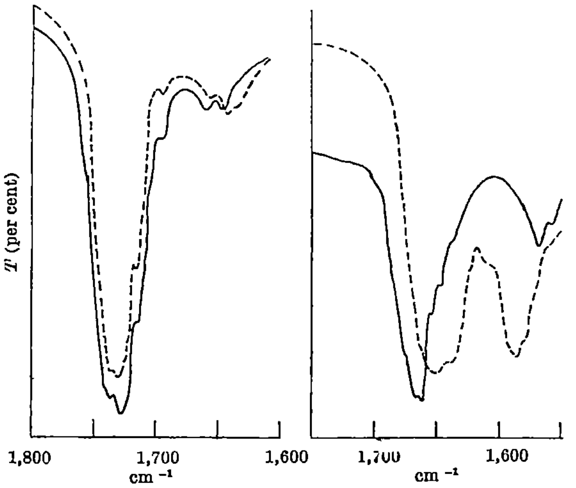
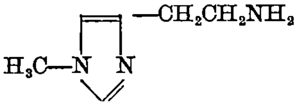


Fig 2 The infra-red absorption spectra of left, methyl dihydrourocanate in CHCl$_3$ (——), methyl dihydrourocanate in KBr (- - -), right, acetylhistamine in CHCl$_3$ (——), acetylhistamine KBr (- - -)

linkage ; or, the ring pyridine nitrogen and the imino hydrogen of the amide group Hydrogen bonding in (II) could result only in the bonding of the hydrogen of the pyrrole nitrogen and the carbonyl of the ester group

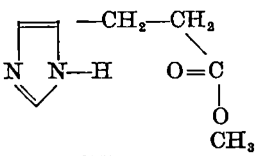
The infra-red spectra of acetyl histamine, methyl dihydrourocanate, imidazole and 1,4-methylhistamine (1-methyl-4-(β-amino-ethyl)-imidazole) in the region 2,600–3,600 cm $^{-1}$ and 1,600–1,800 cm $^{-1}$ are given in Figs 1 and 2 The 2,900–3,100 cm. $^{-1}$ region of the methyl dihydrourocanate and acetyl histamine spectra is complicated by the coincident absorption of CH, CH$_2$ and CH$_3$ The contribution of this absorption, however, was determined in the absence of imidazole NH (bonded) absorption by examining the spectrum of 1,4 methylhistamine, structure III, in chloroform solution



(III)

As shown in Fig 1, there is a strong absorption band at 2,967 cm $^{-1}$, (CH$_3$), a weak band at 2,878 cm $^{-1}$ and a shoulder at 3,007 cm $^{-1}$ The two weak absorption bands at 3,373 and 3,310 cm. $^{-1}$ are due to the NH vibration of the NH$_2$ group[4]

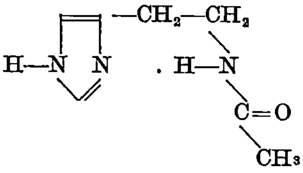
Examination of the methyl dihydrourocanate spectrum in chloroform solution reveals a spectrum similar to that of 1,4 methylhistamine with a strong band at 2,957 cm $^{-1}$ and a weaker band at 2,878 cm $^{-1}$ Furthermore, it is unlike the imidazole spectrum in chloroform solution since the strong imidazole band at 3,048 cm $^{-1}$ is completely absent and leads to the postulate that intermolecular hydrogen bonding does not occur due to the existence of the internally bonded structure (IV).



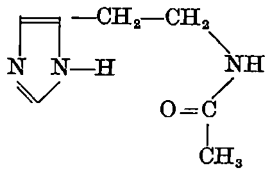
(IV)

The behaviour of the absorption band of the ester carbonyl stretching vibration in solution and in the solid state lends support for this structure Compounds such as *N*-methylacetamide in the solid state in which intermolecular hydrogen bonding occurs, absorb at a lower frequency (C=O bonded, 1,655 cm $^{-1}$) than in their dilute solutions (C=O unbonded, 1,695 cm $^{-1}$)[5,6]. However, methyldihydrourocanate absorbs at 1,730 cm. $^{-1}$ in dilute and concentrated chloroform solutions and in the solid state, Fig 2. The expected frequency shift did not occur since the hydrogen bond in this compound, whether inter- or intra-molecular, involves the same atoms That intermolecular hydrogen bonding occurs in the solid state between NH (imidazole) and C=O (ester) is indicated in its spectrum in Fig 1 Here, broad absorption bands exist at 3,390 and 3,216 cm $^{-1}$ This is characteristic of intermolecular bonded hydrogen in amides, although the amide bonds are at 3,300, 3,275 and 3,070 cm $^{-1}$ (ref 6)

The absorption spectrum of acetylhistamine in chloroform solution is also similar to that of 1,4 methylhistamine It has a strong band at 2,986 cm $^{-1}$, a weak band at 2,900 cm $^{-1}$ and it lacks the bonded NH (imidazole) band at 3,048 cm $^{-1}$ The absence of bonded NH (imidazole) absorption in this region indicates intramolecular hydrogen bonding which could result from two possible structures (V) and (VI)

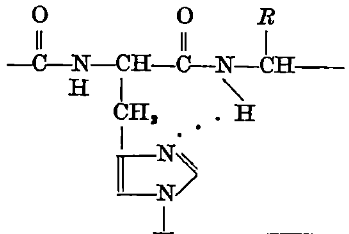


(V)

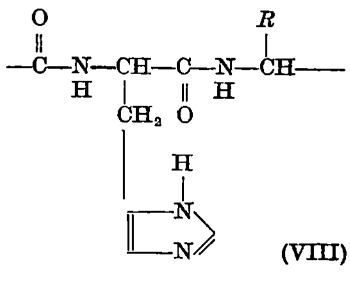


(VI)

Structure (V) is favoured because it consists of a six-membered hydrogen-bonded ring which is considerably more stable than the eight-membered ring of structure (VI)[7] Also, there is a frequency shift in the carbonyl absorption from 1,660 to 1,650 cm $^{-1}$ in going from chloroform solution to the solid state (Fig 2) As would be expected, the shift is small compared with the shift observed in completely unbonded and bonded amides



(VII)



(VIII)

The smaller frequency shift is explained on the basis of a change from intra-molecular hydrogen bonding V to intermolecular hydrogen bonding in which the NH (amide) and C=O (amide) participate. Absorption at 3,266, 3,083 and 3,035 cm.$^{-1}$ and characteristic of NH $\cdots$ O=C absorption in amides are observed in solid acetyl histamine (Fig. 1). An analogous structure to that proposed by Mizushima[8] for *N*-methylacetamide is suggested.

Several interesting consequences with respect to naturally occurring substances follow from these considerations. In so far as imidazole may participate in protein hydrogen bonding, two configurations would be possible (besides those which may involve bonding with other amino-acid residues).

Both structures contain seven-membered rings; however, structure (VIII) would be favoured. If such structures do exist in proteins, they might help provide an explanation for marked differences in reactivity of various histidine residues such as exist in ribonuclease[9].

Intramolecular hydrogen bonding in the dipeptide carnosine (β-alanyl-histidine) may exert an effect in facilitating the dissociation of the amide imino hydrogen atom during the formation of the copper carnosine chelate[10,11], also it may contribute to the base strength of the imidazole of carnosine (*pK* (*Im*) = 6.83) which is a considerably stronger base than that in histidine (*pK* (*Im*) = 6.00)[12].

I am indebted to Dr. R. W. Schayer, Merck Institute for Therapeutic Research, Rahway, New Jersey, for supplying the 1,4 methylhistamine.

This investigation was supported by a research grant from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service grant No. *A*-3066.

Research on this project was conducted at Adelphi College, Garden City, N.Y.

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PROPERTIES OF SOME COPPER COMPLEXES

Five-Covalent Copper(II) Complexes containing 1,10-Phenanthroline and 2,2′-Bipyridyl

THIS preliminary communication reports the preparation and properties of an extensive series of five-covalent copper(II) complexes by the reaction of *bis*(1,10-phenanthroline)copper(II) and *bis*(2,2′-bipyridyl)copper(II) perchlorates with various neutral and negatively charged ligands.

The addition of halide ions (Cl^-, Br^- and I^-) to nitrobenzene or nitromethane solutions of $[Cu(phen)_2](ClO_4)_2$ leads to the formation of variously coloured solutions (blue, green and brown respectively) containing monohalogeno-*bis*(1,10-phenanthroline)-copper(II) ions due to the reaction

$$[Cu(phen)_2]^{2+} + X^- \rightarrow [Cu(phen)_2X]^+$$

This 1 : 1 reaction has been confirmed both by conductometric titrations and spectrophotometric continuous variation studies. Also, by working in aqueous acetone solutions, a series of coloured crystalline compounds of the type $[Cu(phen)_2X]ClO_4$ (where $X = Cl^-$, Br^-, I^-, SCN^-, NO_2^-, $HCOO^-$, CH_3COO^- and $C_6H_5COO^-$) has been isolated. These compounds possess molecular conductivities in the ranges 27–29 mho and 87–90 mho at 25° in 10^{-3} *M* nitrobenzene and nitromethane solutions respectively. These values are comparable with those of other uni-univalent electrolytes in these solvents[1] and practically half those of the diperchlorate $[Cu(phen)_2](ClO_4)_2$ (Table 1). The *bis*(2,2′-bipyridyl) compound $[Cu(bipy)_2](ClO_4)_2$ also reacts readily with halide ions in the above two solvents and compounds of the type $[Cu(bipy)_2X]ClO_4$ (where $X = Cl^-$, Br^-, I^- and SCN^-) have also been isolated. These compounds possess similar conductivities to their phenanthroline analogues (see Table 1).

Table 1. MOLECULAR CONDUCTIVITIES AND MAGNETIC MOMENTS OF VARIOUS 1,10-PHENANTHROLINE AND 2,2′-BIPYRIDYL COMPLEXES OF BIVALENT COPPER

Compound	Molecular conductances (mho) in 10^{-3} *M* solutions at 25°		Magnetic moment per Cu atom in B.M.
	$C_5H_5NO_2$	CH_3NO_2	
$[Cu(phen)_2](ClO_4)_2$	43.1	176	1.94
$[Cu(phen)_3](ClO_4)_2$			1.91
$[Cu(phen)_2X]ClO_4$ ($X = Cl^-$, Br^-, I^-, SCN^-, NO_2^-, $HCOO^-$, CH_3COO^- and $C_6H_5COO^-$)	27–29	87–91	1.83–1.96
$[Cu(phen)_2X]X$ (Cl^-, Br^-, I^-, SCN^-, $HCOO^-$ and CH_3COO^-)	24–27	82–93	1.86–1.97
$[Cu(phen)_2L](ClO_4)_2$ ($L = NH_3$ and C_5H_5N)		176–199	1.84–2.00
$[Cu(bipy)_2](ClO_4)_2$	54	186	2.03
$[Cu(bipy)_3](ClO_4)_2$	44.5	181	1.93
$[Cu(bipy)_2X]ClO_4$ ($X = Cl^-$, Br^-, I^-, and SCN^-)	28–31	89–91	1.89–1.97
$[Cu(bipy)_2X]X$ ($X = Cl^-$, Br^-, I^-, and SCN^-)	11–30	79–85	1.81–1.96
$[Cu(bipy)_2NH_3](ClO_4)_2$		199	1.92
$[Cu(bipy)Cl_2]$		~1	1.96

Attempts to prepare a monocyano-derivative of the $[Cu(phen)_2]^{2+}$ ion lead to the isolation of the cyano-bridged complex

$$[(phen)_2Cu - C \equiv N - Cu(phen)_2](ClO_4)_3 \cdot H_2O$$

in which the cyanide group is linking two $[Cu(phen)_2]^{2+}$ ions.

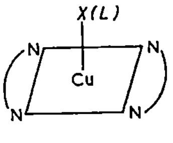
The *bis*-complexes $Cu(phen)_2X_2 \cdot nH_2O$ ($X = Cl^-$, $n = 3.5$, $X = Br^-$ and I^-, $n = 1$, $X = SCN^-$, $n = 0$, and $X = HCOO^-$ and CH_3COO^-, $n = 6$) and $Cu(bipy)_2X_2 \cdot nH_2O$ ($X = Cl^-$, $n = 6$; $X = Br^-$, $n = 2$; $X = SCN^-$ or I^-, $n = 0$) were also prepared and their properties examined. These complexes are also uni-univalent electrolytes in nitromethane solution (Table 1) and contain the $[Cu(chelate)_2X]^+$ ion. In the more weakly polar solvent nitrobenzene they undergo some re-arrangement due to the reaction

$$[Cu(chelate)_2X]^+ + X^- \rightleftharpoons [Cu(chelate)X_2] + chelate$$

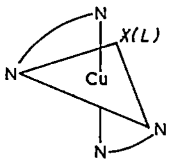
This equilibrium, which is independent of concentration, has been studied in detail by means of accurate conductivity measurements over a concentration range and will be reported later[2]. Mono-complexes of the type [Cu(bipy)X_2] (where X = Cl^-, Br^- and SCN^-) were prepared and examined during the course of this work. For example, the dichloro-complex [Cu(bipy)Cl_2], which is readily obtained by adding the ligand to excess copper(II) chloride in ethanol, is virtually a non-electrolyte in nitromethane solution (Table 1).

The reaction of neutral ligands with $[Cu(phen)_2]^{2+}$ and $[Cu(bipy)_2]^{2+}$ has also been studied and the stable mono-ammine complexes $[Cu(chelate)_2(NH_3)](ClO_4)_2$ were obtained from aqueous ammonia solutions. The *bis*(1,10-phenanthroline) complex also forms the mono-pyridine complex $[Cu(phen)_2(py)](ClO_4)_2$ as well as the aquo-derivative $[Cu(phen)_2(H_2O)](ClO_4)_2$. These compounds function as di-univalent electrolytes in nitromethane solution (see Table 1).

There is a number of possible structures for the five-covalent ions $[Cu(chelate)_2X]^+$ and $[Cu(chelate)_2L]^{2+}$ (where X is a negative ion and L is a neutral ligand). They could, for example, possess the square-pyramidal arrangement I, or the trigonal bipyramidal one represented in II.



(I)



(II)

In this regard it is of considerable interest that Barclay and Kennard (see following communication) have shown that the structure of iodo-*bis*(2,2′-bipyridyl)copper(II) iodide contains an approximately trigonal bipyramidal arrangement about the copper atom in the $[Cu(bipy)_2I]^+$ ion. This type of arrangement confers the possibility of mirror-image isomerism on the copper atom and the unit cell contains both isomers. The X-ray data reveal that the Cu – N and Cu – I distances correspond to normal covalent bond distances whereas the stereochemistry of bivalent copper is usually such that the copper atom is surrounded by four atoms at normal covalent bond distances in the plane with one or two more elongated bonds normal to the plane.

The magnetic moments of the 5-covalent complexes in the table all fall in the range 1·8–2·0 B.M. These values are similar to those of copper(II) complexes in general.

The magnetic moments of $[Cu(phen)_2Br]ClO_4$ and $[Cu(phen)_2Br]Br$ have been reported previously as being essentially independent of temperature over the range 90–300° K (ref. 3).

C. M. Harris
T. N. Lockyer
Hanneke Waterman

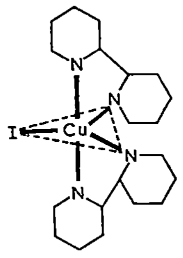
The Trigonal-Bipyramidal Configuration of the Iodo *Bis*(2-2′-Bipyridyl)Copper(II) Ion

The compounds $Cu(dipy)_2X_2$ and $Cu(phen)_2X_2$ formed between 2,2′-bipyridine or *o*-phenanthroline and copper(II) salts have been shown to have co-ordination numbers of five in non-aqueous solutions (see previous communication). The purpose of this communication is to record preliminary results of an X-ray structure determination of one of them, *bis*(2,2′-bipyridyl)copper(II) iodide.

The lustrous brown crystals are triclinic (a = 10·66 ± 0·01, b = 14·37 ± 0·01, c = 7·44 ± 0·01 Å, α = 93 ± 1°, β = 101 ± 1°, γ = 108 ± 1°). There are two molecules of composition $Cu(C_{10}H_8N_2)_2I_2$ in the unit cell (d_{meas} = 1·99, d_{calc} = 1·98 gm cm^{-3}). The centro-symmetric space group, $P\bar{1}$, was assumed.

One-thousand-four-hundred independent intensities were estimated visually from equi-inclination Weissenberg photographs taken with copper radiation. The co-ordinates of the atoms were determined from three-dimensional Patterson and Fourier syntheses. With a disagreement index (Σ| |F_{obs}| – |F_{calc}| |/ Σ|F_{obs}|) of 0·18, the estimated standard deviations of the atomic positions were I, 0·003, Cu, 0·005, N, 0·03, C, 0·05 Å. The structure is being refined further in an attempt to locate the light atoms more precisely. However, it is quite clear that the copper atom has an unusual stereochemistry.

The compound contains a positively charged iodo-*bis*(bipyridyl)copper(II) ion and a negatively charged iodide ion. The copper atom is surrounded by four nitrogen atoms (at a distance of 2·02 Å) and an iodine atom (at 2·71 Å) at the corners of a distorted trigonal bipyramid. The iodine atom and two nitrogen atoms (from different bipyridine molecules) are in the same plane as the copper atom. The other two nitrogen atoms lie on a line (passing through the copper atom) which makes an angle of about 9° with the normal to this plane. The arrangement is shown in III.



(III)

All five atoms are at normal covalent bond distances from the copper atom. The trigonal bipyramidal configuration for the copper atom differs in this respect from the usual stereochemistry of bivalent copper which involves four bonds of normal covalent lengths with a fifth, and sometimes a sixth, at a greater distance. As the iodo-*bis*(2,2′-bipyridyl)-copper(II) ion possesses neither a plane nor a centre of symmetry, the two ions in the unit cell are related to one another as optical isomers.

Terpyridylcopper(II) chloride dihydrate is isomorphous with terpyridylzinc chloride, which has a similar trigonal bipyramidal configuration about the metal atom[4].

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THERMAL REACTION BETWEEN TWO LIGHT REACTIONS IN PHOTOSYNTHESIS

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ALLEN and Franck[1] showed with the alga *Scenedesmus* that a single flash of less than 1 msec in duration resulted in no detectable oxygen evolution from photosynthesis If two flashes were given, separated by a suitable dark interval, a measurable yield was obtained Alternatively an increased yield was obtained when a single flash was superimposed on a continuous background of low intensity Whittingham and Brown[2] confirmed and extended these observations with the alga *Ankistrodesmus* They interpreted their results in terms of two photochemical reactions, the first of which was thought to activate an essential catalyst of photosynthesis, the second being concerned in photosynthesis directly.

More recent work has suggested the existence of two photochemical reactions in the photosynthetic process This has been postulated to explain the enhancement (or 'Emerson') effect on photosynthesis obtained with a number of algæ excited with light of two different wave-lengths[3]. Govindjee *et al* [4] showed that the enhancement effect was also observed for the photoreduction of quinone by *Chlorella* (Hill reaction) Duysens *et al* [5] showed with *Porphyridium* that light, absorbed by the phycobilins, preferentially resulted in the reduction of cytochrome *f*, whereas light at 680 mμ absorbed by chlorophyll predominantly oxidized the cytochrome Losada *et al* [6] independently suggested, on the basis of work with isolated chloroplasts, that one pigment system was concerned in the liberation of oxygen from water and the transfer of an electron to an acceptor and a second pigment system was concerned with the acceptance of the electrons from this first acceptor They produced evidence to suggest that the second system was that in which photophosphorylation was concerned and deduced that cytochromes were probably concerned here

Our earlier observations with flash illumination can also be interpreted in terms of a mechanism involving two light steps In this article are further results on the effects of flash illumination with isolated chloroplasts which further clarify the mechanism involved

Ankistrodesmus was cultured in the manner described in ref 2 Chloroplasts were isolated from *Beta vulgaris* (perpetual spinach, Thompson and Morgan, Ltd) grown either in the field or in a greenhouse without close control of growth conditions The chloroplasts were isolated at 0° C by grinding in a medium containing 0 35 *M* sodium chloride, 0 17 *M tris* and 0 03 *M* $MgCl_2 6H_2O$ at *p*H 7 4, washed once in the grinding medium, and then re-suspended in the same medium diluted one in ten. The chloroplasts were kept on ice throughout the period before measurement A reaction mixture containing 10 μmoles $Fe_3(CN)_6$, 6 μmoles $MgCl_2 6H_2O$, 70 μmoles sodium chloride and 40 μmoles *tris* at *p*H 7 4 was made up to 2 5 ml , and when required 2 μmoles adenosine diphosphate and 40 μmoles inorganic phosphate added. For the purposes of uncoupling phosphorylation 10^{-2} *M* ammonium chloride was added In other experiments ferricyanide was replaced by 0 5 μmole phenol-indo 2,6-dichlorophenol Chloroplasts prepared in this way and kept at 0° C were found reasonably stable during the period required for the experiment Measurements could not begin for 40 min after preparation of the chloroplasts, since it was necessary to remove oxygen from the reaction mixture Two types of light flash were used a 'Mazda' discharge tube *FA*4 giving a flash of approximately 0 2 msec duration (short flash) and a Philips photoflux bulb *PF*60 approximately 30 msec (long flash) All other conditions of measurement were as described before except that the reaction vessel was maintained at 4° C

Whittingham and Brown found that the maximum yield from a pair of flashes was obtained with *Ankistrodesmus* when the short flash preceded the long flash by a dark interval of 0 7 sec at 25° C Some influence of the short flash on the yield from the long flash was detectable even with a dark separation of up to 15 sec Further work has shown that when the temperature was reduced to 4° C the yield from the long flash alone was much decreased , but the absolute maximal yield for a long flash preceded by a short flash was of the same order of magnitude as the maximum yield for the same two flashes at 20° C , except that the time between flashes for maximal yield was now 16 sec (Fig 1) A similar value was found for the time of separation of the two flashes for optimal production of oxygen from ferricyanide by chloroplasts isolated from spinach beet It is suggested that a dark thermal reaction, interposed between the two photochemical processes, is necessary for some conversion of the products of process 1 to become the reactants for process 2 The duration of the short flash is so brief that during the flash none of the product of the first reaction has been converted by the second Witt *et al* [7] have shown that the absorption changes in *Chlorella* at −150° C indicate that only one photochemical process predominates at low temperature Experiments reported here indicate a significant effect of temperature even within the physiological range The results in Fig 1, which compare the yield for two flashes as a function of their separation in time at 4° and 20° C , suggest that the thermal reaction proceeds more slowly but that its product has a greater half-life at the lower temperature This is consistent with the observations of Emerson *et al* [8], who showed that at lower temperatures photosynthetic efficiency was increased at longer wave-lengths in the far red consistent with an increased life-time at low temperature of the product of a reaction characteristic of shorter wave-length absorption

When phenol-indo 2,6-dichlorophenol was used instead of ferricyanide, the optimal separation between flashes was considerably shorter, being less than 5 sec at 4° C This indicates that dichlorophenol indophenol can result in production of oxygen without

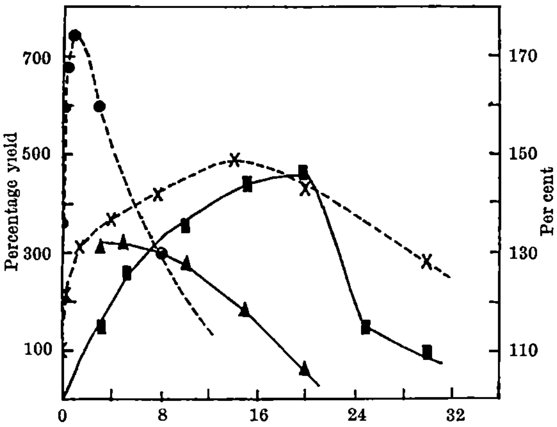

Fig 1 Relationship between the combined yield of oxygen for a short and long flash as a function of the dark time separating the two flashes The yield is expressed as a percentage of the sum of the yields from the two flashes given simultaneously ●, *Ankistrodesmus* cells in bicarbonate buffer at 20° C , ■, Isolated beet chloroplasts with ferricyanide at 4° C , ▲, Isolate chloroplasts with ferricyanide and ammonium chloride at 4° C (all right-hand ordinate), ×, *Ankistrodesmus* cells in bicarbonate at 4° C (left-hand ordinate)

proceeding through the same thermal reaction as is required for ferricyanide reduction Addition of catalytic amounts of indophenol (0 01 µmole) in the presence of ferricyanide (10 µmoles) changed the optimal time between flashes from that characteristic of ferricyanide alone to that characteristic of dye alone This is consistent with the results observed in continuous illumination[6], which Losada *et al* interpreted as indicating that reduction of dye could take place as a result of only one photochemical reaction

The difference in time of separation between flashes for optimal yield for ferricyanide and dye may be related to the fact that reduction of ferricyanide is accompanied by phosphorylation whereas that of dye is not Addition of adenosine diphosphate and inorganic phosphate to a system containing ferricyanide did not result in any significant change in the time of flash separation for optimal yield, but in the presence of ammonium chloride, which has been shown to uncouple the reduction of ferricyanide from phosphorylation[9], the separation in time was significantly shortened It became of the same order of magnitude as that in the presence of indophenol alone

The two types of flash used differ in spectral composition Further experiments, using monochromatic light flashes, have shown that with a long flash of 692 mµ a maximal effect is obtained with weak continuous illumination of 674 mµ A full investigation of the whole action spectrum is now in progress

These results are consistent with a mechanism of photosynthesis involving two light reactions separated by a thermal dark process The thermal dark process is apparently accelerated when phosphorylation does not accompany transfer of electrons Phosphorylation may be concerned directly in the thermal stage as previously suggested or if it accompanies the second photochemical step the rate of transfer of electrons from process 1 to process 2 must be slower in the presence of phosphorylation than in its absence

We are grateful to the Royal Society for a Government grant-in-aid of this investigation One of us (P M B) holds a postgraduate studentship of the University of London We are also grateful for a grant from the Central Research Fund of the University of London

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SULPHATE TRANSPORT AND METABOLISM BY *DESULPHOVIBRIO DESULPHURICANS*

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LITTLEWOOD and Postgate[1] stated that the micro-organism *Desulphovibrio desulphuricans* is not freely permeable to anions such as sulphate and chloride They suggested that sulphate reduction occurs either outside the osmotic barrier or that the penetration of sulphate into the organism is regulated in such a manner that at equilibrium the ratio of the internal concentration of sulphate ions to the external concentration is so low that the analytical procedure used in their work could not detect penetration of the ion even at high external sulphate concentrations Skarzynski and Ostrowski[2] claimed that *Thiobacillus thioparus* takes up only the outer sulphur atom of thiosulphate, leaving the inner sulphur atom of thiosulphate outside the cell as sulphate ion

These two papers give an impression that the inorganic sulphur compounds are metabolized on the surface of the cells, even though sulphate and thiosulphate are the main energy-yielding substrate for these organisms

It was of interest to elucidate whether these organisms, which utilize inorganic sulphur compounds, actually metabolize their substrates at the surface of the cell outside the osmotic barrier

This communication is concerned with the problem of sulphate accumulation and metabolism in the cells of a strain of *Desulphovibrio*, which was isolated from a muck paddy field in Japan this organism plays a very important part in the reduction of sulphate to sulphide in the soil

The transport of sulphate into the cells was examined using a moderately thick suspension of cells (about 27 5 mgm. dry weight of cells was added to each reaction mixture , this corresponded to about 0 1 ml of packed cells per 2 ml of reaction mixture), as it was found that the ordinary thick suspension method[3] was not suitable for substances which are metabolized very quickly by the cell The cells were incubated with radioactive sulphate in Warburg vessels at 37° C in a *tris* buffer of *p*H 7 2, using hydrogen in the gas phase After incubation, the contents of each vessel were rapidly cooled with ice and then centrifuged at 18,000*g* for 14 min. in a refrigerated centrifuge The supernatant was removed by decantation for the estimation of sulphate and sulphide. The packed cells were rinsed three times with ice-cooled water very quickly to remove radioactive sulphate adhering to the wall of the centrifuge tube and to the surface of the packed cells

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For the estimation of sulphate in the supernatant, an aliquot of the supernatant was placed in a test-tube and to it was added one drop of *N* hydrochloric acid Then the test-tube was put in a boiling water-bath for 2 min so as to eliminate hydrogen sulphide The solution was diluted ten times with distilled water, 0 5 ml of the diluted solution was placed on an aluminium plate and dried by an infra-red lamp The radioactivity on the plate was measured by a Geiger–Muller counter and expressed as the amount of sulphate in the reaction mixture

For the estimation of sulphide in the supernatant, 1 ml of the original supernatant was strongly acidified by hydrochloric acid in a distillation apparatus and the hydrogen sulphide evolved was driven into a zinc acetate solution, contained in a volumetric flask, by bubbling nitrogen gas through the apparatus for 1 hr The sulphide content of the zinc acetate solution was estimated by St Lorant's methylene blue method[4] The radioactivity of sulphide-sulphur was estimated by measuring the radioactivity of methylene blue adsorbed on carbon powder

The packed cells were extracted with 10 ml of 5 per cent trichloroacetic acid solution at 0° C An aliquot of the extract was dried on a steam-bath so as to drive off the hydrogen sulphide and almost all the trichloroacetic acid, then the residual organic matter in it was oxidized completely by hydrogen peroxide The residues were dissolved in a known amount of water and applied to the estimation of sulphate in the packed cells For this estimation an aliquot of the solution thus prepared was put on an aluminium plate and dried The radioactivity on the plate was measured and it was expressed as the amount of sulphate in the packed cells

For the estimation of sulphide in the packed cells, they were transferred into the distillation vessel using about 2 ml of water and the suspension strongly acidified by hydrochloric acid The hydrogen sulphide thus evolved was treated by the same procedure as was the supernatant for the estimation of the radioactivity of the sulphide-sulphur

In this article, the term 'transport' will be used for the description of inflow and outflow It should also be mentioned that the term 'accumulation' will be used to indicate the result of both transport and reduction

A typical result of sulphate accumulation in the cells is shown in Fig 1*a* The concentration of sulphate in the packed cells was found to be higher than that of the medium at a certain period of the reaction At the peak of this accumulation, the sulphate concentration in the packed cells was calculated to be 145 per cent greater than that of the reaction medium at that time, or 28 per cent greater than the initial sulphate concentration This indicates that there is a distinct accumulation of sulphate in the cells

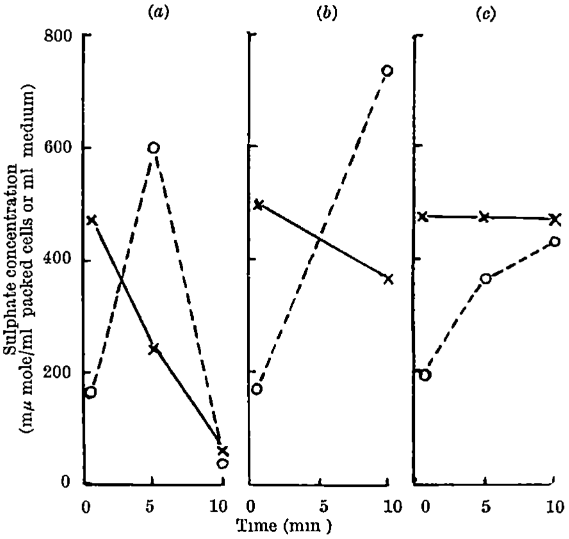

Fig 1 Sulphate accumulation in the cells ○---○, Sulphate concentration in the cells (mμ mole/ml packed cells) ×—×, Sulphate concentration in the reaction mixture (mμ mole/ml reaction medium)

(*a*) without selenate, (*b*) with *M*/16,500 selenate, (*c*) with *M*/1,100 or *M*/5,500 selenate

Each vessel contained 1 0 ml of *M*/20 *tris*-hydrochloric acid buffer (*p*H 7 2), 0 1 ml of *M*/1,000 phosphate buffer, 0 1 ml of magnesium chloride, 0 5 ml of cell suspension (corresponds to 0 1079 ml packed cell volume), 0 3 ml of water, 0 1 ml of *M*/100 sodium sulphate-^{35}S, and 0 1 ml of sodium selenate (*M*/750, *M*/250 or *M*/50 corresponding to *b*, *c* or *c*, respectively) In the centre well 0 3 ml of potassium hydroxide–pyrogallol solution was placed Total volume was 2 2 ml Sodium sulphate and sodium selenate were placed in a side-arm and were added to the reaction mixture at zero time

The mean velocity of sulphate transport, which corresponds to the mean velocity of disappearance of sulphate from the medium, and that of accumulation of sulphate in the cells, were calculated from the values obtained after 30 sec and 5 min in Fig 1*a* The mean velocity of sulphate transport was calculated to be 1,022 mμ mole/ml packed cells/min, dividing the amount of sulphate which disappeared from the medium (470 − 244 = 226 mμ mole/ml reaction mixture) by time (4 5 min) and the volume of packed cells (0 0491 ml), which corresponds to 1 ml of the reaction mixture On the other hand, the mean velocity of sulphate accumulation was calculated to be 96 6 mμ mole/ml packed cells/min, dividing the amount of sulphate accumulated in the cells (435 mμ mole/ml packed cells) by the time (4 5 min) These figures indicate that the sulphate transport is about eleven times more rapid than sulphate accumulation, that is, almost all the sulphate transported into the cells is reduced quickly and only a small part of the sulphate is left in the cells It must be borne in mind that the amount of sulphate accumulated in the cells is the result of two phenomena transport and reduction

It might be possible to show much more accumulation of sulphate in the cells if a way could be found to diminish the velocity of sulphate reduction without affecting the velocity of sulphate transport As a trial, the hydrogen gas phase of the reaction vessel was replaced by helium The helium gas was passed through for 2 min, with a consequent reduction of the partial pressure of the hydrogen gas Much greater accumulation of sulphate in the cells was observed with a helium–hydrogen gas phase than in the pure hydrogen phase, even though both velocities of sulphate reduction and transport were diminished It was also observed that sulphate, once accumulated in the cell, passes into the medium rapidly after sulphate reduction has stopped due to the lack of a hydrogen donor

More detailed experiments were conducted concerning the fate of the sulphide The concentration of sulphide in the cells was found to be always two to six times higher than that in the medium, even though it was much lower than that of sulphate The highest concentration observed in the packed cells was about 100 mμ mole/ml packed cells Changes in the concentration of sulphide in the cells follow that of sulphate

Selenate is known as a competitive inhibitor of sulphate reduction[5] It is also known that selenate competes with sulphate by the formation of a very unstable transient compound with adenosine triphosphate in the presence of sulphurylase[6] The effect of selenate inhibition was examined at different concentrations When *M*/16,500 selenate solution was used, a higher accumulation of sulphate in the cells was observed than in the cells without selenate, even though the velocity of sulphate accumulation was lowered (Figs 1*b* and 1*a*) The inhibition of sulphate transport and sulphate reduction calculated from the Figs 1*a* and 1*b* were 80 per cent and 85 per cent respectively. These values indicate that sulphate transport and reduction were inhibited by selenate, and the difference between the rates of inhibition of both reactions by selenate was very slight When the concentration of selenate was high enough to stop the reduction almost completely (95 per cent inhibition of sulphate reduction), no accumulation of sulphate in the cells was observed (Fig 1*c*) From these results the following possibilities are suggested. either (*a*) sulphate uptake is associated with sulphate reduction, or (*b*) selenate inhibits transport of sulphate directly.

To test whether sulphate transport is associated with sulphate reduction, experiments with oxygen were carried out Oxygen is a strong inhibitor of the metabolism of this organism When oxygen is used in the gas phase, the accumulation of sulphate in the cells and the reduction of sulphate are inhibited completely Further confirmation is seen in the results of diffusion experiments carried out at 0° C It was observed that 30 per cent of the packed-cell volume took up sulphate within the first 30 sec, but no marked increase of sulphate in the cells was observed in the following 30 min This figure, 30 per cent of the packed cell volume, corresponds to the inter-cellular volume of packed cells of *Desulphovibrio* (27 3–33 6 per cent as described by Littlewood and Postgate[1]) Even though there was observed a slight increase in permeable volume in 30 min, the final observed value of 36 8 per cent is still far from the percentage of the water content of the packed cells (76 3 per cent w/v) Therefore, little diffusion of sulphate into the cells occurs at 0° C and it is negligible compared with the velocity of sulphate reduction

The suggestions of Littlewood and Postgate[1] already cited seem to be only partially true There was no evidence for the idea that sulphate reduction might occur outside the osmotic barrier of the cell The main differences between our experimental conditions and those of Littlewood and Postgate are the presence of a hydrogen donor necessary for the reduction of sulphate and the low concentration of sulphate used in the reaction mixture (*M*/2,200) When a higher concentration of sulphate (*M*/220) was used, it was very difficult to observe any distinct accumulation of sulphate in the cell, probably because the concentration of sulphate in the medium was of the same order as the saturation concentration of the cells, the difference between the two being too small to be detected

My results show that *Desulphovibrio* is not freely permeable to sulphate, this being indicated by the results obtained using the following experimental procedures (*a*) inhibition by selenate, (*b*) oxygen inhibition of sulphate reduction, and (*c*) diffusion at 0° C Thus it must be concluded that (1) a significant accumulation of sulphate occurs in the cell, (2) sulphate must be metabolized inside the cell; and (3) the transport of sulphate is associated with the reduction of sulphate

I thank Prof J H Quastel for his valuable advice and interest in this work, and also the U S National Science Foundation for financial aid which made this work possible

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ANTIGENICITY OF PENICILLIN AND ITS RELATION TO ALBUMIN BINDING

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ANAPHYLACTOID reactions to penicillin are most commonly observed among severe immediate hypersensitivities to antibiotics[1] Reagin type of antibody was reported to cause these anaphylactoid reactions[2]. However, experimental hypersensitivity to penicillin has not been established by actual demonstration in animals In this article we present a method to induce active and passive anaphylaxis to penicillin in guinea pigs and discuss the mechanism of antigenic binding of penicillin to albumin

The method used to induce active anaphylaxis in guinea pigs to penicillin was as follows Equal volumes of 100,000 U/ml of penicillin *G* and 10 mgm/ml of recrystallized egg white albumin or horse serum albumin were incubated at 37° C for 24 hr The precipitates obtained by incubation were centrifuged and dissolved in saline to the original volume and adjusted to *p*H 7 6–7 8 For the sensitization 0 1 ml of this solution of penicillin–albumin complex was injected subcutaneously into guinea pigs twice weekly for a total of 5–10 times After 3 weeks

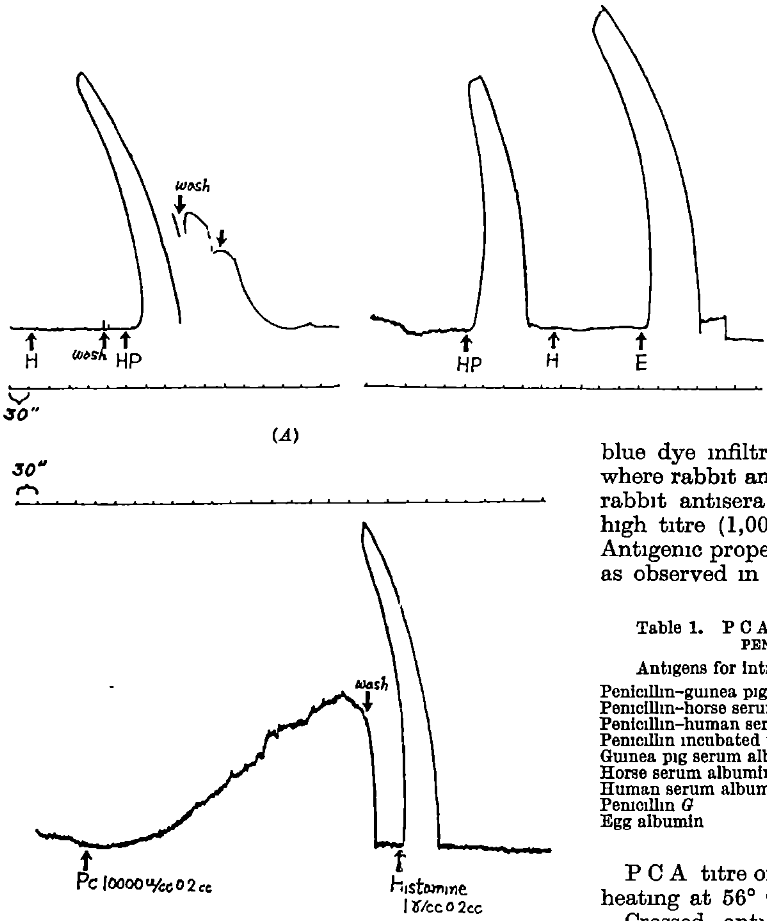

Fig 1 Schultz-Dale's ileum contraction test in guinea pig sensitized with penicillin–egg albumin complex (*A*) *H*, horse serum albumin, *HP*, penicillin–horse serum albumin complex, *E*, egg albumin, (*B*), *PC*, penicillin

of incubation, as shown in Fig 1*A*, the ileum of guinea pig sensitized with penicillin *G*–egg albumin complex showed a typical anaphylactic contraction as a result of contact with penicillin-horse albumin complex and egg albumin, but such contraction was not caused by horse albumin Penicillin-guinea pig serum albumin complex also can be used for the sensitization and the *in vitro* challenge

Plain penicillin *G* did not cause the anaphylactic contraction, or an atypical slow contraction was observed as shown in Fig 1*B* However, after pre-incubation with penicillin *G* any contraction caused by penicillin *G*–albumin complex was inhibited By use of the intravenous challenge method positive results were obtained only with penicillin–albumin complex and not with plain penicillin From these results penicillin appears to be a simple haptene and needs to be bound with albumin in order to gain a complete antigenicity.

Albumins to be combined in this method are not different according to their origins, in other words, they are exchangeable for sensitization and challenge.

Passive Cutaneous Anaphylaxis in Guinea Pigs

Passive cutaneous anaphylaxis (P C A) in guinea pigs by use of Ovary's method[3] was applied to test antigenicity of penicillin and closely related substances 3 ml of penicillin–egg albumin complex, 1 ml of dehydrated lanolin and 8 ml of liquid paraffin were mixed to make antigen for the sensitization of rabbit A total 0 2 ml of the mixture was injected into each foot pad and thereafter 1 ml subcutaneously once a week for a total of 10 times One-tenth ml of arbitrary diluted rabbit antiserum was injected intradermally into shaved abdominal area of guinea pigs After 5–6 hr , 1 ml of penicillin–albumin complex (2 5 times dilution of the complex antigen above described) and 1 ml of 0 5 per cent Evans's blue were injected into jugular vein Within 30 min blue dye infiltration was observed in the skin area where rabbit antiserum was previously injected All rabbit antisera of 8–10 weeks sensitization yielded high titre (1,000 times dilution) of P C A reaction Antigenic properties of penicillin were simple haptene as observed in the ileum contraction test (Table 1)

Table 1. P C A IN GUINEA PIGS WITH RABBIT ANTIBODY TO PENICILLIN–EGG ALBUMIN COMPLEX

Antigens for intravenous challenge	Results
Penicillin–guinea pig serum albumin complex	+ + +
Penicillin–horse serum albumin complex	+ + +
Penicillin–human serum albumin complex	+ + +
Penicillin incubated with human gamma globulin	—
Guinea pig serum albumin	—
Horse serum albumin	—
Human serum albumin	—
Penicillin *G*	—
Egg albumin	+ + +

P C A titre of rabbit antibody was unchanged after heating at 56° C for 4 hr

Crossed antigenicity to various penicillin derivatives and degradation products were tested by the P C A method. Penicillin *O* (allylmercaptomethyl-penicillin), penicillin *V* (phenoxymethyl-penicillin) and benzethicillin (dimethoxyphenyl-penicillin) gave the same positive results as penicillin *G* Exceptionally, phenethicillin (DL-phenoxyethyl-penicillin) needed acidification to make combination with albumin in order to cause positive P C.A reaction

Degradation products such as penicilloic acid, penicillic acid and penicillenic acid incubated with albumin gave no P C A reaction against the antibody to penicillin *G*–albumin complex, while inhibition was observed to result from antiserum pre-incubated with these degradation products.

Mechanism of Antigenic Binding of Penicillin to Albumin

After incubation of penicillin *G* and albumin at 37° C for 24 hr , the *p*H of the medium decreased from 6 2 to 4 6–4 8 By making the medium alkaline the P C A titre was almost completely lost On the contrary, by acidification the binding process was accelerated The relation between decrease of *p*H and P C A titre is shown in Table 2

An incubated mixture of penicillin *G* and serum did not induce P C A but after acidification and dialysis positive reaction was obtained Tissue homogenate such as liver, spleen, lung and skin gave no positive result, with the exception of the exudates, which usually have acid reaction From these observations it is obvious that penicillin may block alkaline

Table 2 RELATION BETWEEN DECREASE OF *p*H AND TITRE OF P C A

Antigens	*p*H before incubation	*p*H* after incubation	Titre of† P C A
Penicillin *G* + Albumin	6 2	4 4	1,000
Penicillin *G* + Albumin (acidified)	4 6	4 6	1,000‡
Penicillin *G* + Albumin (alkalized)	7 2	5 8	1
Phenoxyethylpenicillin + Albumin	6 2	5 6	0
Phenoxyethylpenicillin + Albumin (acidified)	4 6	4 6	1,000
Penicillin *G* + Normal serum	7 2	7 0	0
Penicillin *G* + Normal serum (acidified)	5 0	4 4	100
Penicillin *G* + Dialysed serum	6 4	5 0	1,000
Penicillin *G* + Pleural exudate	6 2	5 2	100

* 24 hr incubation at 37°C
† maximum dilution of antiserum
‡ 30 min incubation

radicals of the albumin molecules from the antigenic binding, whereas acidification accelerates this reaction By means of penicillin *G* labelled with sulphur-35 the number of molecules of penicillin bound to one molecule of the albumin complex antigen was estimated as 44 5 (mean of 4 experiments) In autoradiographs of paper electrophoresis with serum or exudate, penicillin was found to be bound to albumin and not globulin

A Peculiar Protein found in the Serum of Atopic Patients

The serum from patients with anaphylactoid reaction or angioneurotic œdema had strong capacity for antigenic binding without acidification This binding occurred within 30 min and was independent of the presence of any reagin to penicillin (Table 3)

Table 3. CAPACITY OF ANTIGENIC BINDING OF SERUM FROM NORMAL SUBJECTS AND PATIENTS

	Antigens	Duration of incubation	Terminal *p*H	Titre of P C A
1	Normal serum + Penicillin *G* (5 cases)	24 hr	6 8–7 2	0
2	No 1 serum* + Penicillin *G*	24 hr	7 0	1,000
	+ Penicillin *G*	30 min.	7 0	100
	„ + Penicillin *V*	24 hr	7 0	1,000
	„ + Phenoxyethyl-	24 hr	7 0	1,000
	„ penicillin	24 hr	7 0	1,000
	„ + Penicilloic acid	24 hr	5 8	0
	Heated serum (56° C , 4 hr) + Penicillin *G*	24 hr	7 0	1,000
3	No 2 serum† + Penicillin *G*	24 hr.	6 8	1,000
4	No 3 serum‡ + Penicillin *G*	24 hr	7 0	1,000

* Patient No 1 with anaphylactoid reaction to penicillin, reagin to penicillin (+++)

† Patient No 2 with anaphylactoid reaction to sulpha drug, reagin to penicillin (—)

‡ Patient No 3 with angioneurotic œdema, reagin to penicillin (—).

From these results and observations we assumed the existence of a peculiar protein in such patients which has strong capacity of antigenic binding to some kind of haptene so that atopic patients acquire hypersensitivities very easily to many kinds of haptenes

Physicochemical properties of this protein and implications respecting haptenes other than penicillin remain to be investigated

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INHERITED CASEIN VARIANTS IN COW'S MILK

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PAPER electrophoresis has been used successfully to demonstrate the occurrence of genetically distinct types of β-lactoglobulin[1,2] and α-lactalbumin[3] in cow's milk With the aid of the same method, evidence has now been obtained for the existence of gene-controlled variants of the β- and γ-components of the principal milk protein, casein

Paper electrophoresis of casein in conventional buffer systems presents difficulties which stem from the anomalous behaviour of α-casein Zhdanova and Vlodavets[4] and Libbey and Ashworth[5] have shown that this fault can be eliminated by adding urea to the buffer, but they did not obtain a clean separation of β- from α-casein Such a separation has now been achieved by working with a buffer system made up of constituents with which casein is associated under natural conditions, namely, citrate and phosphate Briefly, the technique consists of electrophoresis at 4° C in a buffer of *p*H 7 15, made up of 0 913 gm citric acid monohydrate, 3 56 gm disodium hydrogen phosphate and 360 gm urea a litre Using the horizontal type of apparatus described by Kohn[6], a constant 180 V is applied for 5 hr to two sheets of Whatman 3 *MM* paper (10 × 20 cm), each carrying three or four samples deposited across the centre of the sheets Samples are prepared by isoelectric precipitation of the casein from 10 ml of skim milk, addition of 2 gm. urea to the centrifuged and washed casein, and dispersion with water to a final volume of about 5 ml of a slightly opaque solution

As shown in Fig 1, the procedure resolves the protein into two α-casein bands, the slower-moving one probably representing the κ-casein of Waugh and von Hippel[7], a single β-band, and two γ-bands which move towards the cathode Contrary to the other components, the γ-caseins—very clearly shown in the chosen examples—vary markedly in strength from milk to milk, as will be discussed later Otherwise rather uniform results were obtained in comparing the casein patterns from the milk of individual cows A departure from the norm emerged during the examination of a series of samples from one-egg twins of different breeds, kept at the Institute milk from each of a pair of cross-bred Jersey–Guernsey cattle (*O*37/38) was found to contain two β-casein

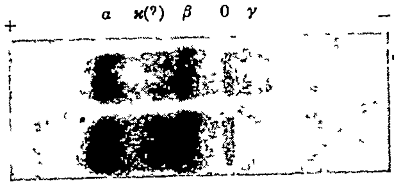

Fig 1. Separation of casein by paper electrophoresis 0 = origin (photographed in transmitted light)

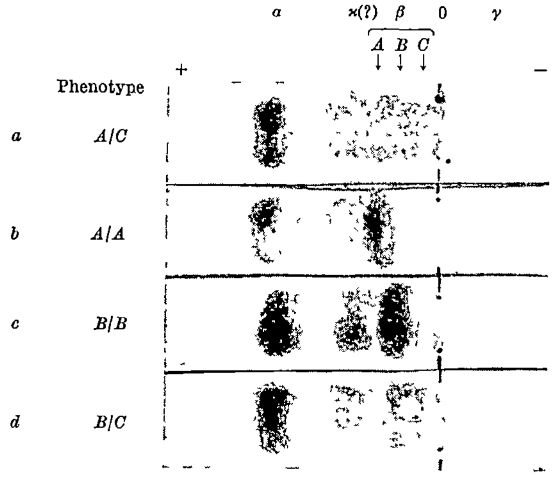

Fig 2 β-Casein variants 0 = origin (photographed in reflected light)

components (*B* and *C*, Fig 2*d*), migrating more slowly than the usual single component (*A*, Fig 2*b*) The fact that the 'anomaly' occurred with both twins, suggested a genetical basis, and led to the collection of samples at the farm of origin with the surprising result that, of 21 cows examined, only 3 produced β-casein *A* Three additional variants, *A* and *C* (Fig 2*a*), *A* and *B* (not shown), and *B* only (Fig 2*c*) were found among the remaining samples The older animals at this farm were Guernseys, the younger ones *F*1-crosses with Jersey sires The male line was traced back to a small pedigree Jersey herd with ten cows in milk four of these produced β-casein *A*, the others *B* or *A* and *B* combined

Examination of the available family data showed the results to be in accord with the concept of the existence of three alleles of a gene (locus symbol β-*Cn*), giving rise to six possible phenotypes of which all but one, *C*/*C*, have so far been observed Some relevant relationships for the twins *O*37/38 are given in Fig 3 They show that all three alleles are present in the Guernsey stock, and that the Jersey sire (III) as well as its sire (II) and grand sire (I) transmit the *B*-allele which, as mentioned here, is prevalent at the Jersey farm

Though still in its early stages, a survey of milk samples from cows of other Channel Island herds has already shown that the observations connected with the twins *O*37/38 are not to be regarded as rare anomalies In tests of samples from seven Guernsey herds, both the *B*- and *C*-alleles were found in one herd and the *B*-allele in another; and in one of three Jersey herds the *B*-allele occurred quite frequently These herds are now under more detailed investigation, and further data on Jersey cows are being collected to ascertain whether the *C*-allele, so far only found in Guernsey milk, occurs in that of Jersey cows as well Information is also required on the possible occurrence of the β-casein polymorphism in other breeds So far only Friesian cows have been examined in sufficient number to suggest that the polymorphism might be restricted to Channel Island cattle This would be highly interesting, since Bangham[8] has shown that hæmoglobin polymorphism among British cattle breeds is confined to Channel Island (and South Devon) animals It is true that during tests on milk from more than a hundred Friesian cows, a few instances were observed in which the *B*-allele was present, but these were so isolated that they might well be merely the consequence of cross-breeding with Channel Island cattle

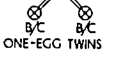

Fig 3 Pedigree of one-egg twins (*O*37/38), showing 5 of the 6 possible phenotypes of β-casein

As mentioned earlier, the two γ-casein bands, obtained from the milk of individual cows, were found to vary considerably in strength, as illustrated by Figs 1 and 2 (The photographic reproduction conceals the fact that weak bands were present in all the examples shown in Fig 2) In some samples, the bands appeared to be completely absent The statement that this variability has a genetical basis is founded largely on the observation that, in comparisons of the strips prepared from the milk of eighteen pairs of one-egg twins, γ-caseins of identical appearance and strength were obtained within each single pair, while the variability between the pairs covered the whole range These findings suggest the existence of a metabolic polymorphism of the kind described by Allison[9] as "that in which one genotype has a particular substance in relatively large amounts while other genotypes have smaller amounts or lack it altogether" There has been no indication that this polymorphism differs from breed to breed. Interpretation of the results is far from easy, when the γ-casein bands are very weak, and detailed study of this polymorphism has been postponed pending the development of a more searching technique producing the weak γ-caseins in higher concentration Such a development seems necessary, as the simple expedient of applying more than the usual amounts of total casein to the filter paper results in the formation of blurred, poorly resolved bands

Casein is present in cow's milk in the form of a colloidal complex It appears from the foregoing that several independent genes are involved in its elaboration by the mammary gland, and that the 'classical' sub-division of the complex into α-, β- and γ-caseins is justified and meaningful

I am grateful to my colleagues of the Dairy Husbandry Department, Dr F H Dodd and W S Mitchell, and to N T Culling and his staff at the Reading Regional Milk Records Office, Milk Marketing Board, for the collection of milk samples and information, and to Miss J E Cooper for skilful technical assistance

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COMPARISON OF TRANSDUCTION VERSUS TRANSFORMATION OF LINKED MARKERS IN *BACILLUS SUBTILIS*

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WITH *Bacillus subtilis*, it has been possible by transformation to demonstrate linkage between mutant sites (markers) responsible for tryptophan and histidine requirements[1,2] and between markers responsible for tryptophan and tyrosine requirements[3]. The degree of the linkage is determined by comparing the frequency with which bacteria which have been transformed to independence of one required metabolite have been simultaneously transformed to independence of the other[4]. Due to the low frequency of transformation in these experiments, cells are rarely transformed simultaneously for two unlinked markers. As a result of the observation of phage-mediated transfer of genetic material (transduction) in *B. subtilis* by Thorne[5], it has become possible to compare the degree of linkage between a given pair of genetic markers introduced either by deoxyribonucleate mediated transformation or by bacteriophage-mediated transduction.

The lysogenic *B. subtilis W*23 *L*–9, carrying a transducing phage, was kindly provided by Dr. C. B. Thorne. Transducing phage suspensions were prepared in broth after confluent lysis in soft agar plates seeded with both the phage and the sensitive wild-type bacterial strain *W*23 (ref. 6). Shaking with chloroform followed by a low-speed centrifugation was sufficient to remove the bacteria from these suspensions. The phage was partially purified by centrifugation at 13,000 r.p.m. for 80 min. Sterile glycerol was added to the phage suspension to make 10 per cent by volume and 1 ml. samples were stored in a freezer cabinet at – 20° C. The phage titre was determined by plating on tryptone agar plates supplemented with 0.5 per cent glucose and 0.1 per cent yeast extract, using the agar layer method[6]. The transduction procedure described by Thorne was modified in the following way. Mutant bacterial cultures were grown in tryptone broth supplemented with 0.5 per cent sodium chloride and 0.5 per cent glucose, for 5 hr. at 37° C., with aeration. To 1 ml. samples, sterile glycerol was added to make 10 per cent by volume, and the cultures were kept frozen at – 20° C. until use. For transduction experiments, the cultures to be transduced were allowed to thaw in an ice bath and either diluted 1/10 into fresh medium and incubated for 5 hr. or centrifuged after being thawed, re-suspended in 1 ml. of fresh broth and incubated with aeration for 60 min. Following either of these treatments, 0.1 ml. of a phage suspension was added and the cultures were incubated with aeration for 45 additional minutes after which aliquots were spread on plates containing minimal medium[2]. Control platings of the phage suspensions showed no bacterial contamination; deoxyribonuclease treatment of these suspensions had no effect on transduction yields. In the transduction tubes, the bacterial densities varied from 2 to 5 × 10^8/ml. Phage densities were between 2 and 3 × 10^8/ml. All incubations were at 37° C. The procedure for transformation has been described elsewhere[2].

It can be seen in Tables 1 and 2 that the frequency with which the respective pairs of markers are transferred jointly is very nearly the same, when they are introduced either by transformation or transduction. This is true for both linkage groups that have been examined, tryptophan–histidine and tryptophan–tyrosine. In Table 3 a comparison has been made between frequencies of transformation and transduction of tryptophan, and a number of markers located in another histidine region showing no evidence of linkage to tryptophan. The four mutant histidine sites represented in the first four strains have been shown by transformation to be

Table 1. LINKAGE BETWEEN HISTIDINE AND TRYPTOPHAN MARKERS AND BETWEEN TYROSINE AND TRYPTOPHAN MARKERS BY TRANSDUCTION AND TRANSFORMATION

Method 1

Recipient bacteria Strain No.	genotype donor	Colonies acquiring his^+			Colonies acquiring $tryp^+$		
		Total examined	his^+-$tryp^+$ found	(Per cent) doubles	Total examined	his^+-$tryp^+$ found	(Per cent) doubles
168-*SB*25	Phage (*W*)	326	238	73 ± 6	297	220	74 ± 5
his_2tryp	DNA (*W*)	357	226	63 ± 7	340	221	65 ± 5
168-31	Phage (*W*)	320	204	64 ± 8	330	235	71 ± 6
his_5-tryp	DNA (*W*)	387	241	62 ± 7	395	248	63 ± 7
		Colonies acquiring tyr^+			Colonies acquiring $tryp^+$		
		Total examined	tyr^+-$tryp^+$ found	(Per cent) doubles	Total examined	tyr^+-$tryp^+$ found	(Per cent) doubles
168-*SB*29	Phage (*W*)	280	157	56 ± 7	354	215	60 ± 9
tyr_1-tryp	DNA (*W*)	335	182	54 ± 7	320	179	56 ± 6
168-*SB*30	Phage (*W*)	325	178	55 ± 4	296	168	57 ± 7
tyr_2-tryp	DNA (*W*)	290	146	50 ± 5	287	150	52 ± 4

The cultures treated with deoxyribonucleate (DNA) or phage were plated on minimal agar supplemented with either histidine (his) or tryptophan (tryp), in the case of the his-tryp mutants, and with tyrosine (tyr) or tryptophan in the case of tyr-tryp mutants. Colonies selected for independence of one metabolite were tested for independence of the other by streaking on unsupplemented minimal agar plates. *W*, wild-type bacterial strain *W*-23.

Table 2. LINKAGE BETWEEN HISTIDINE AND TRYPTOPHAN MARKERS AND BETWEEN TYROSINE AND TRYPTOPHAN MARKERS BY TRANSDUCTION AND TRANSFORMATION

Method 2

Recipient bacteria	donor	his^+ colonies	$tryp^+$ colonies	his^+-$tryp^+$ colonies	(Per cent) doubles
168-*SB*25	Phage (*W*)	1,226	1,280	854	68
his_2-tryp	DNA (*W*)	1,173	1,246	812	67
168-31	Phage (*W*)	696	730	507	71
his_5-tryp	DNA (*W*)	1,120	1,208	782	67
		tyr^+ colonies	$tryp^+$ colonies	tyr^+-$tryp^+$ colonies	(Per cent) doubles
168-*SB*29	Phage (*W*)	499	506	327	65
tyr_1-tryp	DNA (*W*)	838	855	476	57
168-*SB*30	Phage (*W*)	1,237	1,125	623	53
tyr_2-tryp	DNA (*W*)	870	1,081	484	50

The treated cultures were plated simultaneously on unsupplemented and singly supplemented minimal agar plates. The colonies appearing on minimal represent the doubly transformed/transduced cells.

$$\frac{\text{No. of colonies independent of both required metabolites}}{\text{Average number of singly transformed/transduced colonies}} \times 100$$

Table 3 TRANSDUCTION AND TRANSFORMATION OF UNLINKED TRYPTOPHAN⁻, HISTIDINE⁻ MUTANTS

Recipient bacteria Strain No	genotype	*Colonies transduced to his$^+$			*Colonies transformed to his$^+$		
		his$^+$	tryp$^+$	his$^+$-tryp$^+$	his$^+$	tryp$^+$	his$^+$-tryp$^+$
168–26	his$_8$-tryp	157	1,750	0	440	400	0
168–30	his$_9$-tryp	37	536	0	107	85	0
168–(*N*-3)	his$_{10}$-tryp	5	75	0	1,020	850	0
168–*SB*1	his$_1$-tryp	28	130	0	1,020	1,100	0
168–32	his$_6$(?)-tryp	0	220	0	0	150	0

* Number of transduced or transformed colonies on plates, bearing approx 10^7 treated bacteria

closely linked to each other[2] The tryptophan mutation is identical with that of the four mutants in Tables 1 and 2. It can be seen that in transduction, the frequencies of the genetic transfer of the histidine[1,8-10] region are less than 10 per cent of that of the tryptophan marker, whereas by transformation these markers are transferred at about the same frequency as is the tryptophan marker In one mutant strain, 168–32, requiring tryptophan and histidine, neither mutational site has been precisely located This mutant can neither be transformed nor transduced to histidine independence

The results presented show that the ratio of the frequency with which a pair of markers is introduced simultaneously to the frequency of introduction of a single marker is the same, within experimental error, whether these markers are introduced by transformation or transduction A group of histidine markers, shown to be unlinked to tryptophan by transformation, is also found to be unlinked by transduction The frequencies of transfer of these latter histidine markers in comparison with tryptophan were always markedly lower in transduction than in transformation experiments Differences in frequency of transduction of different markers have been described in *S typhimurium*[7]

Thus it has been demonstrated that, for several linked pairs of markers, the process of genetic incorporation as expressed in the degree of linkage, is remarkably similar whether the transfer is mediated by purified deoxyribonucleate or by transducing bacteriophage

We wish to thank Dr R D Hotchkiss for the interest he has shown in this investigation

The investigation was supported by a grant from the Association for the Aid of Crippled Children and grant No *G*/12894 from the National Science Foundation to Dr R D Hotchkiss

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EXTRACELLULAR ESTERASE OF GROUP A STREPTOCOCCI

Esterase in Extracellular Concentrates of Group *A* Streptococci and the Homologous Antibody

A CARBOXYLIC acid esterase sensitive to diisopropylfluoridophosphate (DFP) has been found in extracellular concentrates of group *A* streptococci The existence in various sera of antibodies against this esterase is described These antibodies precipitate the streptococcal esterase but do not block its activity, a situation identical to that found by Uriel[1] with the esterases of human serum and their corresponding horse antibodies

The enzyme was demonstrated by the following method Electrophoresis of ten-fold to a thousand-fold concentrates of the extracellular products of group *A* streptococci was made from holes or slots in agar gel for 75 min in 10 × 4 cm slides by the Grabar and Williams technique[2] or for 45 min by the microscope-slide modification of Scheidegger[2] Following electrophoresis or immunoelectrophoresis, the esterase activity was revealed by a technique described in ref 1 The principle of this method[3] involves the splitting of the substrate β-naphthyl acetate and coupling with the azo dye, diazo blue *B*, the esterase appearing as a red-blue area (Fig 1) So far, esterase activity has been demonstrated in extracellular concentrates of six strains of group *A* streptococci (Table 1) The concentrated uninoculated broths gave negative results

The esterase activity does not appear to be due to the following known streptococcal products, streptolysins *O* and *S*, streptokinase, proteinase, deoxyribonuclease, diphosphopyridine nucleotidase (DPNase), and erythrogenic toxins *A* and *B* Of these the most interesting is proteinase, because it is known that some proteolytic enzymes like trypsin have esterase activity

The electrophoretic mobility of the esterase was similar to that of streptolysin *O* (ref 4) However, the two activities can be differentiated as will be shown here The two products may share another property, as shown in Table 1 Concentrates of lot 32369-26, which had been prepared by filtration of the culture through a Berkefeld filter, showed no esterase activity, a similar adsorption had occurred with the streptolysin *O* of this lot[5]

Investigations of the effects of inhibitors were carried out by the method in ref 1 DFP at 10^{-4} *M* inhibited the esterase activity, while 10^{-4} *M* *p*-chloromercuribenzoate was without effect The opposite results were obtained with streptolysin *O* (ref. 4)

Table 1 ESTERASE ACTIVITY OF VARIOUS STREPTOCOCCAL PREPARATIONS

No	Strain and lot No *	Supernatant (*S*) or filtrate (*F*) and degree of concentration	Esterase activity	Serological type of strain	Main feature of strain
1*a*	32369-22	*S*-1000 ×	+	18	Production of erythrogenic toxin *B*
b	32369-26	*F*-1000 ×	−		
2*a*	Cook *B*-25	*S*-1000 ×	+	Unknown	Production of erythrogenic toxin *B*
b	Cook *B*-27	*S*-1000 ×	+		
3	*NY*5 (*E*14)-2	*S*-1000 ×	+	12	Production of erythrogenic toxin *A*
4*a*	*C*203*S*-9 †	*S*	−	3	DPNase +
b	,,	*S*-30 ×	+		
5*a*	Wilson-1 †	*S*	−		
b	,,	*S*-30 ×	+	1	DPNase −
6*a*	*S*-84-1	*S* (‡)	−	3	Production of high yield of streptolysin *O*
b	,,	*S*-10 ×	+		
c	,,	*S*-30 ×	+		

* Methods of mass culture were used as in ref 5

† Given by Dr A Bernheimer

‡ Given by Dr R Wahl

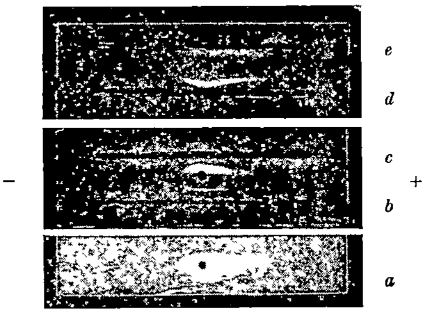

Fig 1 *a*, Electrophoresis of extracellular concentrate Wilson-1 , *b–e*, immunoelectrophoresis of extracellular concentrate Wilson-1, *b*, normal horse serum, *c*, normal human serum No 1, *d*, normal human serum No 2, 1 100, *e*, normal human serum, No 2, 1 1,000

In immunoelectrophoresis, the various sera placed in the trenches included six normal adult sera, a 10 per cent human γ-globulin (Centre de Transfusion, Réanimation de l'Armée), one standard horse antistreptolysin *O* serum (1,500 units/ml Institut Pasteur) and scarlet fever antitoxin (horse), Lederle lot 1570–473 (ref 5) All these sera gave arcs of enzymic activity as shown in Fig 1, due to precipitation of antigen and antibody The arcs of precipitate before staining for esterase were not visible to the naked eye nor could they be observed after staining with azocarmine[6] A rabbit antiserum containing 1,500 units of antistreptolysin *O* and a rabbit antistreptococcal serum (type 1, precipitating) made with killed bacteria gave negative results in immunoelectrophoresis

That normal human serums can react with extracellular streptococcal antigens has been shown by Halbert *et al* and confirmed by others (ref 7), and the present findings confirm another antigen–antibody system

Dr Tom Webb and Efstratios Avrameas of this Service made useful suggestions in the course of the work Sera were given by Drs R Wahl or A Eyquem of the Institut Pasteur Some streptococcal fractions were given by Dr A Bernheimer of New York University and Dr S P Halbert of Columbia University

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Electrophoretic Mobility and Detection of Hæmolytic Activity of Streptolysins O and S in Agar-Gel

While investigating the extracellular esterase of group *A* streptococci, it became necessary to have a direct method for the demonstration of the electrophoretic mobility of streptolysins *O* and *S* A slight modification of the procedure of Uriel[8] for the direct detection of proteolytic enzymes after electrophoresis in agar-gel proved successful and is as follows The streptolysin preparations were placed in slots cut in agar on slides The streptococcal concentrates containing streptolysin *O* were either a crude thousand-fold concentrate of supernatants of broth cultures grown in dialysate broth[5] or a thirty-fold concentrate (as in ref 5) of an Institut Pasteur streptolysin from cultures of strain *S* 84 of Kalbak grown in infusion broth (given by Dr R Wahl, Institut Pasteur, Paris) Both streptolysin *O* preparations contained sufficient lysin in the reduced form, so that addition of cysteine was not necessary for activity, which was fortunate, since this reagent nullified the effect of one of the inhibitors (*p*-chloromercuribenzoate) used in the investigation That streptolysin *O* collected after 12–24 hr can exist in the reduced form without reduction was shown in the pioneering studies of Neil and Mallory[9] The nucleic acid induced streptolysin *S* was given by Dr A Bernheimer of New York University Electrophoresis in agar-gel was carried out for 45 min by the procedure in ref. 2 in a modified microscope-slide method of Scheidegger (see ref 2) Following electrophoresis, the slides were immersed in phosphate-buffered saline *p*H 6 5 (ref 10) for 15 min to convert the gels to isotonicity. The surface of the slides was then dried lightly with filter paper, and overlayed by means of a pipette with 2 ml of a 2 per cent washed human red blood cell suspension in melted 1 5 per cent agar in phosphate-buffered saline at 45–50° C When the overlay had hardened, the slides were placed in Petri dishes and incubated at 37° C In about 30 min the hæmolysis became visible, but the reaction was allowed to proceed for a total of 60 min As shown in Fig 2, streptolysin *O* migrated very slowly while streptolysin *S* exhibited rapid mobility, easily differentiating the two lysins

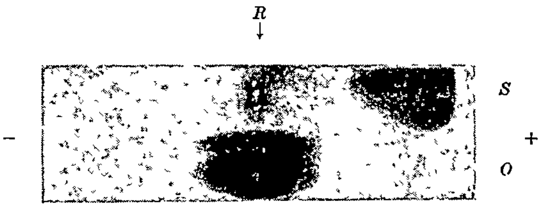

Fig 2 Electrophoresis of streptolysins *O* and *S* *R*, reservoir, top, streptolysin *S*, bottom, streptolysin *O* The dark areas are the hæmolysed zones There is an artefact at the reservoir of streptolysin *S* due to overlay of agar over the reservoir

The mobility of streptolysin *O* observed by the direct method apparently differs from the mobility described by Hanson and Holm[11] with the use of the 'basin overlay' and immunoelectrophoretic techniques and markedly differs with the mobility found by Halbert and Keatinge[7] with an immunoelectrophoretic method By including in the overlay of red blood cells sufficient antistreptolysin *O* (horse, Institut Pasteur), it was demonstrated that streptolysin *O* activity could be inhibited.

Some investigations of chemical inhibitors were carried out using the technique elaborated by Uriel[1] for the study of esterases in human serum Following the electrophoresis of streptolysin *O* or *S*, the slides were immersed in either 10^{-4} *M* di-isopropylfluorophosphate (DFP) or 10^{-4} *M* *p*-chloromercuribenzoate in buffered saline, and overlays of red blood cells in agar were made, but containing 10^{-4} *M* concentration of the inhibitors It was found that streptolysin *O* was inhibited by *p*-chloromercuribenzoate in accordance with the findings by Smythe and Harris[12] of the effect of mercury salts on this lysin, it was not inhibited by DFP The two inhibitors thereby differentiate streptolysin *O* from streptococcal esterase Streptolysin *S* was not inhibited by either *p*-chloromercuribenzoate or DFP To our knowledge these findings have not been reported before

It is planned for the future to carry out electrophoretic work on other bacterial hæmolysins, especially tetanolysin, pneumolysin and the O toxin of *Cl welchii*, all of which were shown by Todd[13] to have an immunological relationship to streptolysin O

This work was aided by a grant from the National Institutes of Health, Bethesda

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A CELL FOR RAPIDLY MEASURING RESTRICTION TO DIFFUSION OF MACROMOLECULES THROUGH AGAR GELS OF VARIOUS PORE SIZES

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RESTRICTED diffusion of small proteins through stretched dialysis tubing has been investigated by Craig *et al* [1] This and other work indicated that restricted diffusion may be an effective tool for (*a*) size estimation of macromolecules in undefined or impure systems, (*b*) fractionation of components in such systems, and (*c*) tests of particle-size homogeneity in purified preparations In view of these possibilities we thought it advisable to extend measurements into the range of pore sizes which would be effective in restricting diffusion of virus particles.

Rapid and reproducible results are obtained by using diffusion cells of the construction shown in Fig 1 The inner chamber is made of glass A thin piece of 'Monofilament Saran' screening (*MS*-904, 51 mesh, 0 008 in yarn diam , National Filter Media Corp) is stretched across its bottom and cemented securely around the edge (The materials mentioned were purchased from the companies designated These sources are presented only for specific identification of the materials used No discrimination is intended and no guarantee of reliability implied) The mesh on the bottom of this inner chamber is then immersed to a depth of approximately 1 mm in a warm agar sol of accurately known concentration Sol is drawn swiftly by surface tension into the interstices of the 'Saran' mesh The chamber is withdrawn from the agar and excess sol is gently scraped from the bottom surface of the 'Saran' and from the outer rim of the chamber A thin film of agar (about 0 03 cm thick) is left in the mesh across the lower surface of the chamber This film is allowed to gel at room temperature and forms a continuous, sturdily supported membrane The cell is assembled with a rubber sleeve, a flat cover, a screw clamp, and an outer chamber of the dimensions shown The inner chamber is filled with buffer solution and closed securely with the screw clamp The membrane assembly is placed in contact with buffer in the outer chamber and allowed to 'mature' for 48 hr in a cold constant-temperature chamber The buffer in the inner chamber is then replaced by a buffered solution of the molecular species the diffusion of which is to be measured That in the outer chamber is replaced by fresh buffer at successive timed intervals and concentrations of the diffusates are assayed

Integration of Fick's first law for the boundary conditions of this system leads to equation (1) (ref 2)

$$\frac{DA}{l} = -\frac{V_i V_0}{t(V_i + V_0)} \ln \frac{(C_i - C_0)f}{(C_i - C_0)i} \qquad (1)$$

D is the diffusion coefficient l and A are the effective length and total cross-sectional area of pores in the

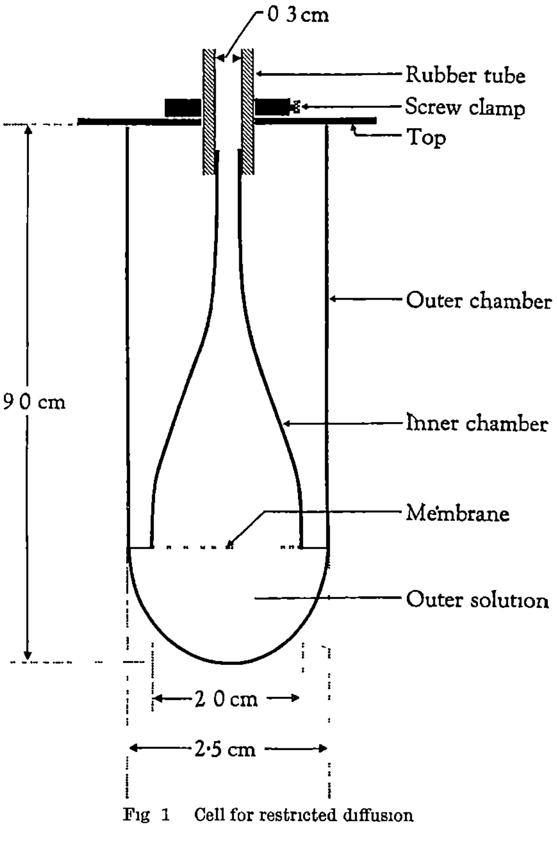

Fig 1 Cell for restricted diffusion

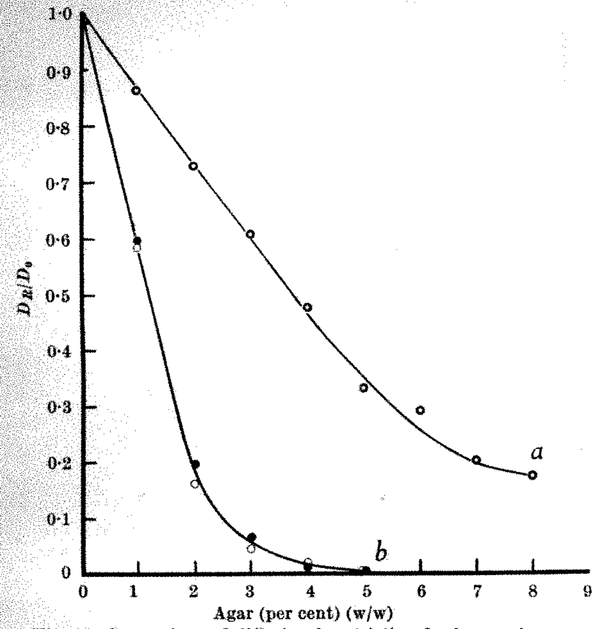

Fig. 2. Comparison of diffusional restriction for human hæmoglobin and southern bean mosaic virus particles. The restricted diffusion coefficients are plotted as percentages of the extrapolated diffusion coefficients against gel concentration. *a*, Human hæmoglobin; *b*, southern bean mosaic virus; ●, assay by ultra-violet absorbance; ○, assay by infectivity

membrane. V_i and V_0 are the respective volumes of the inner and outer solutions the concentrations of which are C_i and C_0; i and f refer to initial and final conditions; t is the duration of the experiment. In practice the quantity A/l is evaluated for each membrane by diffusion of a small molecular species the diffusion coefficient of which is known.

Representative data (averages of 4 determinations) for restricted diffusion of purified southern bean mosaic virus (0·27 mgm./ml.) and 1 per cent human hæmoglobin (2 × cryst., 9019, Nutritional Biochemicals Corp., Cleveland, Ohio) both in 0·0667 *M* phosphate buffer (*p*H 6·7) through a series of 'Ionagar No. 2' (Oxoid) membranes of different gel concentrations are shown in Fig. 2. The agar was made up at each concentration (w/w) in distilled water and autoclaved 15 min. at 15 lb./in.^{2} pressure. The cells were assembled and calibrated with 0·1 *M* sodium chloride (using $D_{20,w} = 1{\cdot}36 \times 10^{-5}$ cm.^{2}/sec.) (ref. 3). Behaviour of all cells conformed closely to equation (1). This indicates that transport through the gel under the experimental conditions used is at least predominantly diffusional.

Diffusion was carried out in a constant-temperature chamber at 8° ± 0·5° C.. Suspensions were assayed by ultra-violet absorbance at 2600 Å. (for southern bean mosaic virus) and 2800 Å. (for hæmoglobin). The southern bean mosaic virus diffusates were assayed also by infectivity tests. Results of these measurements agree closely with the absorbance values as shown in Fig. 2.

Values for restricted diffusion coefficients were calculated by using equation (1) for the two species of particles at each gel concentration. When these coefficients (corrected to water at 20° C.) were plotted against agar concentration and the curves extrapolated to zero gel concentration, the values obtained were $1{\cdot}23 \times 10^{-7}$ cm.^{2}/sec. for southern bean mosaic virus and $6{\cdot}6 \times 10^{-7}$ cm.^{2}/sec. for hæmoglobin. These values are in good agreement with the reported values of $1{\cdot}39 \times 10^{-7}$ cm.^{2}/sec. and $6{\cdot}9 \times 10^{-7}$ cm.^{2}/sec. (ref. 4). Fig. 2 shows the comparative diffusional restriction encountered by particles of these two types as the gel pore size is decreased with increasing agar concentration. In this graph, ratios of D_R (the restricted diffusion coefficient) to D_0 (the extrapolated coefficient) for the two particle-species at each gel concentration are plotted against the gel concentrations. It is clearly seen that the larger southern bean mosaic virus particles (28·0 mμ diam.[5]) were restricted to a much greater extent in their migration through the gel matrix than were the smaller (6·16 mμ diam.[6]) hæmoglobin particles.

Results of this investigation suggest that restricted diffusion by use of agar-gel membranes should be a particularly useful tool for the study of viruses. For example, although the hæmoglobin molecule diffuses only 4·5 times as fast as the southern bean mosaic virus particle under free conditions with only hydrodynamic restriction, it was found to diffuse 63 times as fast through a 4 per cent agar-gel membrane. With such separation between virus particles and other macromolecular components of tissue extracts, it should be possible to effect a high degree of purification by using a limited number of countercurrent steps under properly selected restrictional conditions.

We wish to express our appreciation to James M. Parsons for help in the initial stages of this work.

A part of this work was carried out in the laboratories of the Harding College Chemistry Department, Searcy, Arkansas, under the support of a Brown Hazen grant obtained through Research Corporation, New York.

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AUTORADIOGRAPHY USING MELTED NUCLEAR EMULSION

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AUTORADIOGRAPHY of tissue sections using melted emulsion may be carried out in two ways: the sections may be stained first, coated with nitrocellulose and emulsion and then processed, or the sections may be stained after photographic processing. During an investigation of rabbit uterus (labelled with carbon-14) by the first method, it was found that sections became de-stained in spite of considerable overstaining, and black deposits, ranging from a few silver grains to large aggregates, appeared

Table 1

Slide No.		Mean No. of grains ± stand. error in 1,380 μ^2		Mean No. of aggregates ± stand. error in 5,520 μ^2	Mean No. of grains ± stand. error over one nucleus
		Background	Over tissue		
1	Unstained sections covered with thin nitrocellulose coat	8·4 ± 0·59	16·0 ± 1·95	15·4 ± 2·05	
2	Unstained sections embedded in a thick nitrocellulose coat	6·0 ± 0·32	7·7 ± 0·76	none	
3	Sections stained in hæmatoxylin and covered with thin nitrocellulose coat	7·4 ± 0·71	18·1 ± 1·1	63·4 ± 5·71	15·75 ± 1·92
4	Sections stained in eosin and covered with thin nitrocellulose coat	7·1 ± 0·61	10·8 ± 0·76	22·2 ± 1·8	several

All sections were of non-radioactive rabbit uterus.

Grains were counted under oil immersion, in 1,380 μ^2 at a time, at least 10 such areas being counted for a sample. Aggregates were counted in 5,520 μ^2 at a time, four such areas being counted for a sample.

on slides. Discrete grains, indistinguishable from those caused by ionizing events, occurred in greater density near the aggregates.

The formation of aggregates has been reported by others[1,2], but discrete grains which may occur with or without aggregates do not seem to have received as much attention. Stains and other solutions have been shown to remove radioactivity from tissue[3] and silver grains from images[4]. Several substances have been shown to cause chemical fogging[5], but a protective film between tissue and emulsion is considered to prevent such artefacts. The observations reported here indicate that stains may be involved in the formation of artefacts when a thin nitrocellulose film is used and that artefacts as well as bleaching of stains may be prevented by embedding sections in a hydrophobic layer.

Strain-induced artefacts. Slides with 8μ thick sections of non-radioactive rabbit uterus were deparaffinized and covered with a thin or thick coat of nitrocellulose. Kodak *NTB*3 emulsion was applied over these unstained sections, the slides sealed in light-tight boxes and processed after 24 hr. in the refrigerator (Fig. 1 ; Table 1, slides 1 and 2). Slide 1 showed several aggregates and the grain count over tissue was twice the background count $(P < 0\cdot001)$, whereas slide 2 showed no aggregates and the grain count over tissue was not significantly different from background. Among slides with a thin nitrocellulose coat, appreciably fewer aggregates were found on those that were dried flat after dipping in nitrocellulose than on slides which were dried vertically.

The thin coat was applied by two dips in 1 per cent nitrocellulose, the slide being dried for 15 min. after the first dip and overnight after the second. The film which resulted was about 0·3μ thick and the emulsion formed a layer varying in thickness from 3 to 10μ. The thick coat was obtained by pipetting 1·5 ml. of 2 per cent nitrocellulose on the slide and drying in a slow stream of air for about 18 hr. The sections became embedded in a 9μ thick layer of nitrocellulose and the emulsion applied on its even surface formed a uniform layer about 3μ in thickness.

It has been suggested[2,4] that emulsion layers of uneven thickness may become reduced due to physical strains during drying or processing. Evidence confirming this view was obtained here when clean slides, slides with coverslips cemented on them and slides with depressions (5, 8, 10 and 15μ deep) ground on them were coated and processed as in the case of slide 1. Aggregates and grains were found at the edges of coverslips and depressions. It may be mentioned here that such strain-induced artefacts occur with 5μ or thicker sections ; they are less common or absent with thinner sections. Tissue sections with few spaces or small ones (less than about 30μ across), such as liver and spleen sections, also do not produce many artefacts.

Chemically induced artefacts. In order to determine whether stains could reduce the emulsion when they were separated from it by a thin nitrocellulose film, a drop each of Mayer's hemalum and 1 per cent eosin were dried on a slide, the slide was coated with

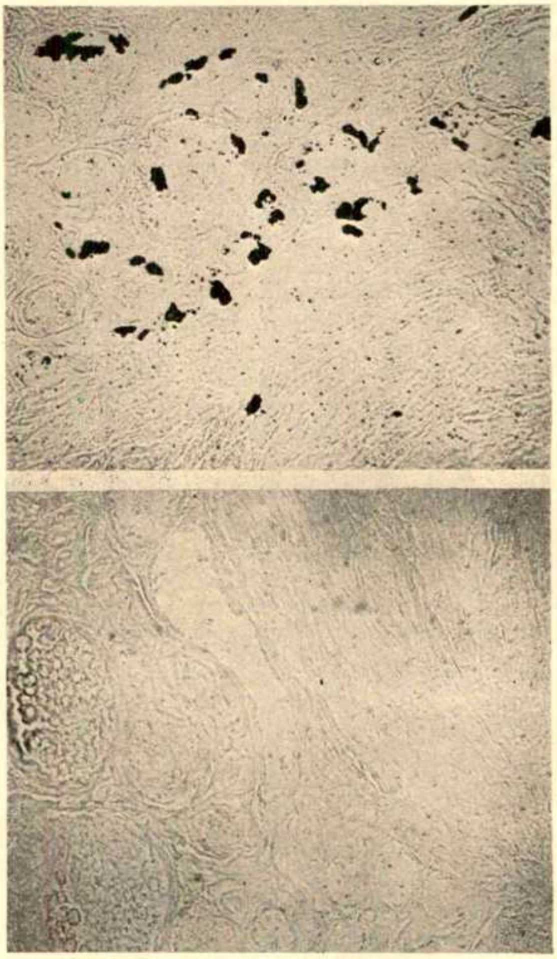

Fig. 1. Non-radioactive unstained section of rabbit uterus covered with a thin nitrocellulose coat (above) showing strain-induced aggregates and grains. Similar section embedded in a thick nitrocellulose coat (below) showing absence of aggregates ; grains over this section appear as out of focus images due to the thick nitrocellulose coat. (× 300)

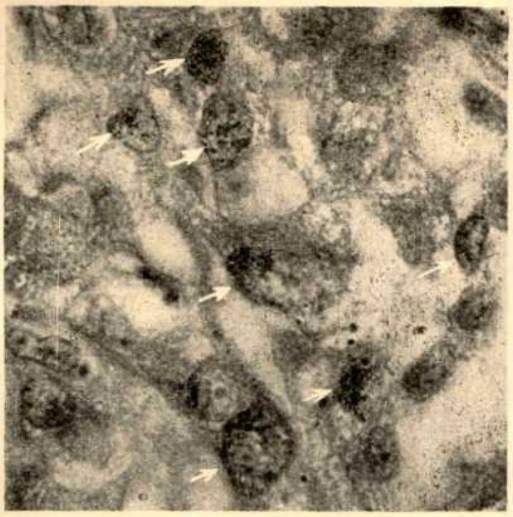

Fig. 2. Grain clumps (arrows) over non-radioactive nuclei (slide 4, Table 1). (× 1,000)

nitrocellulose and emulsion and processed. The grain counts over hemalum, eosin and background were $24 \pm 6{\cdot}1$, $12{\cdot}3 \pm 0{\cdot}86$ and $7{\cdot}2 \pm 0{\cdot}75$ respectively. Counts around the hemalum spot were 1·5–2 times background count, indicating some lateral diffusion of the reaction. When non-radioactive sections stained in hemalum were treated similarly, counts over tissue and background were 18·1 and 7·4 respectively (Table 1). The mean number of aggregates was 63·4 and the largest of them was 262μ. Corresponding results with sections stained in eosin were 10·8 and 7·1 grains over tissue and background, mean number of aggregates 22·2 and the largest of them measuring 210μ. All grain counts are significantly above background ($P < 0{\cdot}001$, except slide 4, where P is 0·03). The difference between aggregate counts over unstained sections and those stained with eosin is also significant ($P < 0{\cdot}02$). Aggregates on these slides were not confined to tissue spaces and edges as the strain-induced ones were. Slides on which sections were stained for 5, 10 and 15 min. in hemalum showed increasing grain count over tissue with longer staining time (5·9 : 13·3 : 14·9, $P < 0{\cdot}01$). Similar sections counterstained with eosin for 10 min. also showed an increase (8·7 : 10·8 : 15·3, $P < 0{\cdot}01$). The increase of grain count with longer staining time was abolished by a thick coat of nitrocellulose. These experiments show that stains, either directly or from tissues, may reduce the emulsion in spite of a thin nitrocellulose film.

Observations such as the effect of a thick nitrocellulose coat in reducing artefacts, and association of aggregates and grains with bleached areas of stained sections, indicated that the photographic developer and fixer (Kodak *D*19 and 'Acid Fixer'), diffused across the thin nitrocellulose film, reacted with the stains on sections and as a result stains were bleached and the emulsion reduced. Slides processed in a less alkaline *D*19 (made up with 3 gm. of sodium carbonate instead of 48 gm./l.) and 5 per cent sodium thiosulphate showed a grain count over tissue similar to background count and bleaching of stains was almost eliminated. Thus, these chemically induced artefacts seem to be the result of interaction between the stains and *D*19 and 'Acid Fixer'.

One other observation of grains appears worth recording. Slides 3 and 4 (Table 1) showed several nuclei over which grains occurred in clumps (Fig. 2). The mean of grain counts over 20 nuclei on slide 3 was 15·75. These artefacts are indistinguishable from autoradiograms of nuclei labelled with tritiated thymidine.

Protective coating. Systematic work[6-9] on protective films has tested a wide variety of substances. None of them seems to give complete protection and yet is satisfactory in other respects[10]. More recently 'Plexiglas' solution and acrylic membranes have been tried[11,12]. 'Harleco Synthetic Resin' (HSR), the routine mounting medium for histological preparations in this laboratory, was found to be a satisfactory protective coating. The sections may be coated after staining, dehydration and clearing in xylol in the following manner. Each slide is removed from xylol, the under surface and edges wiped dry and placed on a previously levelled metal platform. A volume of 2 per cent HSR in xylol that will produce a layer as thick as the sections is pipetted on the surface before the sections become dry. The linear relation between volume of 2 per cent HSR and final thickness of dry coat is obtained by prior calibration. When all slides are ready a 'Plexiglas' cover is placed over the platform and a stream of air drawn over the slides through an outlet on top of the cover using a squirrel cage blower. The slides are dried overnight and may then be coated with emulsion. The sections become embedded in the HSR layer, which presents a smooth and even surface to which emulsion will adhere.

Sections stained with toluidine blue, phosphotungstic hæmatoxylin and with two triple stains (Heidenhain's azan and by the method of Himes and Moriber[13]) were fully protected by HSR. The resin does not seem to reduce the emulsion by itself; after exposure of emulsion to the resin for 3 weeks, grain counts were not significantly different from background. HSR has excellent optical qualities[14] and is readily available (Hartman Leddon Co., Philadelphia). Since sections are embedded in HSR by this method, they never become dry during the entire process. Solvents like xylol cannot be used in permanent mounting of these autoradiograms; but the slides may be mounted in 4 per cent polyvinyl alcohol directly after photographic processing, thereby eliminating the dehydration and clearing used as a rule.

I am grateful to Dr. J. D. Ebert and Dr. B. G. Böving for their advice. I wish to thank Mr. W. H. Duncan for his suggestions concerning histological procedures.

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LETTERS TO THE EDITORS

COSMOLOGY

Dirac's Cosmology and Mach's Principle

THE dimensionless gravitational coupling constant

$$\frac{Gm_p^2}{\hbar c} \sim 5 \times 10^{-39} \qquad (1)$$

with m_p the mass of some elementary particle, for definiteness taken as the proton, is such a small number that its significance has long been questioned. Thus Eddington[1] considered that all the dimensionless physical constants, including this one, could be evaluated as simple mathematical expressions. Dirac[2] considered that such an odd number must be related to other numbers of similar size, characterizing the structure of the universe. However, most physicists seem to believe that a dimensionless constant, such as (1), is provided by Nature, cannot be calculated, and is not in any way related to other numbers.

Dirac noted that most physical and astrophysical dimensionless constants are of the order of magnitude of integral powers (positive and negative) of the number 10^{40}, where such numbers as $m_p/m_e \sim 1{,}800$ and $\hbar c/e^2 \sim 137$ are said to be of the order of unity, the zero power of 10^{40}. He considered unlikely the accidental correspondence of the apparently unrelated, enormous numbers, and he suggested some unknown causal connexion.

One of these large numbers is:

$$T\,\frac{m_p c}{\hbar} \sim 10^{42} \qquad (2)$$

with T the Hubble age of the universe. For an evolutionary universe, T varies with time. This suggested to Dirac that all the large numbers vary with time, with numbers of the order of $(10^{40})^n$ varying as T^n. Dirac[2] constructed a cosmology based on this idea, and Jordan[3] produced a proper relativistic theory of Dirac's cosmology.

The three principal large numbers are given by equations 1 and 2, and by the mass of the universe to its visible limits, expressed as:

$$\frac{M}{m_p} \sim 10^{80} = (10^{40})^2 \qquad (3)$$

According to Dirac's hypothesis, these three numbers should vary as the -1, 1, and 2 power of the time respectively.

It should be noted that it is necessary to assume more than a connexion between the three numbers (1), (2), and (3) to justify Dirac's hypothesis. In addition, one must assume that the apparent interconnexion between the three numbers is independent of time. This assumption could be supported by a statistical argument. If the present value of T were to be considered conceptually as a random choice from a wide range of possible values of T, the present 'choice' would have had a small *a priori* probability, and an accidental correspondence of the type exhibited by the three numbers would have been unlikely. In view of the inexactness of the interrelation between the three numbers, a very wide range of possible values of T and a small *a priori* probability must be assumed if Dirac's hypothesis is to receive support from this type of argument.

It will be shown that, with the assumption of an evolutionary universe, T is not permitted to take one of an enormous range of values, but is somewhat limited by the biological requirements to be met during the epoch of man.

The first of these requirements is that the universe, hence galaxy, shall have aged sufficiently for there to exist elements other than hydrogen. It is well known that carbon is required to make physicists.

It is known that the galaxy was formed initially from hydrogen only. Hence, the minimum time for the start of the epoch of man is set by the age of the shortest-lived stars, for elements, other than hydrogen, are formed in the interior, and distributed at the death, of the star.

An upper limit for the epoch of man is set by the requirement that he has a hospitable home in the form of a planet circling a luminous star. This time is set by the maximum age of a star capable of producing energy by nuclear reactions. For stars less massive, gravitational contraction is halted by the onset of electron degeneracy pressure, before central temperatures rise high enough for the onset of nuclear reactions. The mass of the longest-lived stars can be computed assuming that electron degeneracy occurs at the nuclear reaction temperature. This gives for the lower bound on M_s, the mass of the star:

$$\frac{M_s}{m_p} \sim 10^{-3}\left(\frac{\hbar c}{Gm_p^2}\right)^{3/2} \qquad (4)$$

where m_p is again the proton mass. According to the above definition of order of magnitude, 10^{-3} is to be regarded as equivalent to unity and this number is of the order of magnitude of $(10^{40})^{3/2}$.

The life-span of a star of this mass can be calculated, assuming that the emission and opacity are determined by free–free transitions (the bremsstrahlung radiation and absorption process). This lifetime is:

$$T_{\text{max.}} \sim \left(\frac{m_p}{m}\right)^{5/2}\left(\frac{e^2}{\hbar c}\right)^3\left(\frac{Gm_p^2}{\hbar c}\right)^{-1}\frac{\hbar}{m_p c^2} \qquad (5)$$

after dropping numerical factors, where m is the electron mass. Dropping the 'unity factors', this becomes:

$$\frac{m_p c^2}{\hbar}\,T_{\text{max.}} \sim \left(\frac{Gm_p^2}{\hbar c}\right)^{-1} \qquad (6)$$

in agreement with equations (1) and (2). In similar fashion, T_{min}, as determined by requirements for stellar stability, is of the same order of magnitude. Thus, contrary to our original supposition, T is not a 'random choice' from a wide range of possible choices, but is limited by the criteria for the existence of physicists.

Two problems remain. Why is the gravitational coupling constant so small? Why does the square root of the number given by equation (3) agree with the reciprocal of (1)? Both of these questions are answered by introducing Mach's Principle. According to the interpretation of Mach's Principle sometimes made[4-6], the gravitational coupling constant is not fixed but is determined by the mass distribution of the universe in such a way that:

$$\frac{GM}{c^3 T} \sim 1 \qquad (7)$$

Combining equations (7) and (6) gives the expression obtained by combining equations (1) and (3)

The reason for the smallness of the gravitational coupling constant is the enormous amount of matter in the universe This may not be a very satisfactory answer, and it cannot be completely satisfactory until the problem of mass creation is understood

The statistical support for Dirac's cosmology is found to be missing However, the existence of physicists now and the assumption of the validity of Mach's Principle are sufficient to demand that the order-of-magnitude relations between the three numbers, given by equations (1), (2) and (3), be satisfied

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Dicke discusses the three cosmological numbers (1) which determines the gravitational constant, (2) which determines the Hubble age of the universe, and (3) the number of particles in the universe They are related in that (1) is roughly the reciprocal of (2) and (3) is roughly the square of (2) I assumed that these relations correspond to something fundamental in Nature With an evolutionary universe (2) varies with time, and then (1) and (3) would also have to vary with time

Dicke considers that there is a fundamental relation between (1) and (3), following from Mach's Principle, but that (2) is independent, so that (1) and (3) may be constant while (2) varies with time He then shows that (2) would have to have roughly its present value when habitable planets exist On this assumption habitable planets could exist only for a limited period of time With my assumption they could exist indefinitely in the future and life need never end

There is no decisive argument for deciding between these assumptions I prefer the one that allows the possibility of endless life One may hope that some day the question will be decided by direct observation One would have to measure (1) to an accuracy of 1 in 10^{10} and then repeat the measurement after a few years and see whether the result has altered

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SPACE SCIENCE

Direct Measurement of Interplanetary Dust Particles in the Vicinity of Earth

Additional knowledge about interplanetary dust particles has been obtained from the direct measurements made with rockets, satellites, and space probes The results obtained with the microphone type of dust particle sensor carried by the U S satellites, *Explorer I* (1958 Alpha) and *Vanguard III* (1959 Eta), the U S space probe, *Pioneer I*, and the Soviet vehicles, *Sputnik III* (1958 Delta 2), Space Rockets I and II, and the Interplanetary Station (1959 Theta), have already been reported In addition, a definitive set of data has recently been obtained with the U S satellite, *Explorer VIII* (1960 Xi) A series of corrections has been completed which allows our results from a series of seven successful high-altitude rockets instrumented at Oklahoma State University to be used in quantitative discussions of interplanetary dust particles We would like to present the preliminary results (including a new and very interesting curve for the mass distribution and spatial density) from an analysis of the available direct measurements of interplanetary dust particles

The best calibrations of the microphone system that are at present possible are performed with particles ranging from large glass beads elastically impacting at low speeds to small iron spheres inelastically impacting at speeds up to approximately 10 km sec^{-1} Such calibrations indicate that the microphone system is sensitive to a quantity that is closely related to the momentum of an impacting dust particle An average velocity, relative to a satellite, of 30 km sec^{-1} has been assigned to the dust particles in order that the results may be used in a determination of the mass distribution of the interplanetary dust particles

The three datum points obtained with *Explorer VIII* are plotted (together with those from *Vanguard III*, *Explorer I* and the series of rockets) as the distribution curve shown in Fig 1 The equation of a straight line that approximately fits the datum points is

$$\log I = -17\ 0 - 1\ 70 \log m$$

where I is the influx-rate of dust particles and m is the particle mass holding approximately for m in the range 10^{-10} gm $\lesssim m \lesssim 10^{-6}$ gm The data from *Vanguard III*, *Explorer I* and the rockets fit remarkably well on the curve defined by the data from *Explorer VIII* In fact, all other available direct measurements also can be made to look fairly reasonable when plotted as in Fig 1 if proper allowances are made for the type of experiment, the confidence that can be placed in any particular measurement, the shielding effect of Earth, and the actual fluctuations in the spatial density that have been observed with *Explorer I*, *Vanguard III*, and *Explorer VIII*

The curves obtained from an extrapolation of the tabulation by Watson[1] and from the extrapolated tabulation by Whipple[2] of results from meteor observations are shown as reference curves in Fig 1 Both the extrapolated curves follow the constant mass per unit visual magnitude relationship common to discussions of results from meteor observations and roughly serve as the limits of uncertainty encountered in placing such results on a mass distribution curve This uncertainty arises because of the uncertainty in relating particle mass to photographic, visual, or radar magnitude, and the uncertainty in assigning a mass density of meteoroidal particles

Either of the extrapolated curves can be forced to pass through any one of the given direct measurements datum points by adjusting the mass to magnitude relationships and the mass density for meteoroidal particles The three datum points obtained with *Explorer VIII* define a slope for a limited portion of the distribution curve in addition to defining the influx-rates for dust particles in three different ranges of particle mass, showing that the curve determined by direct measurements departs significantly from the extrapolated curves

Caution must be exercised in extrapolating the new distribution curve out of the range of particle mass for which direct measurements have actually been

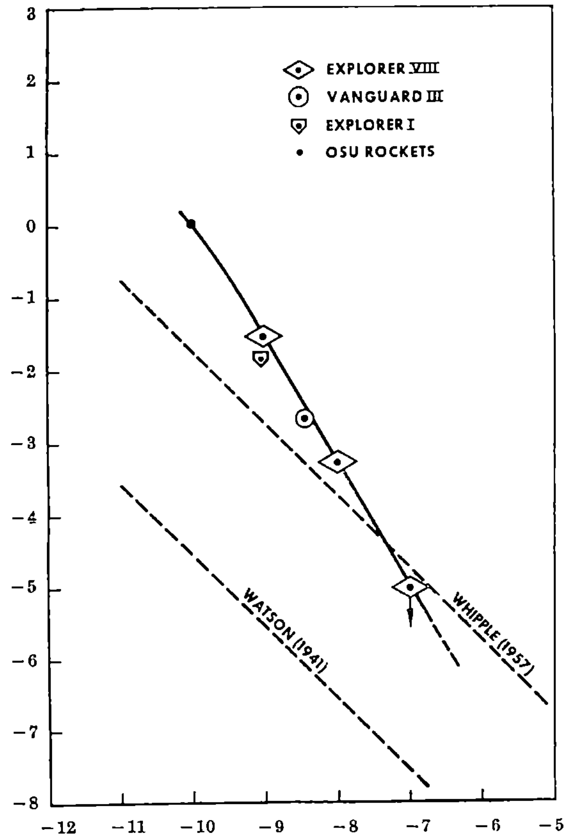

Fig 1 Distribution curve for interplanetary dust particles in the vicinity of Earth

obtained A considerable change in the shape of the distribution curve is bound to occur between the direct-measurements range of particle size and the range of particle size where the radiation pressure control becomes dominant unless there is a most prolific source of very small dust particles somewhere near or inside the orbit of Earth The new distribution curve can be joined smoothly to the extrapolated curve labelled "Watson" at a size of particle smaller than the faintest radar meteors, but junction with the curve labelled "Whipple" is rather difficult

An interesting consequence of the new distribution curve is that the daily influx-rate of interplanetary dust is approximately 10^4 tons a day on Earth, meaning that dust particles in the direct-measurements range of particle size dominate the accretion process The meaning of the new distribution curve with regard to theories of the zodiacal light and observations of atmospheric aerosols bears further investigation

The question whether the direct-measurements results show a dependence on altitude of the spatial density of dust particles has not been resolvable, on the basis of results obtained with the microphone type of impact detector, prior to obtaining the results of *Explorer VIII* The reason for this difficulty was that the rocket measurements were of higher sensitivity and at lower altitudes while the reliable satellite measurements were generally of lower sensitivity and at higher altitudes The satellite measurement at high sensitivity and at a large geocentric distance made by *Pioneer I* extended over only a short sampling interval and may not be of much use in establishing a model average distribution Any one of several interpretations could be given to the available direct measurements, depending on the choice of a model distribution to which they were compared

By assuming that the extrapolated curve labelled "Whipple" represented the true distribution, Whipple[3] has deduced that the direct measurements have confirmed the existence of a geocentric "dust belt" If the empirical curve defined by the data from *Explorer VIII* is used as a basis for an analysis of other direct measurements results, then no discernible dependence on altitude of the spatial density of dust particles exists The conjectural geocentric concentration of dust particles under discussion here should not be confused with concentrations of dust-particles possibly suspended in the atmosphere of Earth at and slightly above the temperature inversion layer at about 80 km altitude

In conclusion, it should be emphasized that while the data at present available show no evidence for the existence of an appreciable geocentric concentration of dust particles, the data are really not of such a nature that they can be used in conclusively settling the question If such a concentration does exist, then it certainly has not been convincingly measured by rockets and satellites The direct measurements are quite useful in determining a new model of the average dust distribution, with the direct measurements results showing remarkable self-consistency when displayed as in Fig 1

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W M Alexander
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ASTRONOMY

Exact Determination of the Lunar Mass by means of Selenoid Satellites

As is well known, the astronomical determination of the mass of the Moon has so far been accomplished by methods based on the observation of the terrestrial nutation of the motion of the centre of the Earth around the centre of mass of the Earth–Moon system, and from the perturbation of the asteroid Eros

The numerical value 0 01230 times the terrestrial mass is known to four significant figures, with an accuracy of about 4 parts in a thousand It has been suggested[1,2] that this accuracy can be improved by observation of the motion of what we have called a 'selenoid satellite', that is, a satellite placed in the general vicinity of one of the sextile libration points of the Earth–Moon system The reasons for believing that an improved accuracy would result by means of such a method are based on the fact that, in the first place, a prolonged time of observation would, in this case, be available, secondly, that the motion of such a body would be accurately predictable

More specifically, the motion of a selenoid satellite could be qualitatively described[1,2] as a superposition of what we might call free and forced oscillations The free oscillations would be those which the selenoid would perform if the Earth–Moon system were isolated in space If the relative orbits of the Earth and the

Moon were exactly circular, the period of these oscillations would be respectively of 28 54 and 91 35 sidereal days. Owing to the effects of non-linear terms and eccentricity of the Earth–Moon orbits, additional periodic terms having as period all possible linear combinations of the above periods as well as of the period of the unperturbed Earth–Moon system will make their appearance The forced oscillations will be due mainly to the action of the Sun, which will directly influence the orbiting satellites as well as the remaining components of the Earth–Moon system The most conspicuous of the above oscillations will presumably be the free ones as well as those having periods equal respectively to the sum and difference of one year and the above free periods

The proposed method of determination of the lunar mass is based on establishing, by means of a periodogram analysis of the libratory motion executed by the selenoid satellite, its free periods The latter depend solely on the ratio of the masses of the Earth and the Moon which can thus be directly calculated The determination of such periods could be accomplished with considerable accuracy on the basis of straightforward astronomical observation of the position of the selenoid satellite on the celestial sphere When the above method was originally suggested, the use of artificial selenoid satellites (in the form of large inflated balloons) was contemplated Quite recently, however, K Kordylewski, of the Cracow Observatory, has reported the discovery of two (cloud-like) natural selenoid satellites orbiting in the vicinity of the trailing libration centre of the lunar orbit[3] On the basis of the data communicated by Kordylewski, the amplitude of the libration of the two bodies can be estimated to be of the order of a few degrees, which appears adequate for a determination of the periods as described above It is hoped that a sufficient number of sufficiently accurate data will be available in the near future, thus permitting application of the proposed method It should be noted that a complete description of the motion of the selenoid satellites is redundant for the purpose discussed here, since the information required can be extracted from the knowledge of the dependence on time of any appropriate co-ordinate

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PHYSICS

Radioactivity in Rainfall during September 1961 following the Russian Nuclear-Weapons Tests of September 1961

The collection and analysis of rainwater are a part of the environmental survey programme of this Laboratory Simple analysis of the total β-activity, consisting of evaporation and counting the residue, using anti-coincidence low-level techniques, has been carried out over the past two years and specific isotopic analysis has been performed when this was of particular interest, as at the present time

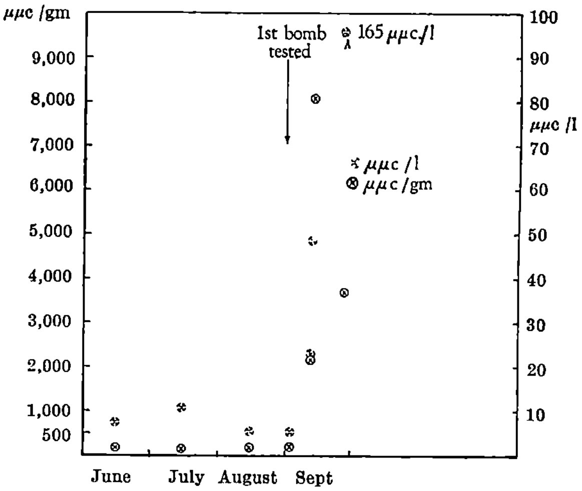

Fig 1 Total β-activity of rainwater and rainwater residue from June to September 31

From the analyses for total β-activity it is evident that the residue from rain falling after September 2, 1961, that is, following the commencement of the Russian nuclear tests, had an activity which was of a distinctly higher order (up to 8,000 μμc /gm) than that of previous months rainfall (about 160 μμc / gm) The activity of the rainwater in this period was correspondingly greater (48 4 μμc /l) compared with previous months (about 5 1 μμc /l).

The results of these and subsequent samples are presented in Table 1 The build-up of activity in the rain can be seen from a graphical interpretation of these results (Fig 1)

Table 1 TOTAL β-ACTIVITY OF RAINWATER AND RAINWATER RESIDUE

Date of sample collection	Date of sample analysis	Rainwater* (μμc /l)	Rainwater residue* (μμc /gm)
1/8/61–31/8/61	12/ 9/61	5 1	157
2/9/61– 4/9/61	5/ 9/61	3 4	188
12/9/61–13/9/61	20/ 9/61	22 7	2,160
13/9/61–14/9/61	18/ 9/61	48 4	8,050
27/9/61–28/9/61	3/10/61	165 0	2,360

* Activity at date of analysis

In view of the complex nature of the uptake of solid matter during and after cloud formation, both activities per litre of rainwater and per gram of residue have been given The uncertainty in both these figures is conservatively estimated at ± 20 per cent, so that the increases after September 12 are highly significant

Specific Isotopic Analysis To confirm that the excess activity was due to fission products, specific isotopic analysis was carried out for iodine-131 This isotope was chosen partly because of its biological importance, but it was also convenient as a tracer because of its characteristic 0 36-MeV. γ-ray The half-life of 8·05 days was sufficiently long to permit precise measurement but not long enough to permit iodine contamination from previous tests to cause confusion.

Chemical separation of iodine was made and the resulting samples were counted for β-activity using anti-coincidence methods The activity was attributed

Table 2 IODINE-131 IN RAINWATER (ACTIVITY)

Date of sample collection	Date of sample analysis	Activity at date of analysis μμc /l of iodine-131
13/9/61–14/9/61	14/9/61	9 8
14/9/61–18/9/61	18/9/61	95 5 82 0*
27/9/61	28/9/61	14 2

* By γ-ray spectra analysis

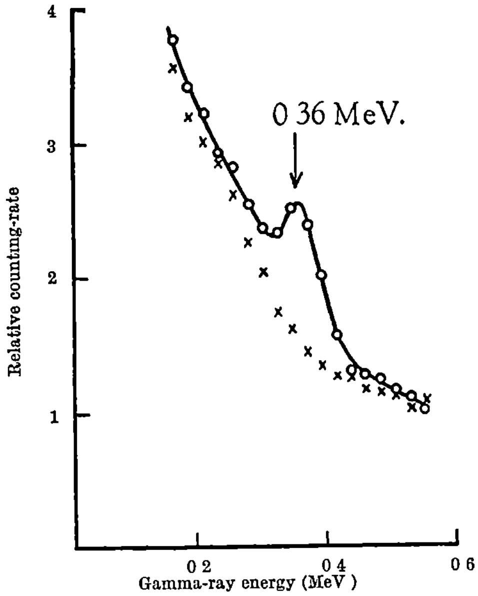

Fig 2 Gamma spectrum of iodine-131 in rainfall O, Source, ×, background

to iodine-131 in view of the comparatively short half-lives of the other iodine fission products A confirmatory measurement on one of the samples was made by γ-ray spectrometer analysis for iodine-131 Good agreement was obtained, as can be seen in Table 2 The spectrum obtained is shown in Fig 2

The confirmatory test of the γ-ray spectrometer on the second sample yielded a result of 82 μμc /l of iodine-131 compared with 95 5 μμc /l by β-analysis

Although these preliminary results indicate clearly the onset of the Russian testing programme, it is impossible at this stage to attempt a more detailed correlation with individual tests

I would like to thank Mr R A Pope for measuring the γ-ray spectra, and the staff of the Radiological Protection Unit for the preparation and analysis of samples I would like to express my special thanks to Dr S E Hunt for his helpful advice and comments Thanks are due also to Dr T E Allibone, director of the Laboratory, for permission to publish this communication

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Nuclear Spin of Protoactinium-231 from Hyperfine Splitting

The nuclear spin of protoactinium was first determined by Schuler and Gollnow in 1934[1] They examined the spectrum of protoactinium-231 in the region 4300–5600 Å and obtained the nuclear spin $I = \frac{3}{2}$ from hyperfine splitting

More recently the nuclear spin of protoactinium-233 has been determined[2] from paramagnetic resonance, and the nuclear spin of tetravalent protoactinium-231 from an investigation of paramagnetic resonance and hyperfine structure[3] Both authors obtain $I = \frac{3}{2}$

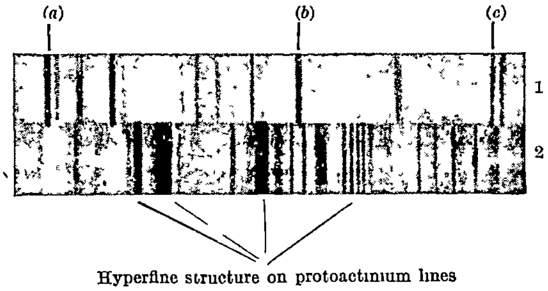

Fig 1 Protoactinium-231 spectrum with thorium comparison spectrum 1, Thorium comparison spectrum, 2, protoactinium spectrum, *a*, thorium, 5869 847 Å , *b*, thorium, 5874 981 Å , *c*, thorium, 5878 922 Å

So far, however, the work of Schuler and Gollnow has not been confirmed

The atomic emission spectrum of protoactinium-231 was recently photographed in the region 4000–9000 Å using the 21-ft grating spectrograph at Harwell, and also recorded in the region 0 8–2 5μ The light source was an electrodeless discharge tube containing about 3 mgm of protoactinium iodide and excited by microwaves The spectrum shows pronounced hyperfine structure, the majority of lines being split into four components This confirms the value $I = \frac{3}{2}$ obtained by previous workers

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Rotational Analysis of $^{65}Cu^{35}Cl$ Bands

Although many extensive band systems are known for the CuCl molecule[1], our knowledge of their rotational structure has been meagre Apart from being heavy, natural cuprous chloride is a mixture of four isotopic species with not very different relative abundances Very high dispersion and resolution are therefore needed to resolve, even partially, the rotational structure of the bands

With the availability of compounds containing separated copper and chlorine isotopes, it has become possible to prepare copper chloride with any desired copper and chlorine isotopes Milligram quantities of $^{65}Cu^{35}Cl_2$ have been thus prepared and the spectrum of $^{65}Cu^{35}Cl$ excited in an electrodeless discharge tube by a 2 450 mc /sec Raytheon microwave oscillator[2] All the known band systems of cuprous chloride were obtained with high intensity The bands were photographed mostly in the fifth order of the Argonne National Laboratory 30-ft concave grating spectrograph (dispersion 0 3 Å /mm) Bands for which rotational analysis has been done are given in Table 1 Spectroscopic data obtained from the present work are given in Table 2 All values in this table, except those of r_e, are in cm $^{-1}$ units

Table 1

System	Bands analysed						
$B - X(^1\Pi - {}^1\Sigma)$				(1,0),	(0,0),	(0,1)	
$C - X(^1\Sigma - {}^1\Sigma)$				(1,0),	(0,0),	(0,1)	
$D - X(^1\Pi - {}^1\Sigma)$		(3,1),	(2,0),	(1,0),	(0,0),	(0,1),	(0,2)
$E - X(^1\Sigma - {}^1\Sigma)$	(4,1),	(3,0),	(2,0),	(1,0),	(0,0),	(0,1)	
$F - X(^1\Pi - {}^1\Sigma)$		(3,0),	(2,0),	(1,0),	(0,0),	(0,1),	(0,2)

Table 2. Molecular Constants of $^{65}Cu^{35}Cl$

State	T_e	ω_e	$\omega_e x_e$	$\omega_e y_e$	B_e	$\alpha_e \times 10^4$	$q \times 10^3$	$D_e \times 10^7$	r_e (10^{-8} cm)	Observed transitions Designation	$\nu_{0,0}$
F	25,285.30	384.94	1.65	—	0.1608	9.1	—	1.2	2.148	$F \longrightarrow X$	25,270.11
E	23,074.33	403.08	1.49	−0.03	0.1663	10.8	—	1.0	2.112	$E \longleftrightarrow X$	23,068.25
D	22,970.04	392.94	1.73	—	0.1677	8.4	1.6	—	2.103	$D \longleftrightarrow X$	22,958.83
C	—	—	—	—	0.1691	8.9	—	1.2	2.094	$C \longleftrightarrow X$	20,621.78
B	—	—	—	—	0.1685	9.3	0.7	1.2	2.098	$B \longleftrightarrow X$	20,476.07
X	0	415.29	1.58	—	0·1764	10.0	—	1.3	2.050		

The rotational analysis indicates that the upper states of the five systems are either $^1\Pi$, $^1\Sigma$, etc., as shown in Table 1 or states which 'behave' like these. The Λ-type doubling in the F state is negligible, whereas that in the D and B states is large. This is apparently due to the circumstance that whereas there is no perturbing state near the F level, the levels D and B have each a perturbing state nearby.

In a recent note, Lagerqvist *et al.*[3] have given the results of their rotational analysis of the (1,0), (0,0) and (0,1) bands of the B–X system and the (1,0) band of the C–X system of $^{63}Cu^{35}Cl$ recorded in absorption. The rotational constants of $^{63}Cu^{35}Cl$ calculated from the present $^{65}Cu^{35}Cl$ data are in excellent agreement with those given by them.

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Electron Paramagnetic Resonance and Antiferromagnetism in $LaCrO_3$

The presence of antiferromagnetic ordering in $LaCrO_3$ has been verified before by both neutron diffraction[1] and static magnetic susceptibility[2] techniques. In the former case, a Néel temperature of approximately 320° K is reported, while the susceptibility results indicate $T_n \approx 295°$ K. Above T_n, $LaCrO_3$ is paramagnetic and hence amenable to investigation by standard electron paramagnetic resonance techniques. We have carried out the experimental resonance investigations over the temperature-range 200–450° K.

The powder was prepared by heating reagent grade Cr_2O_3 and La_2O_3 at 1,600° C for 2 hr. Our X-ray diffraction results indicate a typical perovskite with small distortions from cubic symmetry. Assuming a cubic structure, we find the lattice parameter to be 3.870 Å in reasonable agreement with the value 3.874 Å published in ref. 3.

The pertinent features of our results are summarized in the figures. All data were obtained at 9.1 kMc/s using a Varian V–4500 electron paramagnetic resonance spectrometer with d.c. magnetic field varied continuously from 0 to 10,000 gauss. The resonances were singly peaked, with g values equal to 1.98, at all observable temperatures.

Our results are consistent with previous resonance investigation of similar compounds[4]. In the antiferromagnetic state anisotropy and exchange fields effectively 'drive' the resonance from the region of observation[5]. Line-widths exhibit the behaviour predicted by the calculations in ref. 6. With antiferromagnetic ordering each Cr^{3+} ion is indirectly coupled to the six nearest similar ions with spin orientation opposite to that of the central ion. Since the spins 'lock' into this configuration, the time-averages of the dipolar fields at a particular spin site approach the instantaneous value. Hence line-broadening results. Extinction of resonance below the transition appears to rule out impurities as a major contributor to line-width.

In the paramagnetic region, it is expected that peak intensities will be proportional to the magnetization and hence the susceptibility[7]. However, it has been argued that peak intensity should depend on both magnetic susceptibility and the existence of some residual short-range order close to, and above, T_n (ref. 8). The transitions observed here are relatively abrupt when compared with similar results for other compounds[8]. This is interpreted as indicating a minimization of short-range order effects above and close to T_n. Hence from the maximum in relative intensity we obtain $T_n \approx 295°$ K. Our numerical agreement with the susceptibility results is, no doubt, fortuitous. The two results are, however, taken to be in approximate agreement. In any event, the present results differ appreciably from that of the neutron diffraction experiments. In this respect it is perhaps significant to note that Jonker[2] prepared his sample in a manner somewhat similar to ours, using the same compounds as starting materials. On the other hand, Koehler and Wollan[1] used as their starting

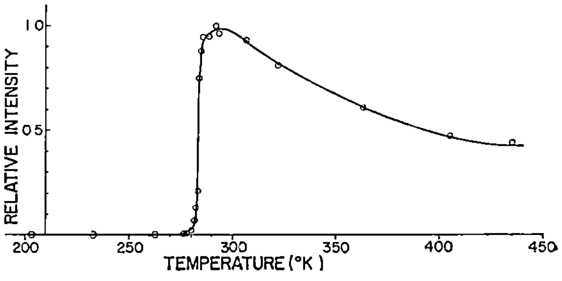

Fig. 1. Relative intensities versus temperature for electron paramagnetic absorption in $LaCrO_3$

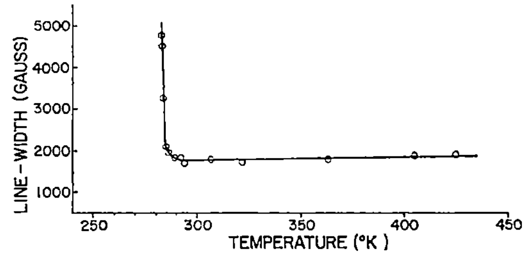

Fig. 2. Line widths, at half-maximum, versus temperature for electron paramagnetic absorption in $LaCrO_3$

materials a lanthanum compound in stoichiometric mixture with the transition metal

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GEOLOGY

Potassium-Argon Ages at Broken Hill, Australia

WE have obtained three results which contribute something to an understanding of the more recent events in the Broken Hill district of New South Wales. Field evidence[1,2] indicates that the main lead lodes lie conformably with gneisses of the Willyama Series, thought to have been formed from sediments of older Precambrian age These have been strongly folded, the metamorphism and folding having taken place before deposition of the younger Torrowangee Series, which is regarded[3] as equivalent to the Adelaide System of Upper Precambrian age At some subsequent time, possibly in the early Palæozoic, greenschist facies assemblages were developed (White, A. J R , personal communication) in the Torrowangee Series, suggesting that temperatures were reached adequate for the loss of a considerable proportion of any argon (if not all) accumulated up to that time[4]. Although there is comparatively little evidence for this heating (Carruthers, D S , personal communication) in the earlier Willyama rocks, it is inconceivable that they can have been completely unaffected This inference is supported by our results.

The Broken Hill samples analysed were

GA 113	Upper granitic gneiss (Formation No 13), New Broken Hill Consolidated, Ltd	Age 504 m y
GA 114	Sillimanite biotite garnet gneiss (Formation No 4), No 21 level, Zinc Corporation, Ltd	Age 526 m y

These results are to be compared with the age determined on a transgressive-type granite (*GA* 212) from Encounter Bay, in the southern part of the Adelaide Geosyncline The date obtained here, 457 m y , can be regarded as being related to the more recent events in the Adelaide Geosyncline, thus constituting a possible upper limit to the time of any movement of the Torrowangee at Broken Hill Thus the Cambrian ages obtained for the undoubtedly older gneisses suggest that the event affecting the Torrowangee happened between 500 and, say, 460 m y. ago, with the implication that the effect on the older Willyama rocks may not have been sufficiently intense to cause total loss of argon. There is inadequate evidence to relate these results with the two 'model ages' derived from lead isotope measurements by Russell *et al* [5], of 1,600 m y for the conformable leads, and "less than 1,000 m y " for the event causing the formation of the transgressive Thackaringa-type deposits Further measurements are in progress, and more complete details of these experiments are to be published elsewhere

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METALLURGY

Difference between High- and Low-Stress Fatigue

RECENT papers by Porter and Levy[1] and Benham and Ford[2] have suggested that the conventional *S/N* curve is composed of two separate curves, a discontinuity occurring at some particular stress-level. The former authors concluded from a large number of rotating bending tests on copper that the discontinuity occurred at about ± 21,500 lb /sq in., for lower stresses fatigue cracks originating in slip bands; for higher stresses cracks developing from L-shaped nuclei Kemsley[3] also found that with copper specimens tested at ± 15,000 lb /sq in (giving fracture after long endurances) cracks formed in slip bands and were transcrystalline, while at ± 25,000 lb /sq in. (giving fracture with low endurances) slip bands were virtually absent from the specimen and cracks appeared to be intercrystalline Benham and Ford[2], who tested mild steel in direct stress, found for large stress amplitudes that the specimen gauge-length extended permanently each cycle and a 'necked-out' fracture resulted They termed this phenomenon 'cyclic creep' and such failures occurred while the stresses corresponded to the upper portion of the composite *S/N* diagram For stresses in the lower portion of the *S/N* diagram typical fatigue fractures occurred and they suggested that, if the fatigue mechanism could be suppressed as the cyclic stress decreased, the upper curve might flatten out to a 'cyclic creep limit'

Low-endurance fatigue tests have been carried out by many workers, the usual procedure being to apply strain cycles rather than stress cycles of constant amplitude, the work of Coffin[4] is typical Coffin suggested the results of such tests could be represented by the equation

$$N^{\frac{1}{2}} \varepsilon_p = C$$

(where N = number of cycles to failure, ε_p = cyclic-plastic strain amplitude and C = material constant). The endurances over which this relationship applies vary with material Coffin found for copper it was about 10^2 cycles, for aluminium about 10^5 cycles

On the other hand, when the cyclic stresses are in the vicinity of the fatigue limit, no relationship exists between the width of the hysteresis loop and life of specimen. For such tests, metallographic examination of the specimen surface shows, in general, that slip bands form early in the test from which micro-cracks eventually develop Indeed, both Hempel[5] and Wadsworth[6] have observed that, with mild steel, cracks may form in slip bands at stresses just below the fatigue limit Crack initiation may thus be considered as a consequence of surface roughening within broad slip bands[7,8]

Holden[9] has proposed a mechanism of failure, distinct from that responsible for surface crack initiation, occurring when the material is subjected to cyclic strains such as those occurring in the material ahead of a growing fatigue crack From examination, by means of an X-ray micro-beam technique, of the fractured faces of a material through which a crack had grown and of plain specimens subjected to large cyclic-plastic strains, he found for mild steel and aluminium the structure consisted of well-defined sub-grains. The sub-grains became progressively more misorientated as the test proceeded and he suggested that cracks could form in the sub-grain boundaries

Sub-grains have been observed metallographically on the surface of fatigue specimens by, for example, Forsyth[10] and Jacquet[11], although, in their case, the sub-grain structure did not contribute to failure presumably because the cyclic stress was insufficient to misorientate the sub-grains through sufficiently large angles, and failure arose from surface roughening as mentioned above

It could thus be argued that, when plain specimens are subjected to such nominal stresses that failure occurs in a small number of cycles, failure is due to the formation of sub-grains in the bulk of the specimen and cracks forming in the sub-grain boundaries In such cases the life of the specimen is related to the plastic strain amplitude, indeed, Holden suggested that the random movement of dislocations into arrays leads to a relationship of the type suggested by Coffin. As the nominal stress amplitude is reduced a stress-level will be reached at which either the sub-grain structure does not form or, if sub-grains do form, they are unable to misorientate through sufficiently large angles to cause cracking This stress-level may be considered as the 'true plain fatigue limit' although, of course, its value is much higher than the conventional plain fatigue limit For a plain specimen to fail at lower stress-levels it is necessary for surface roughening phenomena to produce surface grooves or cracks. The sub-grain structure will then form at the groove root due to the local strain concentration and the crack may grow across the specimen Whether or not cracks of macroscopic dimensions formed in notched specimens grow has been discussed by me[12]. It is not unreasonable to suppose that the behaviour of micro-cracks in surface grooves will be similar. Thus, at stresses below the 'true fatigue limit' a specimen fails because surface roughening phenomena effectively turn the plain specimen into a notched one. The life and nominal cyclic-plastic strain are now no longer subject to the same relationship as that which applies when stresses are above the 'true plain fatigue limit' The cyclic stress below which the specimen will not break (the conventional plain fatigue limit) is that value of the cyclic stress which is just unable to produce surface grooves or cracks to a sufficient depth to enable them to grow

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CRYSTALLOGRAPHY

Lattice Rotation at Slip Band Intersections

INTERSECTION of a slip band by an active slip band of different slip direction results in the formation of a step in the first band. Slip band intersections in magnesium oxide crystals suggested that the distortion of the intersected band was not merely a shear due to lattice translation, this was supported by the observation that subsequent widening of the intersected band (as described by Gilman and Johnston[1]) added to it a margin that followed the step This indicated that there was a change of lattice orientation at the intersection Imaging X-ray photographs of the Berg–Barrett[2,3] type confirmed the change of lattice orientation Fig 1 shows a compressed magnesium oxide crystal, afterwards etched to reveal dislocations; the dislocation etch-pits make the bands appear dark. Fig 2 is an X-ray image of the same crystal; the slip bands appear dark because of the reduction of primary extinction The slip band inter-

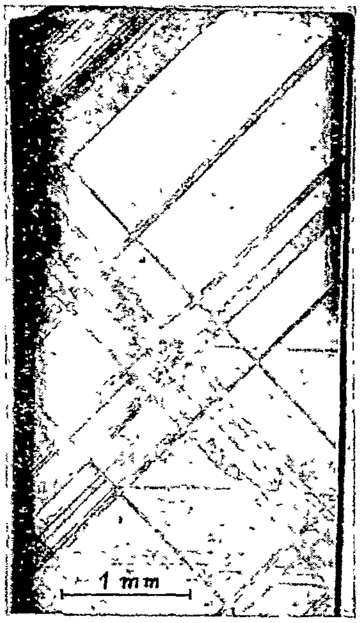

Fig 1 Etched surface of compressed magnesium oxide crystal, reflected light

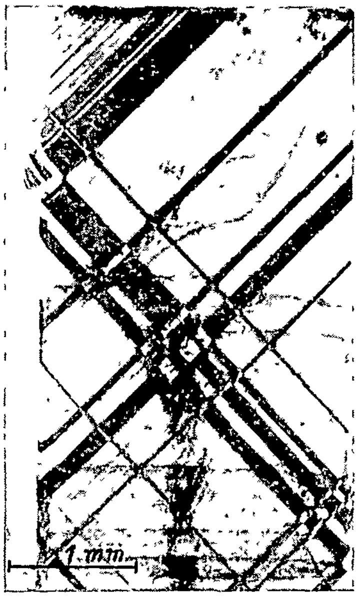

Fig 2. X-ray back reflexion image of same surface as in Fig 1, copper K_α radiation, reflexion from (210) plane

sections are white, and the reflexions from them can be found in more or less displaced positions, showing that there is a macroscopic change of orientation of the lattice at the intersections Such a change can arise in the way shown in Fig 3 Since the intersection is full of edge dislocations of both slip directions [110] and [$1\bar{1}0$], and the shear stress is the same in both slip systems, the shear strain in the active slip band may be due to slip and kinking[4,5] (= slip on the planes of the intersecting band) in the area of intersection In Fig 3 all strain in the intersection is assumed to have been the result of kinking

In Fig. 3, the parallelogram of the intersection is bounded by edge dislocations arranged in the same sense of rotation, because the dislocations of th intersecting band have arrived from outside, those of the intersected band (the kink dislocations) from inside the parallelogram Evidently, the lattice rotation due to conversion of slip into kinking at the intersection has the direction shown by X-ray asterism of the microscopic type (asterism of the macroscopic type is due to bending of the slip lamellæ[6], it is a different phenomenon) Conversion of slip to kinking is possible whenever slip bands with different slip directions intersect, it may also occur between parallel slip bands in sodium chloride[7], and it seems to be the cause of microscopic asterism generally

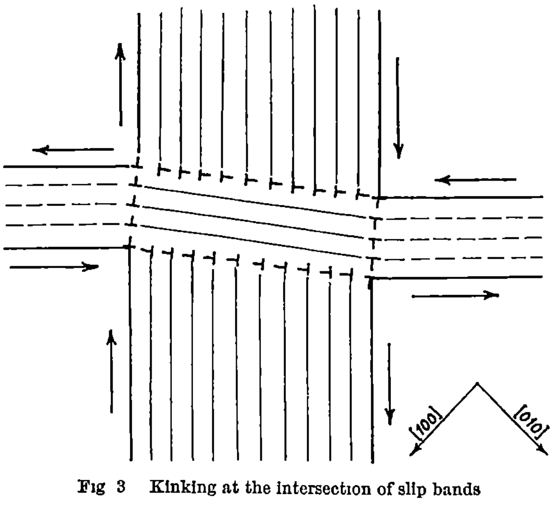

Fig 3 Kinking at the intersection of slip bands

The array of dislocations forming the low-angle boundary of the intersection is an obstacle to further slip and a possible cause of strain hardening in the post-easy-glide region Kink conversion, therefore, may perform the same task in the theory of strain hardening as Lomer–Cottrell dislocations, with the advantage of being a more general and observationally less elusive process Fig 3 indicates that it is a reversible process which may be connected with the Bauschinger effect and associated reversal phenomena Since the kink-conversion areas oppose slip in one direction more than in the opposite one, they may play a part in fatigue and extrusion phenomena[8] The stresses due to kink-conversion may lead to crack formation in several ways, explaining the observations of Stokes, Johnston and Li[9] which could scarcely be attributed to crack nucleation by reactions between individual dislocations

This work was sponsored by the Wright Air Development Division through the Armour Research Foundation

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CHEMISTRY

Requirements of an Ice Nucleus

THE striking similarity between the crystal lattices of silver iodide and ice led Vonnegut[1] to the conclusion that the efficiency of a substance as an ice nucleator depended on the closeness of fit between the ice lattice and the lattice of the nucleating substance This view persisted for some years, but it has become increasingly apparent[2] that nucleation is not governed by lattice spacing only and that a good nucleator must also possess some other characteristic By taking into account the changes in entropy involved in growing ice on a polar substrate, Fletcher[3] has predicted that the second necessary characteristic of a good nucleator is that exactly half the water molecules comprising the layer immediately adjacent to the nucleating surface should have a proton directed towards this surface As a first approximation this requires that a uni-univalent nucleator such as silver iodide should present a surface comprised of equal numbers of positive and negative ions, thus the surface should be at its isoelectric point, and any departure from this point due to the adsorption of potential-determining ions would be expected to decrease the nucleating efficiency

In what follows we present what we believe to be the first experimental demonstration that ice nucleation is indeed a function of the surface charge of the nucleating substrate

The surface charge of silver iodide particles in contact with an aqueous solution is readily controlled by the concentration of silver ions and iodide ions in the solution[4] Therefore the freezing point of a small droplet of such a solution in contact with silver iodide particles reflects the effect of surface charge, providing that there is no interfering electrolyte effect, and that each droplet contains a uniform quantity of silver iodide To meet these requirements, monodisperse sols of constant particle size and concentration were prepared by diluting, with water, a saturated solution of silver iodide in 0 2 *M* potassium iodide A uniform deposit of sol particles was centrifuged on to glass coverslips which had previously been rendered hydrophobic by treatment in chlorsilane vapour The particle-coated slips were washed thoroughly in water, sprayed with the required solution by means of a spinning disk atomizer, transferred to a microscope cold stage, cooled at a rate of 2 deg C per min, and the freezing point of each droplet noted To avoid undue scatter the freezing point of only the droplets between 30μ and 60μ in diameter was recorded, the average number of particles per 50μ droplet being 200. In each experiment about 50 droplets were observed, and generally all the droplets froze within a range of 4 deg C

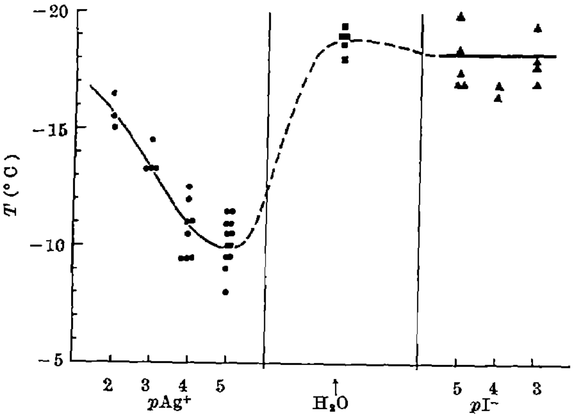

Fig 1 The variation of T with the composition of the droplets ●, Silver nitrate solutions, ▲, potassium iodide solutions, ■, distilled water

Fig 1 shows how T, the temperature at which 50 per cent of the droplets had frozen, varied with the composition of the droplets

It is apparent that, as Fletcher's theory would suggest, nucleation is most efficient in the vicinity of the isoelectric point (10^{-6} *M* silver nitrate[4]) and that quite extreme supercooling is required when the surface is charged either positively or negatively That iodide ion should be the more effective in this regard is consistent with the known tendency of silver iodide to adsorb iodide ions in preference to silver ions

These results are unaltered by the presence in all solutions of 10^{-2} *M* potassium nitrate, therefore the variations in T are not due to changing ionic strength

It is of considerable practical importance that the negative charge which silver iodide acquires in pure water is sufficient to hinder nucleation This suggests a new approach to the search for the best cloud-seeding substance—namely, that the substance should be at the isoelectric point under the conditions prevailing within a super-cooled cloud droplet

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Evaluation of Boundary Layer Thickness in Electrolytic Systems from Decay of Anode Concentration Polarization

The boundary layer produced in the vicinity of a dissolving anode in an electrolytic system is maintained by a supply of ions from the electrode, which supply balances the migration away from the layer due to the combined effects of electrical transport, diffusion and convexion On interruption of current, the excess concentration of the existing boundary layer is reduced by convexion and diffusion into the bulk of the electrolyte The convexion conditions may be very complex since a natural convexion mode occurs as a result of changes in solution density in the immediate vicinity of the electrode

Two extreme limiting conditions may be established for the decay of the excess concentration in the boundary layer on current interruption First, it may be assumed that there is zero relative motion between the fluid of the layer and that of the bulk electrolyte This 'pure diffusion' extreme is analogous to the levelling of a concentration excess from a zone within a solid to the remainder of the solid. Secondly, it may be assumed that 'perfect stirring' corresponds to the decay of an excess concentration within a zone with no accompanying change in the spatial dimension of the zone, together with a continual maintenance of the bulk concentration at the outermost extremity of the zone In the 'perfect stirring' case, natural convexion is considered to contribute negligibly to the establishment of the thickness of the boundary layer, this layer being chiefly a function of externally imposed stirring conditions

Intermediate to the extreme conditions must lie 'mixed' control of collapse of the boundary layer by both stirring and natural convexion in addition to the ever-present diffusion

The concentration contour of the boundary layer may be approximated by a straight line tangent to the slope of the actual contour at the electrode ($x = 0$), thus denoting an effective boundary layer thickness at the point of intersection of this line with the bulk concentration

For any given effective boundary layer thickness δ, the concentration (strictly, activity) at $x = 0$ is given, where zero time occurs at current interruption for 'pure diffusion' as

$$\frac{C-C_b}{C_0-C_b} = \operatorname{erf}\frac{1}{2\tau^{1/2}} + \frac{2}{\pi^{1/2}}\tau^{1/2}\left[e^{-1/4\tau}-1\right] \qquad (1)$$

and for 'perfect stirring' as

$$\frac{C-C_b}{C_0-C_b} = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}e^{-\frac{(2n+1)^2\pi^2\tau}{4}} \qquad (2)$$

where $\tau = Dt/\delta^2$, D = diffusion coefficient of ionic species, cm ^{2} sec $^{-1}$, t = time after current interruption, sec , δ = effective boundary layer thickness (cm), C_0 = initial concentration of ionic species (involved in the electrode reaction) at the electrode-electrolyte junction ($x = 0$) , C = concentration of ionic species at $x = 0$, $t > 0$, C_b = bulk concentration of ionic species in electrolyte

Equations 1 and 2 are re-arranged forms of solutions to related problems in heat conduction[1] Equation 2 may be rendered in an alternative form which is useful for computation when τ is small, namely

$$\frac{C - C_b}{C_0 - C_b} = 1 - 2\tau^{1/2} \sum_{n=0}^{\infty} (-1)^n \left\{\frac{1}{\pi^{1/2}} e^{-n^2/\tau} - \right.$$
$$- \frac{n}{\tau^{1/2}} \left[1 - \operatorname{erf} \frac{n}{\tau^{1/2}}\right] - \frac{1}{\pi^{1/2}} e^{-(n+1)^2/\tau} +$$
$$\left. + \frac{n+1}{\tau^{1/2}} \left[1 - \operatorname{erf} \frac{n+1}{\tau^{1/2}}\right]\right\} \tag{3}$$

For very small τ, equation (1) may be approximated as:

$$\frac{C - C_b}{C_0 - C_b} = 1 - \frac{2}{\pi^{1/2}} \tau^{1/2} \tag{4}$$

Similarly, equation 3 may be reduced in form for small τ since all terms become zero for $n > 0$ The result is identical with equation 4 Thus, for small τ, the time behaviour of concentration at $x = 0$ is essentially independent of conditions at the outermost part of the boundary layer, that is, irrespective of the presence or absence of stirring

Because of the equality of the solutions given by equations 1 and 2, 3 for small τ, it is suggested that this result may be utilized for the evaluation of boundary layer effective thickness in electrolytic systems This may be accomplished by analysis of the decay of concentration polarization upon current interruption By the Nernst relation

$$\frac{C - C_b}{C_0 - C_b} = \frac{\exp[ZF\eta/RT] - 1}{\exp[ZF\eta_0/RT] - 1} \tag{5}$$

where η_0 = concentration polarization at time zero, η = remaining polarization at $t > 0$, F = Faraday constant, R = gas constant, T = °K, Z = number of electrons in the electrode reaction The approximation to equation 1 given in equation 4 is valid provided

$$\operatorname{erf} \frac{1}{2\tau^{1/2}} \approx 1 \text{ and } e^{-1/4\tau} \approx 0$$

Arbitrarily choosing erf $1/2\tau^{1/2} = 0\ 99$ yields an upper limit of $\tau = 0\ 075$, whereas $e^{-1/4\tau} = 0\ 01$ sets a limit of $\tau = 0\ 054$ Taking a limit of $\tau = 0\ 05$ together with $D = 10^{-5}$ cm ^{2}/sec , it may readily be seen that for the very thin boundary layers encountered in stirred systems ($\delta \approx 10^{-3}$ cm), the overvoltage measuring device should be able to resolve time to better than 5 msec., suggesting an oscilloscopic technique For thicknesses of about 0 5 mm , encountered in natural convexion systems, time resolution need only be better than about 10 sec , which is well within the range of chart recorders

In the dimensionless time $\tau = 0\ 05$, the concentration (activity) fraction has dropped from unity to about 0 75 The fractional decay of polarization depends on the initial value before current interruption, according to equation 5 At 25° C and for $z = 2$, an initial polarization of 30 mV will have decayed to about 14 6 mV in the interval $0 < \tau < 0\ 05$ From the relationship of equation 5 with equations 1–4, it is clear that for any hydrodynamic conditions, such as stirring or its absence, all anode concentration polarization data can be normalized according to equation 5 and fitted to a master curve (equation 4) provided the real time-scale (in sec) is multiplied by D/δ^2 Since D is generally known, a graphical method may be used to cause a plot of the function of η, η_0 given in equation 5 versus time to be superimposed on a master plot the abscissa of which is rendered in dimensionless time τ, such as that given by equation 4 for $0 < \tau < 0\ 05$

From equation 4, it may be seen that the time required to attain a given fractional decay varies as the square of the boundary layer thickness δ, indicating that the proposed graphical method will permit a good resolution of the actual magnitude of δ Equations 1–4 may also prove useful as a means for separating concentration polarization from other types of overvoltage

Work is in progress to prepare analytical solutions similar to equations 1 and 2 for a concentration contour which is better than the linear approximation for actual systems. Functional tables for equations 1 and 2 over a wide range of τ and tables of the general term of equation 5 are in preparation

This work was supported by the U S Office of Naval Research

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Pyrolytic Carbon Formation from Carbon Suboxide

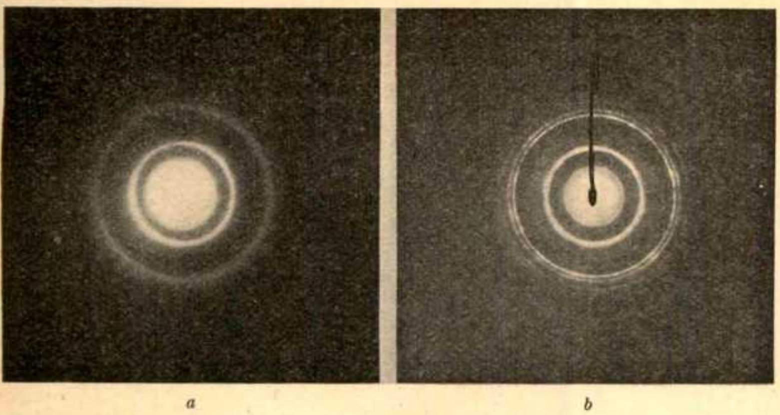
ALMOST all the extensive work done on the formation of pyrolytic carbon films has consisted of the decomposition of a hydrocarbon on non-metallic substrates like porcelain or artificial graphite Work is in progress in our laboratory investigating the kinetics and mechanism of carbon film formation on glazed porcelain from carbon suboxide (C_3O_2)[1] In the course of this work, it was of interest to look briefly, initially, at the effect of some different substrates on the qualitative rate of carbon formation and more particularly on the nature of the film formed

Very pure C_3O_2, entrained in pre-purified helium, was pyrolysed at 713° C for about 3 hr over porcelain, electrolytic nickel and copper foils, and reagent grade platinum foils The concentration of C_3O_2 at the pyrolysis temperature was about 6×10^{-6} mole/l Tenacious and hard carbon films were obtained, with the rate of film formation on nickel being roughly four times that on the other substrates

The carbon films were separated from the metal substrates by acid treatment prior to studying their structure by transmission electron diffraction and

Table 1. SPACINGS OF (hkl) LINES OF FILM CARBON DEPOSITED FROM C_3O_2 ON TO A NICKEL SUBSTRATE AT 713° C

(hkl) Graphite	d_g, A	d_{fc}, A	Intensity of experimental halos
002	3 370	3 370	medium
100	2 102	2 091	broad ring with very strong intensity
101	2 036		
110	1 232	1 230	very strong
112	1 150	1 155	strong
200	1 063	1 060	medium
210	0 804	0 799	medium
310	0 709	0 704	medium



Figs. 1 *a* and *b*. Transmission electron diffraction patterns of film carbon deposited from C_3O_2 on to a substrate at 713° C.; *a*, copper substrate; *b*, nickel substrate

transmission electron microscopy. 50-kV. electrons, having a beam width of 10μ, were used. Fig. 1 (*a*) shows the diffraction pattern for the film carbon resulting from deposition over a copper substrate. Diffuse halos, corresponding to the (10) and (11) reflexions of a polycrystalline, turbostratic carbon[2], are observed. The absence of a strong (002) reflexion when the incident electron beam is normal to the film surface is indicative of preferred orientation of the basal planes parallel to this surface. Diffraction patterns very similar to Fig. 1*a* were obtained from the film carbon deposited on a porcelain or platinum substrate.

On the other hand, the diffraction pattern (Fig. 1*b*) of the film carbon deposited on the nickel substrate is characteristic of a material of substantial crystallite size, in which the crystallites have extensive three-dimensional ordering. Table 1 compares the *d*-spacings for the film carbon (d_{fc}) with comparable spacings calculated from the graphite lattice (d_g). Similar diffraction patterns for carbon films deposited on porcelain, copper, or platinum are obtained only when the separated films are ultimately heated in excess of 2,000° C.

Fig. 2 is an electron micrograph of the carbon film deposited on nickel. Variations in the thickness of the films are evident. In addition, the presence of small, white circular spots is noted. It is suggested that these spots were formed by the removal by acid treatment of inclusions of nickel and/or nickel carbide which were originally present, but this has not been verified. From work on the interaction of evaporated carbon films with a similar nickel substrate on subsequent heat treatment, it is known that substantial inter-diffusion of nickel and carbon occurs at 500° C. and above (Banerjee, B. C., and Walker, P. L., unpublished results).

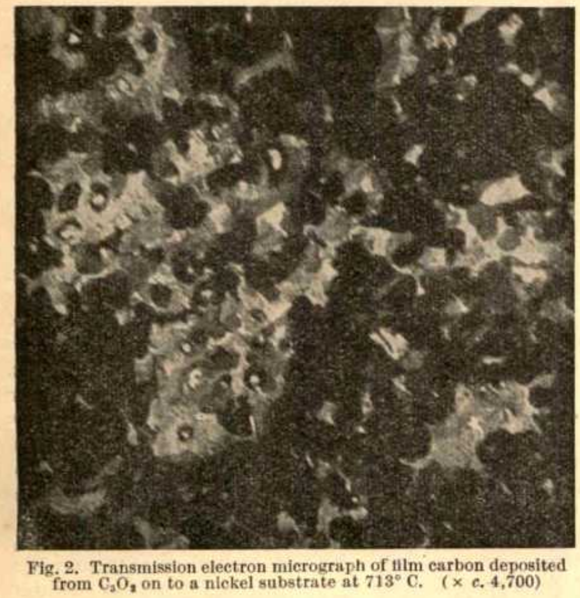


Fig. 2. Transmission electron micrograph of film carbon deposited from C_3O_2 on to a nickel substrate at 713° C. (× *c*. 4,700)

These results clearly suggest that more attention might be paid to the effect of the substrate in work on the formation of pyrolytic carbon films. In particular, substrates of nickel and iron are of interest. They are highly active catalysts for the decomposition of carbonaceous gases, apparently because of the incomplete filling of their 3*d* bands. In addition, they markedly increase the crystallinity of the carbon formed as a decomposition product. The effect of nickel is clearly evident from this work. The effect of iron, both on rate of formation and structure of carbon, has been previously shown for carbon monoxide decomposition[3].

We thank the Atomic Energy Commission, contract No. *AT*(30-1)-1710, for support of this work.

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BIOCHEMISTRY

Changes in Adenine Nucleotides in the Muscles of Some Marine Invertebrates

Hitherto it has been generally accepted that most vertebrate muscles contain an enzyme specific for the deamination of 5′-adenylic acid (AMP) which is formed principally from adenosine triphosphate (ATP). On the other hand, in the case of invertebrates the presence of such an enzyme has been questioned[1].

Recently we have studied the changes in adenine nucleotides in the muscles of some marine invertebrates (prawn, squill, crab, squid, and some sea shells), and observed that those of prawn, squill, and crab contain certain amounts of 5′-inosinic acid (IMP) in addition to AMP, while in others AMP alone is accumulated.

Chopped muscles of each animal were stored in a glass-covered dish at an ambient temperature of − 5° C. in order to minimize bacterial effects. Two gm. of muscle were ground by hand in a mortar in 20 ml. of cold, 4 per cent perchloric acid solution. The extract was filtered and 10 ml. of the filtrate were used for

Table 1. Purine Compounds in the Muscles of Some Marine Invertebrates (μmol./gm. of muscle)

Species	Time (days) elapsed	Inosine and hypo-xanthine	AMP	IMP	ADP	ATP	Total	Muscle
Carp	0	0·01	0·01	1·06	1·41	2·94	5·43	Dorsal
Cyprinus carpio L.	3 (hr.)	0·10	0·24	4·01	0·60	0·25	5·20	
Squill	0	0·46	0·94	0·50	1·24	3·84	6·93	Abdominal
Squilla oratoria De Haan	6	2·12	3·66	0·75	0·29	0·17	6·99	
Prawn	0	0·00	0·94	0·00	2·09	6·61	9·64	Abdominal
Pandalus hypsinotus Brandt	28	6·14	1·31	1·61	0·17	0·18	9·46	
Crab	0	0·00	0·29	0·00	1·75	5·87	7·91	Leg
Erimacrus isenbeckii	35	1·14	3·47	1·98	0·46	0·35	7·40	
Squid *Ommastrephes sloani*	0	0·01	0·55	0·00	1·53	7·48	9·57	Mantle
pacificus Steenstrup	20 (hr.)	1·35	4·70	0·00	2·89	0·83	9·77	
Surf-clam	0	0·00	0·56	0·00	1·03	3·09	4·68	Foot
Spisula	3	0·31	2·96	0·00	0·95	0·42	4·64	
sachalinensis	0	0·00	0·50	0·00	1·10	4·15	5·75	Adductor
Schrank	2	1·08	2·84	0·00	1·12	0·70	5·74	
Scallop	0	0·00	1·56	0·00	1·18	3·48	6·22	Adductor
Pecten yessoensis Jay.	1	2·99	1·52	0·00	1·17	0·92	6·60	
Arkshell	0	0·00	0·41	0·00	1·19	5·72	7·32	Foot
Anadara	10	0·19*	5·74	0·00	0·66	0·51	7·10	
broughtoni	0	0·00	2·23	0·00	3·50	4·15	9·88	Adductor
(Shrenk)	10	0·42*	7·28	0·00	1·38	0·48	9·56	
Abalone	0	0·00	0·20	0·00	0·59	3·48	4·27	Foot
Haliotis discus hannai	45	0·00	3·04	0·00	0·92	0·30	4·26	

Inosine and hypoxanthine fraction is expressed as inosine. * Expressed as adenine.

analysis. Nucleotides, nucleosides, and bases were separated and determined by the hydrochloric acid gradient elution system on 'Amberlite *IRA*-400' or by paper chromatography. The details of the system have been described previously[2]. In Table 1 the concentrations of the compounds found to be present after various time-intervals are shown.

From these results it can be seen that ATP decreased during storage, with rapid accumulation of IMP in the dorsal muscle of carp, while in the case of squill, prawn, and crab IMP was found in small quantity in living muscles and then gradually accumulated. Furthermore, in other muscle samples AMP alone was found in living or post-mortem muscles.

The rates of disappearance of ATP and of ADP were very rapid for squid and were comparable with those for fish, but the other animals tended to require more time for the onset of post-mortem changes. Especially, the changes in the muscle of abalone were very slow. AMP accumulation continued for a long time. Adenosine, inosine that might be formed and the purine bases, adenine and hypoxanthine, that would be released in the hydrolysis or phosphorolysis of nucleosides could not be found in abalone muscle even after 45 days.

Further interesting aspects of the changes are the formation of inosine and hypoxanthine from AMP of squid, surf-clam, and scallop. Inosine was principally found in the muscles of surf-clam and of scallop while hypoxanthine was found in squid.

The occurrence of these compounds might be considered to prove the existence of the deamination of adenosine or adenine. The activity of adenosine deaminase has already been recognized in squid muscle[3]. On the other hand, according to Florkin[4], invertebrates are reported to contain adenase but no adenosine deaminase. If the existence of adenosine deaminase is ascertained in the muscles of surf-clam and of scallop, as in the case of squid, the new evidence will be accepted to support the idea of the deamination mechanism of adenosine in the muscle of invertebrates. This matter is under consideration.

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Effect of Charcoal on the Behaviour of Adenine Nucleotides in Ion-Exchange Chromatography

Charcoal has been used widely for selective adsorption of nucleotides from reaction mixtures and tissue preparations. In experiments reported here three commercially available grades were all found to adsorb quantitatively and to elute reasonably satisfactorily the mono-, di- and tri-phosphates of adenosine; these charcoals were 'Norit', two batches of purified, activated charcoal supplied by Hopkin and Williams, Ltd., and a gift sample of 'Ultrasorb *ZX*' from British Carbo Norit Union, Ltd.

For each trial, between 5 and 10 mgm. of the sodium salts of the individual nucleotides were dissolved together, the solution diluted to about 250 ml. and brought to *p*H 2 with *N* hydrochloric acid. About 4 gm. of charcoal were added, and the mixture stirred vigorously for 15 min. in an ice bath. Under these conditions the nucleotides were quantitatively adsorbed, as indicated both by optical density of the filtrate at 260 mμ and by the orcinol test. The charcoal was then washed with water to neutrality and eluted, under suction on a filter funnel, with several lots of 0·5 per cent ammonia in 25 per cent ethyl alcohol. Using a total eluant volume of 250–300 ml., 80–95 per cent recovery of the nucleotides was obtained. The ammoniacal eluate was subjected directly to ion-exchange chromatography, using the resin in the chloride form, a column of size 1·5 × 3·0 cm., and the elution system of Cohn and Carter[1]. Elution was followed by absorption measurements at 260 mμ of the individual fractions, by pentose estimations, and also (for ADP and ATP) by estimating the 'labile phosphate' liberated by 15 min. hydrolysis at 100° C. in 1·0 *N* hydrochloric acid. The summarized results are as follows.

Provided they had not previously been in contact with charcoal, mixtures of the three adenosine phosphates behaved normally as regards the order and position of elution of their constituents; in other words, they behaved as described by Cohn and Carter (Fig. 1*a*). But after being adsorbed and eluted from any of the charcoals tested, no nucleotide material was removed from the column by the AMP eluant. The ADP eluant, on the other hand, removed quantitatively both the AMP and the ADP, but as a single peak the elution position of which was somewhat behind that normal for ADP (Fig. 1*b*). After suitable

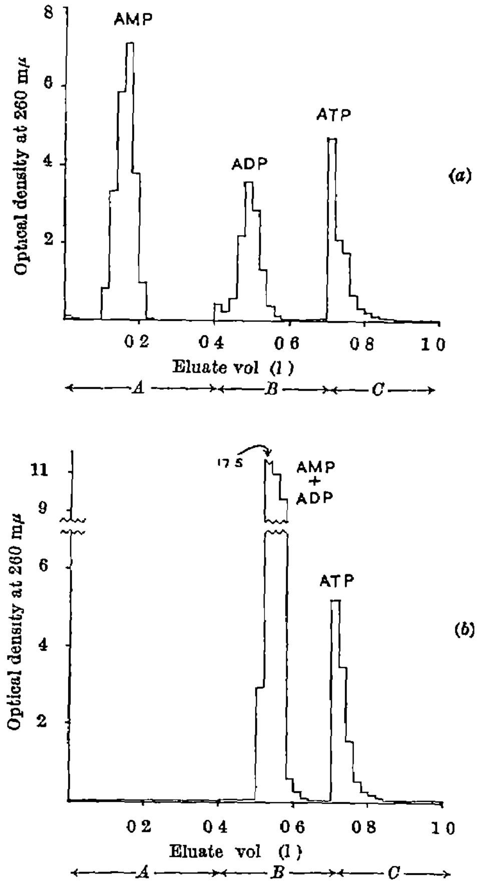

Fig. 1. Effect of charcoal on the chromatographic behaviour of AMP, ADP and ATP. (*a*) Direct chromatography of authentic nucleotides, (*b*) chromatography of nucleotides after charcoal treatment. 'Dowex'-1-chloride, X – 10, 2–400 mesh. Column size, 1·5 cm × 3·0 cm. Eluants: *A*, 0·003 N HCl, *B*, 0·02 N NaCl + 0·01 N HCl, *C*, 0·2 N NaCl + 0·01 N HCl

dilution (to about twice the volume), and adjustment to pH 8–9, the mixed peak was applied to a second column and re-eluted. The two nucleotides then usually separated satisfactorily (Fig. 2), although with the charcoal 'Ultrasorb *ZX*' passage through a third column was needed to obtain full resolution, in this particular case the AMP eluant was used at double its usual strength, but it seems likely that normal strength eluant would have achieved the same result. After adsorption and release of a nucleotide mixture from 'Ultrasorb *ZX*', unsuccessful attempts were made to resolve it on a single resin column by using eluants of increased strength in the first step. Double strength AMP eluant was wholly ineffective, but ADP eluant at half its usual strength removed a peak, this, however, did not consist of AMP alone, since it contained acid-labile phosphate.

AMP was eluted normally when applied alone to a resin column in the ammonia–ethanol solvent without coming into contact with charcoal, whereas, if applied in a quantity of the solvent which had previously been used to wash charcoal (the activated, purified sample), it was no longer eluted with the AMP eluant, but came off only in the position usual for ADP. Prolonged preliminary washing of charcoal with (*a*) 0·5 M ethylenediaminetetraacetic acid (sodium salt), (*b*) 0·1 N hydrochloric acid, or (*c*) the ammonia–ethanol eluant, did not eliminate its influence on the chromatographic behaviour of AMP afterwards adsorbed on it. On evaporation, ammonia–ethanol washings from charcoal left a slight, unidentified solid residue.

These results point to a need for caution in the selection and use of charcoal for nucleotide adsorption, particularly if subsequent resin chromatography is contemplated. None of the samples proved satisfactory, but some were worse than others. The observations indicate very strongly the presence in charcoal of a substance which induces AMP to behave anomalously on chloride resin. Although the substance is soluble in ammoniacal alcohol, even prolonged washing with many changes of the solvent, which continued to extract the substance, did not render the charcoal innocuous. Thus, the substance appears either to be present in relatively large amounts or to exert its effect in very small quantities. Its mode of action is obscure. Should a loose association or complex with AMP be formed, it must be presumed that this is broken up on elution from the first resin column, since the AMP and ADP are then easily separable on a second column. Nevertheless, the AMP still does not behave quite normally, although it now comes away from the column as a single clear peak with the AMP eluant, more elution than normal is required to release it. It seems probable that a much smaller amount of the interfering substance must be applied to the second column than to the first. On the complexing hypothesis, if there were only sufficient of it to involve a part of the AMP present on this second column, it might be expected that the remaining free AMP would come off in its normal position, but the complexed fraction again only with the stronger

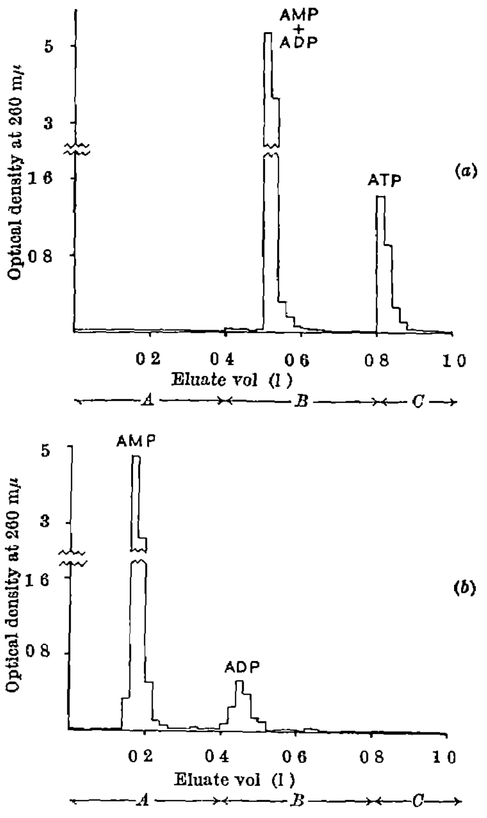

Fig. 2. Passage of a charcoal-treated nucleotide mixture through two successive resin columns. (*a*) Chromatography on first column, (*b*) resolution of the mixed AMP–ADP peak on the second column. Experimental details as in Fig. 1

eluant, so that two peaks should result. Actually there is only one peak, yet a delayed one. Hence on the columns, the AMP always behaves like a single substance the elution point of which is delayed progressively as the amount of the interfering substance increases. No similar interference after charcoal treatment was found when formate columns were used, even though considerably more charcoal was then necessary in the nucleotide isolation step.

Certain anomalies in the chromatographic behaviour of nucleotides resulting from their previous treatment have already been recorded. Lund *et al.*[3] found that treatment of ATP with cation-exchange resins caused it to behave aberrantly when it was chromatographed later on paper, occupying, with some solvents, the position usual for ADP. Laws and Stickland[4] observed that if nucleotides are isolated from yeast by precipitation with barium acetate, the presence of large amounts of water-soluble barium salts causes AMP to behave abnormally during chromatography, on the anion-exchanger 'Amberlite *CG*–400' it is then eluted in a solution intermediate in strength between those usual for AMP and ADP.

This work formed part of a Ph.D. thesis submitted to the University of London; it is a pleasure to acknowledge the guidance and helpful criticism of Dr. F. J. Richards.

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Changes in the Concentration of Ribonucleic Acid during Wound Tissue Regeneration

The metabolism of ribonucleic acid (RNA) in regenerating wound tissue may be expected to be of special importance since the synthesis of protein is one of the principal metabolic activities of this tissue[1,2]. The relative amount of RNA in this tissue at different stages of regeneration should give some insight into the protein metabolism. A complicating factor is the fact that considerable amounts of two quite different types of protein are formed, the intracellular 'metabolically-active' proteins, and collagen.

The concentration of RNA in regenerating wound tissue was determined by analysis of tissue obtained from adult female rats. Because of the marked influence of dietary protein on the rate of regeneration[3,4], these animals were fed identical amounts (8 gm./day) of a protein-free diet for 3 days prior to wounding and for the balance of the experimental period. In this way, the experimental animals served as their own source of nitrogen for the synthesis of protein and nucleic acids. The procedure for making standard wounds has been described previously[2,3]. At intervals after wounding, the regenerating tissue was collected and immediately homogenized by repeated grinding in a mortar with sand and small amounts of cold water. After determining the nitrogen in an aliquot of the homogenized tissue (micro-Kjeldahl), the remainder was fractionated to separate the RNA from the deoxyribonucleic acid (DNA) by the method of Schmidt and Thannhauser[5,6]. The phosphate content of the RNA was measured[7]. Less than 3 per cent of the total phosphate in this fraction was inorganic, this inorganic phosphate may have arisen from the hydrolysis of phospho-proteins during the fractionation procedure[8]. Since phospho-lipids, free nucleotides and inorganic and sugar phosphates had been removed by previous procedures, it was assumed that essentially all the phosphate in this fraction must come from RNA.

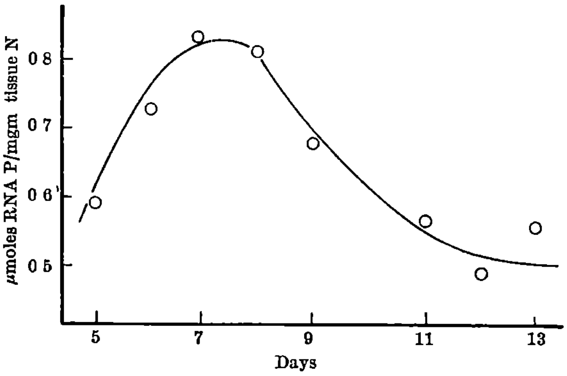

Fig. 1. RNA content of regenerating wound tissue in μmoles of phosphorus per mgm. of tissue nitrogen at various times after wounding. Each point represents the data from the analysis of tissue from 5–6 animals

The level of RNA in the regenerating tissue at various intervals after wounding is shown in Fig. 1. The highest concentration appears to be reached in about 7–9 days after wounding, thereafter it decreases to a lower and relatively constant level. In part, this decrease is due to the accretion of nitrogen in the wound tissue with time. However, even when calculated on the basis of tissue weight, there is still an appreciable decrease in the amount of RNA.

If the types of RNA molecules being synthesized by cells can be shown to change, it might be considered to be indirect evidence that new or different proteins are being synthesized. A change in the type of RNA, or the proportion of the various kinds of RNA produced by the cell, may be signalled by a change in the proportion of purines to pyrimidines of the RNA fraction of the tissue. In a further experiment, it was possible to obtain a sufficient quantity of regenerating tissue to enable both the ribose and phosphate content of the RNA fraction to be measured. The amount of ribose in the RNA fraction was determined by the orcinol method[9]. From the data on the analysis of ribose, which is a measure of the purine nucleotides only[10], and that of the phosphate, which is proportional to the total nucleotide content of the nucleic acid, a ratio which approximates the proportion of pyrimidines to purines in the RNA may be calculated (Table 1). During the early phases of regeneration, the RNA in the wound tissue contains about the same amounts of purine and pyrimidine nucleotides, on a molar basis, later the RNA fraction

Table 1. PURINES AND PYRIMIDINES IN RNA OF REGENERATING WOUND TISSUE

Days after wounding	μmoles Ribose per mgm. tissue nitrogen	μmoles Phosphate per mgm. tissue nitrogen	Ratio of purine to pyrimidines*
5	0.25 ± 0.08	0.46 ± 0.11	1.11 ± 0.12
8	0.60 ± 0.11	1.16 ± 0.12	1.05 ± 0.07
12	0.41 ± 0.09	1.01 ± 0.11	0.70 ± 0.07
15	0.33 ± 0.04	0.88 ± 0.12	0.61 ± 0.09

* The standard error was calculated from the purine/pyrimidine ratios for individual tissue samples

appears to contain an appreciably larger proportion of pyrimidine than purine nucleotides

This change in proportion of nitrogen bases might be interpreted to mean that different types of RNA are being synthesized at different stages of regeneration, this, in turn, may presage a change in the nature of the protein synthesized in the regenerating tissue Large amounts of collagen begin to appear in the wound tissue only after the 8th day after wounding[11,12] Since the amino-acid composition of collagen (and presumably its intracellular precursors also) is so radically different from that of the 'metabolically-active' proteins, it does not seem improbable that a type of RNA with radically different nucleotide composition might be involved in the synthesis of the collagen precursors It must not be overlooked that even though much collagen may be formed, some of the 'metabolically-active' proteins are also being formed at the same time Thus, if the change in the nucleotide composition is related to the synthesis of collagen precursor, it may be expected that the actual difference in the types of RNA involved is much greater than is indicated by the above results

This investigation was supported by a research grant from the U S Public Health Service.

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Phosphorylserine as a Constituent of Phosphoproteins from Plasma of the Laying Hen

ALTHOUGH it has been well established that the blood of the laying hen contains phosphorus compounds, the quantities of which are increased immediately preceding and during ovulation[1,2], the nature of some of these substances has only recently been subject to chemical investigation Dilution of plasma from the laying hen results in the precipitation of a complex of lipid and a phosphoprotein, though by far the greater proportion of the total phosphoprotein present in the plasma remains in the supernatant[2]. Designation of these materials as phosphoproteins has rested entirely on the finding that inorganic phosphate is liberated when they are incubated with dilute alkali[3], but the manner in which the phosphorus is bound has not been reported. Phosphoproteins in general and particularly that from egg yolk, contain their phosphorus as phosphorylserine[4,5], and this has now been sought in acid hydrolysates of both the phosphoprotein fractions of the plasma.

Plasma from laying hens was diluted with 9 vol. water[2] and the precipitated lipophosphoprotein collected by centrifugation. The supernatant was precipitated by trichloroacetic acid added to a final concentration of 10 per cent (w/v) and both this precipitate and that of the lipophosphoprotein was extracted with chloroform/methanol (2 1 v/v)[6]. The lipid-free residues were hydrolysed in 2 *N* hydrochloric acid at 100° and the supernatants, containing 80–96 per cent of the total phosphorus of the fractions, were dried over phosphorus pentoxide and potassium hydroxide The residues were fractionated on 'Dowex 50' (× 12)[7] After elution of a fraction containing inorganic phosphate a second major phosphate fraction was eluted at a point corresponding to that at which phosphorylserine was eluted when run through the same column No other fractions were detected The presumptive phosphorylserine fraction contained between 20 and 54 per cent of the organic phosphorus present in the hydrolysate added to the column and was further purified by high-voltage electrophoresis on paper at *p*H 1 5 (ref 8) Under these conditions 80–86 per cent of the phosphorus moved as a single component at a rate identical with phosphorylserine The remaining 14–20 per cent formed a single slower-moving component Both components reacted with ninhydrin When eluted and hydrolysed to yield the constituent amino-acids, two-dimensional paper chromatography showed that the component migrating at the same rate as phosphoserine yielded only serine The molar ratios of phosphorus to serine in two experiments were 1 0 and 1 3 The second minor fraction yielded mainly serine and glutamic acid with traces of alanine and possibly leucine. The total yield of phosphorylserine was 38–39 per cent of the organic phosphorus of the phospholipoprotein residues and 17–20 per cent of that present in hydrolysates of the phosphoprotein derived from the supernatant A specimen of phosvitin subjected to the same procedure yielded phosphorylserine equivalent to 25 per cent of the organic phosphate present in the hydrolysate No phosphoproteins were detected in plasma of the non-laying hen or the cockerel (cf ref 2).

These experiments appear clearly to establish phosphorylserine as being the only major single phosphorylamino-acid detectable in partial hydrolysates of phosphoproteins from plasma of the laying hen The overall recovery, when compared with that obtained with phosvitin, suggests that all the phosphoprotein phosphorus may be bound in this manner.

I thank Dr M E Coates, National Institute of Research for Dairying, Shinfield, Nr Reading, for a generous supply of birds

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Distribution of Ribonucleic Acid in Subcellular Fractions of Various Regions of the Central Nervous System of the Rat

The distribution of ribonucleic acid (RNA) in the subcellular structures of whole brain was studied by Brody and Bain[1], and by Aldridge and Johnson[2], and in the rat brain cortex during development by Keup[3]. We showed previously that appreciable differences exist in the distribution of nucleotides and RNA and in the incorporation of phosphorus-32 in this RNA in various brain regions[4]; we have now studied the RNA content of the subcellular fractions of each region of the central nervous system examined previously and the incorporation of phosphorus-32 in the RNA fractions thus isolated.

Table 1. Distribution of Ribonucleic Acid in Subcellular Fractions of Various Regions of the Rat Central Nervous System

Region	Nuclei			Heavy mitochondria		Light mitochondria		Endoplasmic reticulum (microsomes)		Cytoplasmic fluid		Myelin fraction	
	µgm P/mgm protein	Percentage of total RNA	RNA/DNA	µgm P/mgm protein	Percentage of total RNA	µgm P/mgm protein	Percentage of total RNA	µgm P/mgm protein	Percentage of total RNA	µgm P/mgm protein	Percentage of total RNA	µgm P/mgm protein	Percentage of total RNA
Olfactory lobes	1·27	14·8	0·105	0·99	6·8	1·40	6·9	3·43	30·0	1·35	24·0	0·55	17·4
Cerebral grey matter	1·23	9·0	0·166	0·54	9·3	1·26	8·1	3·55	33·9	1·44	24·0	0·35	15·8
Cerebral white matter	1·05	10·3	0·124	0·55	9·7	1·00	7·3	2·35	29·5	1·00	21·8	0·34	21·0
Hypothalamus	1·69	10·0	0·178	0·56	12·1	1·01	10·1	2·27	32·6	1·06	20·0	0·57	15·0
Cerebellum	1·20	32·4	0·081	1·08	5·3	1·06	5·2	3·49	31·0	0·65	10·6	0·49	15·4
Medulla oblongata	0·98	9·3	0·154	1·01	16·1	1·21	10·9	2·45	31·6	0·68	14·9	0·29	17·2

We have completed six experiments using twenty rat brains on each occasion. The rats received intraperitoneal injections of phosphorus-32 (200 µc/100 gm body-weight) and were decapitated after one, three and five days. The heads were immediately plunged into ice-cold 0·9 per cent sodium chloride and the brains were removed rapidly in a manipulation cabinet at 0° C. Various parts of the brains were separated by dissection: olfactory lobes, grey matter and white matter of the cerebrum, hypothalamus, cerebellum and medulla oblongata. The subcellular fractions were isolated using 0·3 *M* sucrose by the classical method of Hogeboom, Schneider and Pallade[5], as slightly modified by Aldridge and Johnson[2]. The purification of the nuclei did not seem sufficient and we therefore employed the following modification. Centrifuging at 1,000*g* for 10 min with a 0·3 *M* sucrose solution gave a precipitate mixed with capillaries and blood, these forming a lower layer in the centrifuge tubes. The upper layer was separated, re-homogenized with 0·3 *M* sucrose and centrifuged at 1,000*g* for 10 min. The precipitate obtained was mixed with 20 volumes of 1·5 *M* sucrose and centrifuged at 145,000*g* for 90 min in a 'Spinco' model *L* ultracentrifuge. This treatment gave a precipitate consisting of nearly pure nuclei and a supernatant film containing myelinated structures slightly contaminated with their cytoplasm and with axonal fragments. These facts were demonstrated by electron microscopy. It was estimated that about 90 per cent of the supernatant consisted of myelinated structures. This was confirmed by the chemical analysis, which showed that the supernatant contained, on average, about 55 per cent of the total phosphatides for all the different parts, while the cytoplasmic fluid contained only about 2 per cent and the totality of mitochondria 24 per cent[6].

The heavy and light mitochondrial fractions and the microsomes were separated by the method of Aldridge and Johnson[2].

After extraction of the acid-soluble matter by 0·6 *N* perchloric acid, each subcellular fraction was treated with cold ethanol and then, to extract the lipids, treated by the method of Bloor[7]. RNA and deoxyribonucleic acid (DNA) were separated by the procedure of Schmidt and Thannhauser[8] and the RNA fraction was purified by a technique described elsewhere[4]. The specific activity of RNA phosphorus was determined on solutions at infinite thickness in an end-window Geiger counter (mica window 1·8 mgm/cm^2). The results of the experiments are shown in Tables 1 and 2.

The ratio of RNA to protein was found to be lowest (0·29–0·57) in the myelin fraction, higher in the heavy mitochondria (0·54–1·08) and, on average, higher still in the light mitochondria (1·01–1·4), while for the nuclei the values varied from 0·98 to 1·69. For the cytoplasmic fluid the values were of the same order, although rather lower in the cerebellum and the medulla. The highest values were, of course, found in the microsomes. For a given subcellular fraction clear and important differences existed between one part of the brain and another: the highest values for the nuclei correspond to hypothalamus, followed by the olfactory lobes and the grey matter of the cerebrum, the lowest values were for the cerebral white matter and the medulla. The RNA content of the microsomes was fairly constant at about 30 per cent of the total for each region while for the other fraction the RNA content varied appreciably from one region to another. The ratio RNA/DNA, which also gives an indication of the purity of the nuclei, varied between 0·08 and 0·17: the highest values were for the hypothalamus and the cerebral grey matter.

The specific activity of the nuclear RNA at one and three days is clearly higher than that of the other fractions. The same is true at 5 days except for the olfactory lobes and the hypothalamus. A comparison of the results for the specific activity with respect to

Table 2. Specific Activity in Counts/Min µgm of Ribonucleic Acid Phosphorus in Subcellular Structure of Various Regions of the Rat Central Nervous System

Region	Hr	Nuclei	Heavy mito-chondria	Light mito-chondria	Endoplasmic reticulum (microsomes)	Cytoplasmic fluid	Myelin fraction
Olfactory lobes	24	37	24	10	20	33	36
	84	75	39	40	51	57	49
	120	52	—	—	68	69	54
Cerebral grey matter	24	34	9	16	9	13	24
	84	43	29	25	23	30	24
	120	57	32	30	40	41	27
Cerebral white matter	24	26	7	8	8	13	17
	84	46	26	21	27	32	29
	120	49	30	28	37	39	35
Hypothalamus	24	58	24	11	30	19	34
	84	64	36	38	44	47	47
	120	51	51	42	51	88	29
Cerebellum	24	39	9	9	14	16	—
	84	46	34	31	39	55	47
	120	57	—	—	46	64	46
Medulla oblongata	24	50	9	18	15	13	28
	84	56	30	38	36	38	45
	120	71	—	—	53	39	40

time for the nuclear RNA shows that a maximum exists between the first and fifth day for the olfactory lobes and the hypothalamus, while for the other regions the specific activity at five days is either greater than or equal to that at three days For the hypothalamus and the olfactory lobes the specific activity versus time curve for the nuclei crosses those for the cytoplasmic fluid and the microsomes Thus, our results confirm previous data concerning the high RNA content and great metabolic activity of the olfactory lobes and the hypothalamus[4]

The relatively high RNA content of the myelin fraction recalls former investigations concerning the cytoplasm attached to myelin sheaths and the RNA content of axons It should be mentioned that the relatively high specific activity of the RNA of the myelin fraction cannot be entirely accounted for by a contamination of nuclei, since 90 per cent of the nuclei of the various regions, apart from the cerebellum, contains, on average, only about 10 per cent of the total RNA. In addition, 24 hr after injection the myelin fraction has a specific activity superior to that of any other subcellular fraction apart from the nuclei. It is therefore difficult to attribute this activity to a contamination by mitochondria, microsomes or cytoplasmic fluid

Our nuclear fraction contained 70 per cent of the total DNA in the cases of the cerebral white matter and medulla, 75, 86, 89 and 92 per cent respectively for the hypothalamus, cerebral grey matter, olfactory lobes and the cerebellum It should be noted that the ratio RNA/DNA for our nuclear fraction is generally lower than or at the most equal to that found by Chauveau[9] for the liver and is, for three regions, near that found by Georgiev and Mantiewa[10] for the whole brain

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Cystine Content in Hair of Childen as influenced by Vitamin A and Animal Protein in the Diet

RECENTLY, Koyanagi and Odagiri[1] reported that the cystine content of skin of rats dosed with a small quantity of vitamin A is lower than that of rats given a liberal allowance of the vitamin In the presence of methionine, the cystine content increased both with small and large doses of vitamin A We now wish to report on experiments conducted to determine whether the cystine content in the hair of children could be changed by deficiencies of vitamin A in the diet and other factors

Table 1 AMOUNT OF FOODS CONSUMED AND ESTIMATE OF PROTEIN AND VITAMINS PER PERSON PER DAY

Foods	District *A* (gm)	 *B* (gm)	 *C* (gm)
Rice	—	492	300
Barley	150	9	60
Wheat	100	21	100
Japanese barnyard millet	400	—	—
Potatoes	100	22	50
Sugar	—	3	20
Oils and fats	—	6	8
Soybean	54	4	5
Miso (fermented soybean)	100	49	30
Fish, raw	20	100	100
Fish, salted	—	10	10
Tofu (soybean curd)	—	34	80
Meat	—	4	30
Eggs	—	18	30
Milk	—	14	100
Vegetable, green	50	43	100
Vegetable, uncoloured	100	64	100
Vegetable, salted	200	100	70
Fruits	—	9	150
Calories	2,846	2,216	2,549
Protein* (gm)	119 0 (3 8)†	74 6 (27 6)	60 4 (38 5)
Vitamin A (I U)	1,184	1,235	3,572
Riboflavin (mgm)	0 71	0 63	1 40
Thiamine (mgm)	1 97	0 92	1 21

* Digestibility of protein of barnyard millet is very low.

† Amount of animal origin indicated in parentheses

The survey was done on school children aged 8–9 yr , in the following three districts, (*A*) Yamagata village, situated in the mountainous area where people having a small income eat Japanese barnyard millet and very little fish, (*B*) Teshiromori village, situated in the rice field district where people live on rice and eat a moderate amount of fish; school lunches are served, (*C*) Morioka city, where the diets are comparatively well balanced, children have lunch served at school Food articles available for civilian consumption in three districts are shown in Table 1

To determine the cystine content in the hair, 0 1 gm of hair from the back of the head was hydrolysed in 3 *N* hydrochloric acid at 120° C for 4 hr The cystine content in the hydrolysate was analysed by the phosphotungstic acid method[2]

Effect of diet The results (Table 2) indicate that there are striking differences in cystine content in the hair from the three districts, for example, the figures from district *A* show only 8 1 per cent while those from districts *B* and *C* return 11 4 and 12 5 per cent, respectively. The figures thus correlate closely with the consumption of animal protein

Effect of vitamin A and milk Table 2 gives results in districts *A* and *B* obtained by analysing hair of children supplemented for half a year with shark liver oil (2,500 I U). The cystine content is higher in the hair of children in the supplemented group The observations are in agreement with those on the skin of rats reported by Koyanagi *et al* In district *B* some children were allowed to drink milk liberally, that is, 300–400 c c daily a child for half a year. The cystine content of their hair is remarkably higher than that of children without milk

Table 2 CYSTINE CONTENT IN THE HAIR OF CHILDREN IN THREE DISTRICTS

District	Supplement	Height (cm)	Weight (kgm)	Cystine in the hair (per cent)	
A	None	116 3 ± 6 7*	21 9 ± 2 2*	8 1 ± 0 15*	(21)†
	Shark liver oil	122 0 ± 5 2	22 0 ± 2 2	9 5 ± 2 3	(11)
B	None	121 0 ± 3 1	24 1 ± 1 7	11 4 ± 1 8	(13)
	Shark liver oil	124 7 ± 4 5	24 5 ± 2 6	13 0 ± 1 4	(12)
	Milk	128 7 ± 3 1	26 2 ± 0 5	14 4 ± 0 6	(9)
C		127 5 ± 1 8	26 7 ± 1 2	12 5 ± 1 3	(22)

* Standard deviation of the mean

† No of children indicated in parentheses

Effect of age. Hair of 26 infants was collected in district *C* and analysed In this case, in spite of individual variation the higher values were found more often among the younger group and there was no apparent difference between sexes (9 months, 12 1–15 1, 3 yr , 11 9–14 4, 4 yr, 10 6–13 0, 5 yr , 10 5–13 0, 6 yr , 11 0–12 7, adults, 10–12 per cent)

Effect of disease Through the clinical examination conducted on 75 infants in district *A*, 13 cases of active angular stomatitis, 25 cases of fatty liver and 5 cases of severe malnutrition were found It is remarkable that the cystine content in the hair of infants showing undernutrition showed strikingly lower values than in that of the healthy infants in district *C* (2 yr , 6 0–9 2, 3 yr , 5 5–6 6; 4 yr , 5 8–9 2, 5 yr , 7 2 per cent)

In regard to amino-acid content of nails, Hess[3] reported that the molecular ratios of histidine to lysine to arginine for normal nails were 1 · 6 · 13 while for arthritic nails they were 1 : 5 4 13 5 The three basic amino-acids remained practically constant, while the cystine content decreasd 18 4 per cent So it is fair to state that the cystine content in the hair may be influenced by the condition of health, and by disease, as well as through the diet. Observation on the cystine content of hair led to a postulation that the cystine content of hair may be looked on as a sign of nutritional status for children, or as an indication of the presence of some disease

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PHYSIOLOGY

Amounts of Oxides of Nitrogen and Carbon Monoxide in Cigarette Smoke, with and without Inhalation

RECENTLY, Haagen-Smit[1] has referred to the relatively high content of oxides of nitrogen in smoke from cigarettes and cigars It was found that the content of oxides of nitrogen in cigarette smoke varies from 145 to 655 p p m. Furthermore, after inhalation of the smoke into the lungs, no oxides of nitrogen could be detected in the exhaled smoke In view of the very low maximum concentrations of oxides of nitrogen tolerated (Russian workers[2] claim 0 1 mgm /m ^{3} as an average concentration during 24 hr , and 0 3 mgm /m ^{3} as a maximum concentration at one time , for carbon monoxide these concentrations are 2 and 6 mgm /m ^{3} respectively) it was thought interesting to determine how much of these compounds may be taken up as a result of cigarette smoking

Two types of experiments were carried out A smoking apparatus was developed in which normal smoking could be simulated It consists essentially of a spiral washer with xylene for the removal of tars, which may affect the reaction with the Griess-Ilosvay reagent, and of a sampling bottle, a gas meter and finally a magnetically operated valve The latter is connected, through a capillary, with the vacuum line Smoke from a cigarette was drawn in 27-ml. samples into the sampling bottle by opening and closing (2 and 28 sec respectively) the magnetically operated valve The natural smoking process was simulated to such a degree that 15 × 0 5 = 7 5 min were required to smoke a cigarette to a residual length of about 10 mm During this time about 400 ml gas was delivered into the sampling bottle.

In each sample the contents of nitrogen oxides and carbon monoxide were determined by normal analytical methods In a few cases a distinction was made between nitric oxide and nitrogen dioxide From Table 1 it appears that under our smoking conditions approximately equal amounts of nitric oxide and nitrogen dioxide are present The total amount of nitrogen oxides is within the range found by Haagen-Smit for different types of cigarettes.

Table 1 CONCENTRATIONS OF OXIDES OF NITROGEN AND CARBON MONOXIDE IN CIGARETTE SMOKE

(King-size cigarette, relative humidity 45 per cent)

Cigarette	$NO+NO_2$ (p p m)	NO_2 (p p m.)	CO (per cent by volume)	CO_2 (per cent by volume)
1	190			
2	170			
3	180			
4	210			
5	210			
6	180			
7	170			
8	190			
9	190	100	2 2	7 8
10	200	120	2 0	7 0
11	190	80	2 1	

Inhalation experiments were carried out by three separate persons. In each experiment a cigarette was smoked in such a way that 500 ml smoke + additional air were inhaled during each inhalation After exhalation into a 10-litre sampling bottle, the lungs were flushed with four times the amount of air, which was also passed into the sampling bottle. In addition, non-inhalation experiments were carried out by the same persons. The smoke of each pull was kept in the mouth, and then passed into the sampling bottle where it was diluted with air (inhaled through the nose) to a total volume of 2 5 l In both cases 13 × 2 5 = 32 5 l were passed through the bottle, after which the amounts of nitrogen oxides and carbon monoxide were determined

The figures given in Table 2 show that, as a result of inhalation, both the nitrogen oxides and the carbon monoxide are absorbed to a large extent Consequently the amount of these compounds which may be taken up by the body can readily be estimated If 20 cigarettes a day are smoked, 3 0 mgm of oxides of nitrogen (calculated as nitrogen dioxide) and 200 mgm of carbon monoxide may be absorbed These figures are considerably higher than the amounts,

Table 2 CONCENTRATIONS OF OXIDES OF NITROGEN AND OF CARBON MONOXIDE IN CIGARETTE SMOKE, WITH AND WITHOUT INHALATION

Person	CO (per cent by volume)		Absorbed per cent	Oxides of nitrogen $(NO + NO_2)$ (p p m)		Absorbed per cent
	Without inhalation	With inhalation		Without inhalation	With inhalation	
A	2 1*	0 37	82	180	23	87
B	2 1*	0 36	82	240	11	95
B				220	12	95
C	2 1*	0 27	87	180	8	96
C		0 37	82	220	10	96

*The concentrations have been converted to those in the undiluted smoke The dilution factor was calculated from the content of carbon monoxide in the 10-l sampling bottle and the average value of 2 1 per cent as determined from the experiments with the smoking apparatus namely 12 × 0·1 = 1·2 mgm. oxides of nitrogen and 12 × 2 = 24 mgm. of carbon monoxide, regarded as the maximum which can be safely tolerated.

Whether the effect of incidental inhalation of relatively high concentrations of noxious compounds is greater than that from continuous inhalation of a much lower concentration is difficult to establish. It should be remarked, however, that for an evaluation of the health effects of smoking the possibility of synergism[3], caused by the simultaneous presence of both nitrogen oxides and carbon monoxide, should also be taken into account.

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Sodium and Potassium Content in the Muscles of Hibernating Animals

It has long been known that tissues exposed to low temperatures *in vitro* lose potassium while on the other hand there are few published reports regarding sodium exchange during the same conditions[1,2]. In a previous report[3] we have demonstrated that the potassium/sodium ratio in the muscles of fishes caught in cold arctic waters is low when compared with fishes from boreal waters. The very same variations with temperature were found to take place in specimens of the same species both in Nature (*Gadus*) and in the laboratory (*Cottus*).

As we were at the same time working with problems on the physiology of hibernation we were led to suspect that the extreme hypothermia normally occurring during hibernation in these animals might induce even more marked changes in electrolyte composition of the muscle cells.

The animals used were the common European hedgehog, *Erinaceus europaeus* L., and the bat, *Myotis daubentonii* L. The muscle samples were dry-ashed at 550° C and the content of sodium and potassium estimated by flame-photometry.

In the case of the hibernating animals referred to, we found a rather surprisingly high content of sodium in the muscles, compared with that reported in the literature on other mammals, and also confirmed by my own analysis on the rat, Fig. 1. Furthermore, we found the sodium content to be fairly constant throughout the year, despite the extreme changes in body temperature taking place during this time.

Thus, when compared with other homoiothermic vertebrates having about the same plasma composition, the hibernators differ greatly. Whether or not this has something to do with differences in the physiology of the muscle cells, the present results do not demonstrate. It seems to us, however, that the hibernators, which behave much like poikilotherms in the winter, may somehow be prepared by a high sodium-level to stand the effect of low temperatures on the sodium content of the tissue.

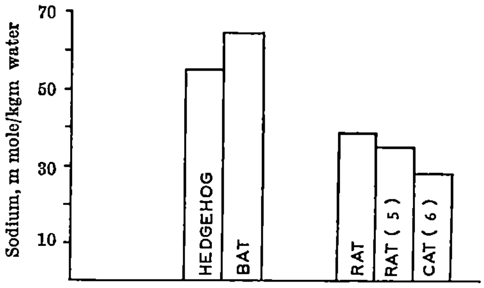

Fig. 1. Sodium content in the muscles of hibernating animals (hedgehog, bat) and some other mammals (refs. 5, 6)

It may be worth considering the same tendency found in arctic fishes, where a high sodium content was encountered at low temperatures. It thus seems that the ability to stand low temperatures may in some way or other be connected with the sodium metabolism of the cell.

Whereas the level of sodium is fairly constant throughout the year, we found distinct changes in the potassium content of the muscles. Muscle samples have been taken from hedgehogs in the period from May to March, while the samples from bats are from November to April only. As seen in Fig. 2, there is an increase in the potassium content of the hedgehog muscles during the first months of hibernation (October–December) and from then on a distinct decrease. In the months October–November the body temperature of the hedgehog varies greatly, changing from the normal level (35° C.) to environmental temperatures (5° – 8° C. in our animal room) in less than 24 hr. In this 'pre-hibernating' period some animals take food, while others are in manition, with no apparent effect on the sodium–potassium level. From about December the animals are in deep hibernation. As regards the bats their body temperatures change in about 10–30 min., and their period of manition begins about September and lasts until about May.

Contrary to the expectations we might have from our results obtained on fish muscles, we thus see that the hedgehog muscles gain potassium in the first part of the hypothermic period. It is rather difficult to explain this considering the temperature effect alone. However, we want to direct attention to certain observations made in this Laboratory[4] on changes in some blood constituents in the 'pre-hibernating' period. In this period we found a marked hæmolysis corresponding to one-third of the total erythrocyte volume. A tentative explanation might therefore be

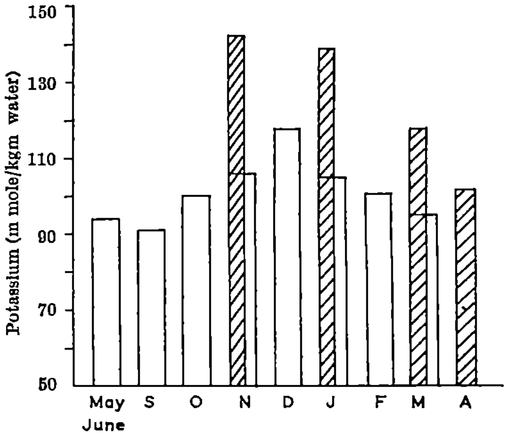

Fig. 2. Potassium content in the muscles of hedgehog and bat at different times of the year. □, hedgehog, ▨, bat

that part of the potassium set free during this hæmolysis is accumulated by the tissue cells Unfortunately we are at present unable to present enough data on the level of potassium in plasma Only small changes in this level are necessary, however, to induce an increase in cellular potassium, due to the effect of the Gibbs–Donnan equilibrium This explanation seems the more reasonable as we found the time of the year to be the important factor no differences in muscle potassium were observed between samples from animals with normal or hypothermic body temperature

The effect of hæmolysis in October–November seems to last until December This lag might be explained on the assumption that the extrusion of salts through the kidney is extremely slow in animals in deep hibernation With the progress of time (December–March) this slow process, however, leads to a reduction of cellular (and plasma) potassium

Regarding our results for bats, these are in accordance with such considerations

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Diuresis due to Stress in Cattle

In the course of investigation of some aspects of renal function in adult dairy cattle, it was noted that there was a marked diuretic response to a painful stimulus The animals used were non-lactating, non-pregnant Ayrshire dairy cows accustomed to experimental procedures, and the stimulus was puncture of the brachial artery by the technique described by Fisher[1], which, despite the use of local anæsthetic, always caused some discomfort Arterial puncture was carried out during continuous urine collection by an indwelling urethral catheter for renal clearance measurements In association with the increased rate of flow after arterial puncture, urine became noticeably paler in colour, and there was a marked fall in urinary bicarbonate concentration Cystometric measurement of bladder pressure showed that there was no variation in intracystic pressure at the onset of diuresis, and, on the basis of these observations, it was assumed that the increase in the rate of urine collection was a true reflexion of increased rate of formation, rather than the expulsion of uncollected urine from the bladder

The discomfort of arterial puncture, in some animals, caused an immediate diuresis of 200–300 per cent of the previous mean rate of urine flow The onset and regression of this response were usually rapid One cow, with a mean rate of urine flow of 9 6 ml /min, showed a diuresis of 28 5 ml /min in the 5 min following arterial puncture, the rate returning to 8 5 ml /min in the next 5 min period After repeated experiments on the same animals, this type of response was preceded by a diuresis associated with the preliminary procedure of clipping, swabbing, and anæsthetizing the site for puncture Fig 1 illustrates the diuresis which occurs on carrying out arterial puncture, preceded by the response to preparation of the site.

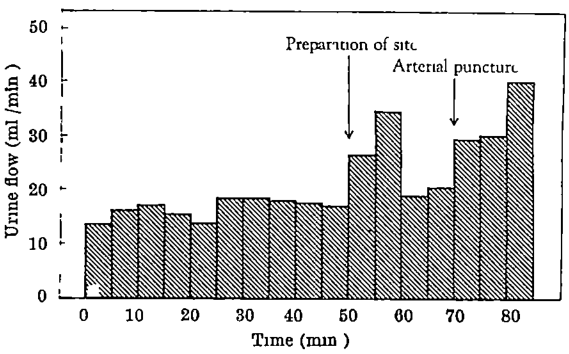

Fig 1 The effect of arterial puncture, and of clipping, swabbing, and anæsthetizing the site on the urine flow of a normal cow

Rydin and Verney[2] demonstrated a marked inhibition of urine flow of hydrated dogs following emotional stress, with good evidence that this response was due to the release of antidiuretic hormone Andersson and Persson[3], however, reported that, in hydrated goats, painful or emotional stimuli did not result in any alteration in the rate of urine formation In man, Miles and De Wardener[4] described an emotional diuresis which occurred on bladder catheterization, or resulted from apprehension of a surgical procedure They attributed the rise in salt excretion which accompanied this diuresis to three possible causes (1) a rise in glomerular filtration-rate, (2) the inhibition of a salt-retaining hormone, (3) a nervous inhibition of tubular function There are, therefore, marked species differences in renal responses to painful or emotional stimuli

The mean blood pressure of cows may rise markedly on experimental interference[5], but whether the observations reported here are the result of alterations in renal hæmodynamics or of inhibition of the antidiuretic hormone is not known It is, however, clear that experiments on cattle which depend on measurement of the rate of urine flow must be carried out with the minimum amount of stress to the subject

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Effects of Thymectomy on the Resistance of Rats to Drowning and Histamine Stress

The thymus has been proposed as a site of hormone production[1] Bomskov *et al*[2] have reported that treatment of normal rats and guinea pigs with calf thymus extract distinctly diminished survival-time of animals exposed to chloroform–air mixtures

The aim of the present work was to determine the degree of resistance conferred by thymectomy on albino male Wistar rats Two procedures were used, one involving resistance to drowning stress and the second resistance to histamine stress

Two hundred and forty male rats averaging 50 gm. were matched by weight and divided into three

Table 1. Effect of Thymectomy on Resistance to Histamine and Drowning Stress

Group	n	Initial body-weight gm. ± S.E.	Final body-weight gm. ± S.E.	LD_{50} mgm./kgm. ± S.E.	Drowning time (sec.) ± S.E.	Percentage difference between two groups	P value
1. Histamine stress							
Group *A* Thymectomy rats	60	52.8 ± 0.4	199.8 ± 2.4	702.9 ± 36.4		A B = + 6.8 A C = + 8.2	0.22 0.13
Group *B* Sham-thymectomy rats	60	52.5 ± 0.5	195.4 ± 2.7	658.4 ± 29.8			
Group *C* Normal control rats	60	52.5 ± 0.4	200.1 ± 2.3	649.4 ± 33.5		*B* C = + 1.4	0.76
2. Drowning stress							
Group *A* Thymectomy rats	19	49.2 ± 0.7	187.6 ± 3.2		541.8 ± 27.8	A B = + 5.1 A C = + 8.4	0.51 0.34
Group *B* Sham-thymectomy rats	19	49.1 ± 0.8	196.8 ± 4.1		515.6 ± 27.3		
Group *C* Normal control rats	20	49.1 ± 0.5	204.5 ± 3.4		499.7 ± 31.7	B C = 3.2	0.71

groups, *A*, *B* and *C*, representing respectively thymectomized rats, sham-operated animals and normal controls. The thymectomies were completed under open-ether anæsthesia according to procedures described by Farris and Griffith[3]. At the conclusion of a 4-week period, 60 animals in each group were selected and equal aliquots tested for histamine resistance. The histamine challenge occurred at four dose-levels, 450, 600, 750 and 900 mgm./kgm. of body-weight. Finney's method of probit analysis was used to calculate the *LD*50 values and dose-response lines[4].

The remaining animals were subjected to drowning stress by immersion in water precooled and maintained at 6° C. Drowning time was determined by recording the time-interval up to the final submergence.

Table 1 presents the *LD*50 results. Comparisons between groups *A* and *B* and between *A* and *C* *LD*50 values indicated that thymectomy induced 6.8 per cent and 8.2 per cent respective increases in the tolerance of the thymectomized rats to histamine stress. Although none of these increases was statistically significant, the low *P* values especially between *A* and *C* approach significance ($P = 0.13$).

Table 1 similarly presents the average time of drowning for the three groups of animals immersed in water maintained at 6° C. Again comparisons between groups *A* and *B* and between *A* and *C* indicate respectively 5.1 per cent and 8.4 per cent increases in the resistance of the thymectomized rats to drowning stress. As indicated by the *P* values, however, none of these increases was statistically significant.

In evaluating the results, it becomes apparent that thymectomy induced similar percentage increases in the resistance of the thymectomized rats to two distinctly diverse types of stress stimuli. The results of this work are thus in accord with previous investigations[2,5]. Although the investigation does not offer conclusive evidence concerning a thymic endocrine function, the results provide support for such a hypothesis.

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Plasma Iron and Iron-binding Capacity in the Pregnant Rat and Rabbit

In the latter half of human pregnancy a decrease in blood hæmoglobin concentration usually occurs and this is associated with a decrease in plasma iron concentration and an increase in plasma total iron-binding capacity[1,2]. The mechanism producing these changes is not fully understood. They may be due partly to iron deficiency[3,4], one of the main causes of which is the rapidly increasing fœtal utilization of iron. If so, the changes might be more pronounced in rats and rabbits, in which the combined demands of the fœtuses would be relatively greater.

Blood for the various determinations was taken from 7 rabbits weighing 1.8–2.5 kgm. immediately before and 7, 14, 21 and 28 days after mating and on the first day after parturition. Control values were obtained on blood from a comparable group of 8 non-pregnant animals. In these rabbits no significant changes occurred in any of the values measured

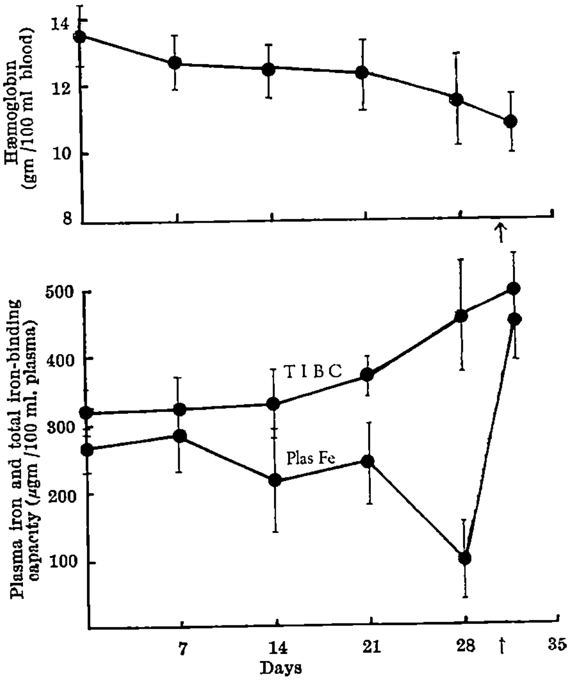

Fig. 1. Changes in blood hæmoglobin concentration, plasma iron concentration (Plas Fe) and total iron-binding capacity (T.I.B.C.) during pregnancy and after parturition in the rabbit. Each point represents the mean of seven rabbits and the vertical lines represent ∓ one standard deviation of the mean. The time of parturition is shown by the arrow.

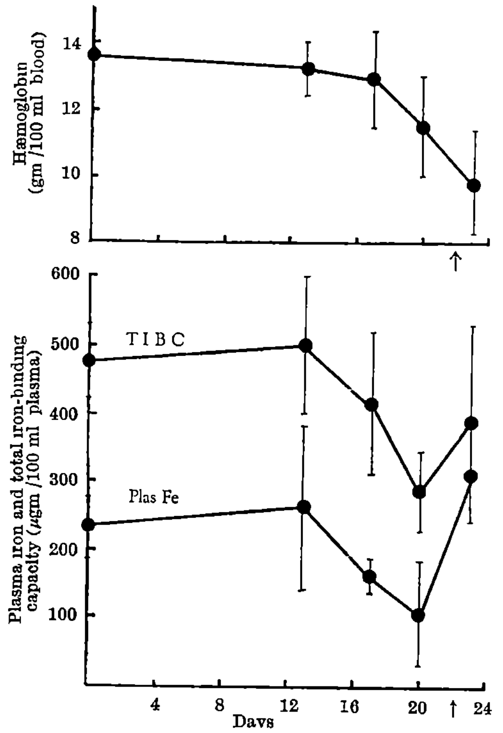

Fig 2 Changes in blood hæmoglobin concentration, plasma iron concentration (Plas Fe) and total iron-binding capacity (T I.B C) during pregnancy and after parturition in the rat Each point represents the mean of six rats and the vertical lines represent ∓ one standard deviation of the mean The time of parturition is shown by the arrow

Twenty-four rats aged 18–22 weeks and weighing 190–220 gm were mated and the day of insemination was determined by daily examination of vaginal smears for spermatozoa The rats were killed in groups of 6 on day 13, 17 and 20 of pregnancy and on the first day after parturition A further comparable group of 6 rats was not mated and determinations were made on these to provide non-pregnant control values The rats were anæsthetized with ether and killed by exsanguination by cardiac puncture

Blood hæmoglobin concentration[5], and plasma iron and total iron-binding capacity[6], were estimated by methods previously described

Both rabbits (Fig 1) and rats (Fig 2) resembled human beings in showing a decrease in blood hæmoglobin and plasma iron towards the end of pregnancy. Both rabbits and rats, however, showed a considerable increase in plasma iron concentration immediately after parturition, while in humans when such a rise occurs it is small[7] In contrast to rabbits and humans, rats showed a considerable decrease in plasma iron-binding capacity in the last few days of pregnancy and a marked rise immediately after parturition

The increase in plasma iron concentration immediately after parturition may be accounted for by the fact that loss of iron to the fœtus is suddenly interrupted Why plasma iron-binding capacity should fall during pregnancy in the rat and rise during pregnancy in man and the rabbit cannot be explained An increase in plasma volume occurs during pregnancy in the rat[8], and this could presumably cause some dilution of the iron-binding protein, transferrin But a similar change in plasma volume also occurs in man[9], where the iron-binding capacity increases during pregnancy[1,2] In the rat, then, it would seem that the decrease of iron-binding capacity could be better explained by alterations in the rates of synthesis and/or destruction of transferrin or by passage of this protein through the placenta or fœtal membranes

This work was carried out during tenure of a fellowship of the National Health and Medical Research Council I am grateful to Miss E Rudeberg for technical assistance

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Importance of Cell Division for Antibody Production in an *in vitro* System

An *in vitro* system for the study of antibody formation has been previously described[1]. In outline, cell suspensions are prepared from rabbit spleens removed two days after anamnestic stimulation with alum-precipitated human serum albumin The synthesis of antibody *in vitro* is measured by the incorporation of leucine labelled with carbon-14 into antibody protein This system has been used to determine whether cell division or cell differentiation plays a major part in providing the cell population which synthesizes antibody after anamnestic antigenic stimulation

The kinetics of antibody synthesis in earlier experiments and the partial inhibition of antibody formation by a thymidine antagonist, 5-bromodeoxyuridine[2], had suggested that 30–80 per cent of the antibody synthesized was made by cells which divided during the 48-hr. incubation period Autoradiographic studies showed that 2–4 per cent of the incubated cells did indeed take up tritiated thymidine during *in vitro* incubation (to be published), but did not, however, establish that these thymidine-incorporating cells synthesized antibody

The following experiment was designed to provide more definite proof. Tritiated thymidine of very high specific activity was added to the medium The dividing cells incorporated, and hence concentrated, the radioactive thymidine into their deoxyribonucleic acid (DNA), which thus resulted in a very selective irradiation of the dividing cells Under these conditions non-dividing cells would be expected to receive only insignificant irradiation The addition of tritiated thymidine at 1 5 c /m mole at the beginning of the incubation period inhibited antibody formation to approximately the same degree as 5-bromodeoxyuridine Higher specific activities (4 4 c /m mole) did not result in any appreciably greater inhibition Dilution of the specific activity of the tritiated thymidine by the additional presence of unlabelled thymidine reduced or prevented the inhibition (see Fig 1) The inhibition was, therefore, a consequence of an intracellular location of the radioactive thymidine and not simply an unspecific effect of irradiation

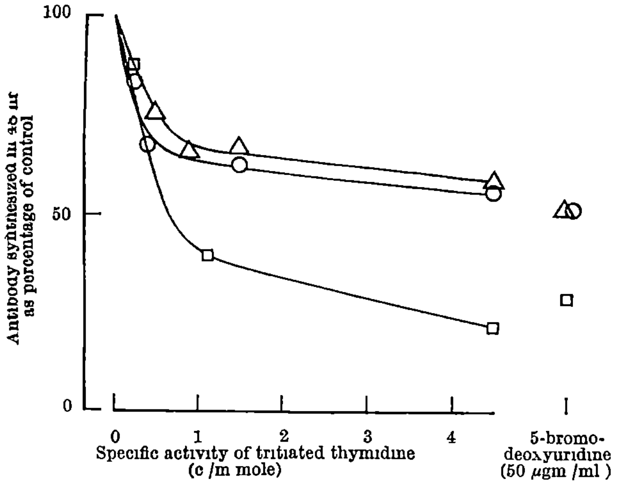

Fig 1 The effect of tritiated thymidine on antibody synthesis The three curves—O, Δ and □—are the results obtained with three separate rabbits The corresponding symbols at the right show the figures for antibody synthesis in the presence of 50 μgm /ml of 5-bromodeoxyuridine for each rabbit

from radioactivity in the extracellular medium A further control was provided by experiments using tritiated water It was found that more than a hundred times as much activity had to be added as tritiated water to produce the same effect as with tritiated thymidine (see Table 1).

That the effect was due to activity concentrated in a most favourable site is supported by a consideration of the calculated doses that the cells received on the basis of various suppositions as to the distribution of the activity (Table 1) By comparison, 200–500 rads of X-irradiation (given at the start of incubation) were required to reduce the antibody synthesis to 50 per cent (to be published) It is not possible to calculate a reliable estimate for the dose received by labelled cell nuclei (see footnote to Table 1) but the figures do provide some basis for comparison.

Table 1 Antibody Synthesis Comparison of Effect of Irradiation from Tritiated Water and Thymidine

	^{3}H water				^{3}H thymidine
Activity in incubation medium, mc /ml	59	5 9	0 59	0 059	0 024
Antibody synthesized in 48 hr as percentage of control	8	37	70	94	42
Dose to cells in rads/hr assuming uniform distribution throughout medium	710	71	7 1	0 71	0 29
Dose to nuclei, rads/hr (see footnote)	(*a*) Assuming synthesis of diploid content of DNA from thymidine at 1 1 c /m mole				*c* 170
	(*b*) Calculated from observed mean grain count over nuclei after autoradiography				*c* 50

In the calculation for the dose-rates to nuclei it has been supposed that the volume of the nucleus was 500 μ^3 and that all the energy was dissipated in this volume In the calculation from grain counts it has been assumed that the overall efficiency of grain development is 5 per cent No allowance has been made for the fact that the activity will not be present in the nucleus until the cell has divided For these and other reasons the figures arrived at can only be taken as a very rough guide to the actual levels of irradiation

Autoradiographs of smears of the cells which had been incubated with thymidine of high specific activity showed very high levels of activity in the nuclei of 2–3 per cent of the cells The rest of the cells showed less than a fiftieth of this activity

Autoradiographs of cells incubated with only tracer-levels (61 mc /m mole) of tritiated thymidine showed proportionately the same grain count per cell. Thus the high specific activity did not appear to affect the uptake of thymidine into the deoxyribonucleic acid (DNA).

There is, therefore, little doubt that the inhibition of antibody formation was due to irradiation from tritiated thymidine incorporated into the DNA of those cells which were dividing

These experiments do not show whether or not a cell can divide after it has started to make antibody In either case it seems likely that most, if not all, of the cells synthesizing antibody have undergone cell division following anamnestic stimulation

These results are thus in agreement with the recent findings of Urso and Makinodan[3] and Baney, Vazquez and Dixon[4], which were obtained by other techniques

This work was supported, in part, by U S Public Health Service grant *E* 2911

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PHARMACOLOGY

Effect of Derivatives of L-Cysteine on Growth of *Leuconostoc mesenteroides* (8042)

In the course of work on the chelating properties of certain amino-acid derivatives reaction products of cysteine with eight different aldehydes were prepared The compounds were probably derivatives of thiazolidine-4-carboxylic acid[1–3] They were prepared by previously described methods of synthesis or modifications of such methods[4–6] In Table 1 the compounds are named simply by adding the name of the aldehyde to cysteine

Table 1 Effect of Derivatives of L-Cysteine on Growth of *Leuconostoc mesenteroides* (8042)

μgm cysteine, equivalent per tube	0 1 *N* sodium hydroxide titre (counts) in a potentiometric titrator					
	0	2	4	6	8	10
Cysteine hydrochloride	13	30	50	67	85	95
Cystine	14	34	55	78	99	112
Cysteine-acetaldehyde	13	27	44	65	80	93
,, propylaldehyde	12	32	51	73	88	94
,, butyraldehyde	13	29	49	69	85	102
,, isobutyraldehyde	16	26	44	66	82	105
,, benzaldehyde	13	26	43	54	73	88
,, formaldehyde	11	19	33	47	64	76
,, arabinose	12	19	33	43	59	65
,, glucose	12	21	33	42	53	71

By the courtesy of O R Brækkan, head of the Government Vitamin Laboratory, Bergen, Norway, I was given the opportunity to have the compounds tested as cysteine sources for *Leuconostoc mesenteroides* (8042) by the method described by G D. Shockman *et al* [7] This organism is widely used for the microbiological determination of both cysteine and methionine The results of one test are given in Table 1

It is seen that the compounds fall into two distinct groups The reaction products of cysteine with acet-, propyl-, butyr- and isobutyr-aldehydes were equally well utilized as were cysteine hydrochloride and cystine, while the products formed with formaldehyde, arabinose and glucose were less well utilized

Cysteine-benzaldehyde was intermediate between these groups The results were confirmed for cystine, cysteine hydrochloride, cysteine-formaldehyde, cysteine-butyraldehyde and cysteine-glucose using the same method, and for all the substances listed in Table 1 by a turbidimetric technique In the latter test the concentrations used were doubled as compared with those used in Table 1

These results probably have some bearing on the problem of determining cysteine in the presence of glucose[8–10], and may partly explain why Shockman *et al* [7] get better results in microbiological cysteine (cystine) determinations when they autoclave the glucose separately before adding it to the sterile medium Cysteine-glucose is readily formed in aqueous solutions[6,11] The present results indicate that other aldehydes may probably also influence the determination of cysteine

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Second-Generation Toxicity of Malathion in Rats

IN a search for evidence of chronic toxicity of malathion, a breeding experiment was performed with rats under continuous exposure of all old and young animals to this insecticide Malathion is an organophosphorus compound and is regarded as a relatively safe member of the anticholinesterases[1,2] so that it is widely used not only in agriculture but also in home gardening

The experimental animals were Wistar rats bred for many years in this laboratory The control rats received ground Purina Fox chow (Ralston Purina Company of Canada, Ltd , Woodstock, Ontario) The experimental animals were given the same food thoroughly mixed with malathion (technical grade, 95 per cent) purchased from the Niagara Brand Spray Co , Burlington, Ontario In preparing the poisoned diet, 9 ml of malathion were dissolved in 45 ml of corn oil and gradually added in a mechanical mixer to 2 65 kgm of the chow, there was thus 4,000 mgm of malathion/kgm of chow and the daily intake approximated 240 mgm of malathion/kgm of rat

The experiment began with rats aged between 70 and 100 days The colony consisted of 160 rats, equally divided between controls and experimental animals, there were thus 40 males and 40 females receiving malathion These rats have now been fed for 5 months They showed a normal rate of growth and at no time showed any sign of intoxication

Ten weeks after the start of the experiment, eighteen females of the controls and eighteen females of the treated group were selected at random for breeding and were isolated from the main colony, twelve males were also taken from each group The mating cages contained three females and one male, the male stayed in the cage for one week and was then replaced by another male that stayed for the same period of time The outcome of this experiment is shown in tabular form (Table 1) Two of the treated rats had only four young each, a rather unusual number for the breed of this laboratory The average litter sizes and their standard errors tended to be different between the groups, but they were statistically not distinguishable

Table 1 RESULTS OF A BREEDING EXPERIMENT IN RATS TREATED CONTINUOUSLY WITH MALATHION MIXED INTO THE FOOD

	Control	Treated	χ^2	P
No of females mated	18	18		
No of females conceived	13	14		
No of new-born	116	107		
Average litter size ± *S E*	10 5±0 47	8 9±0 68		~ 0 07
No of new-born alive after 7 days	105	56	38 54	< 0 001
No of new-born alive at weaning (21 days)	75	34	22 78	< 0 001

With the purpose of conducting breeding experiments of the second generation, all treated animals that weighed less than 30 gm at the time of weaning were discarded This left 26 of 34 surviving animals which were then matched by 26 control animals of approximately equal weight The average weight of both groups was thus close to 44 gm Nine weeks after birth, the average body-weights of the controls was 152 gm , those of the treated rats 136 gm This evidence for the retardation of growth in the treated group is significant at the 1 per cent level

The general state of health of the rat colony was good, since no deaths have occurred among the adult rats since the start of the experiment However, a number of the young rats in the breeding experiment had ring-tail disease A count at weaning time showed that 18 of 75 (24 per cent) of the control rats but 16 of 34 (47 per cent) of the treated rats had this disease These percentages are different, the statistical significance being at the 5 per cent level

This could mean that the drug has merely sensitized the animals to succumb to infection or other noxious influences In any event, the results are not necessarily a direct consequence of cholinesterase inhibition (1) the pseudocholinesterase activity of the plasma of the chronically treated rats was tested and found to be slightly elevated, this agrees with the finding that an accumulation of cholinesters induces cholinesterase formation in tissue culture[3] (2) Malathion is never chemically pure and the purest preparations tend to be the least dangerous in acute toxicity work[4]

Two recent comprehensive reviews[1,2] make no reference to breeding experiments under exposure to organophosphorus compounds The only comparable work reported in the literature seems to be that by Barnes and Denz[5], who found a high rate of death in the third generation of rats constantly exposed to *E* 605 (parathion) The recent observation of a mutagenic effect of organophosphorus compounds in *Drosophila melanogaster* deserves notice[6] Depression of rate of growth by malathion has been seen in chicks[7] There is also some evidence for the inhibition of growth by antagonists of acetylcholine atropine, procaine, and tetraethylammonium prevent the regeneration of the forelimb in newts[8], and an effect of nicotine might be responsible for the decreased size of the babies of mothers who smoke[9]

Even with the present widespread use of insecticides, most human beings are exposed to not more than traces of these agents, while large doses were used in this work on rats, and even more so in the investigation on *Drosophila* quoted[6] Nevertheless, it would appear that broad investigations of growing cells and insecticides are to be encouraged

This work was supported in part by a grant 605–7–146 from the Ontario Department of Health

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5-Hydroxy-3-indoleacetamidine: a New Type of Blocking Agent against 5-Hydroxytryptamine

The possible role of 5-hydroxytryptamine (5-HT) regarding its action on the mammalian nervous system has been discussed repeatedly However, pharmacological investigations were not very successful in clarifying this role The well-known approach of using 'specific' antagonists against the neural effects of 5-HT has been stranded on two grounds (*a*) the commonly known blocking agents of 5-HT are in fact only antagonists of 5-HT on smooth muscles, and (*b*) the actions of 5-HT on nervous receptors, except for a few peripheral sensory and ganglionic receptors, are not adequately explored Recent investigations[1] demonstrate that even those agents which were shown before to block certain hypothetical nervous receptors of the guinea pig ileum[2] are of fairly low potency and/or selectivity to the ganglionic stimulant action of 5-HT The tachyphylaxis observed in the ganglionic action of 5-HT suggested the use of compounds structurally closely related to 5-HT as its possible antagonists One of these compounds, 5-hydroxy-3-indoleacetamidine (5-HTA), which was first described as a 5-HT-like stimulant agent[3], showed the following unique characteristics

(1) In accordance with the data of Woolley and Shaw[3] 5-HTA was found to have pressor action on the nembutalized dog It also stimulated the respiration markedly after intravenous injection of 10–20 μgm /kgm Eighty μgm /kgm intravenously blocked the pressor, reflex-bradycardic and respiratory stimulant actions of 5-HT for a few minutes 5-HT itself, up to 160 μgm /kgm , was unable to influence significantly the actions elicited by 20–40 μgm /kgm 5-HTA

(2) A stimulant action of 5-HTA was found on the pelvic nerve-bladder preparation of the dog, but this action only partly resembled that of 5-HT. Given in doses of 1–4 μgm /kgm intra-arterially 5-HTA evoked a twitch-like rapid contraction. 5-HT in the same dose-range produced a twitch-like reaction at first, followed by a slow contractile second phase[4] The action of 5-HTA and the first phase of the 5-HT action were not inhibited by 0 2 mgm /kgm bromo-lysergic acid diethylamine Morphine, which is known to block nervous receptors sensitive to 5-HT (refs 2, 4, 5), was found to be effective against 5-HT and 5-HTA in doses of 1–2 mgm /kgm No blockage against 5-HT has been observed on the bladder with the small doses of 5-HTA (1–4 μgm / kgm) used

(3) On the isolated rat uterus 5-HTA up to 0 5 μgm /ml did not show stimulatory action, whereas 0 01 μgm /ml 5-HT stimulated the uterus 5-HTA in the concentration mentioned diminished the action of 5-HT only slightly (dose-ratio 4)

(4) Intra-arterial injections of 5–20 μgm. 5-HT and 5-HTA produced contractions of the cat nictitating membrane ('Nembutal' anæsthesia) With temporary occlusion of the external carotid artery, the threshold dose of 5-HT was lowered 2–3 times With this condition the stimulation of the membrane is mainly produced indirectly by stimulating the superior cervical ganglion[5] In contrast, the threshold dose for 5-HTA was rather increased by occlusion, indicating that its stimulant action was chiefly a direct effect on the nictitating membrane 4–20 μgm 5-HTA considerably diminished the ganglionic stimulation produced by 5–10 μgm 5-HT (occluded external carotid artery). The same doses of 5-HTA were less or not effective against the direct stimulatory action of 5-HT on the nictitating membrane (open external carotid artery)

(5) On the inferior mesenteric ganglion preparation of the cat[6] no change or slight increase of the spontaneous post-ganglionic action potentials has been observed by intra-arterial injections of 1–20 μgm 5-HTA The ganglionic stimulant action of 2 5–10 μgm 5-HT, however, was reduced by 1 μgm and inhibited by 5–20 μgm 5-HTA given 30 sec and 4 min before 5-HT The stimulating action of 1,1-dimethyl. 4-phenyl-piperazinium iodide was unaltered by the same doses of 5-HTA

5-HTA is the first compound which shows considerable antagonism against the ganglionic actions of 5-HT Whereas it has stimulant properties on certain organs (urinary bladder, nictitating membrane) it usually lacks stimulant effects on the superior cervical and inferior mesenteric ganglia in the dose-range used So far as its relative potency is concerned, 5-HTA is at least twenty times as potent as either LSD or bromo-LSD, cocaine and morphine against the ganglionic stimulant action of 5-HT It is also more potent than bromo-LSD in preventing the reflex respiratory stimulant action of 5-HT The blocking action of 5-HTA to 5-HT on the rat uterus is weak, inferior to that of LSD, bromo-LSD, chlorpromazine and of many other 'smooth muscle' type 5-HT antagonists The compound seems to have a high degree of selectivity to certain nervous receptors sensitive to 5-HT

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HISTOCHEMISTRY

Variation in Lyoglycogen in Starved and Normal Guinea Pigs

It has been shown that the histochemical staining reaction for tissue glycogen depends on the amount of lyoglycogen present in the tissues[1]. The present communication is a preliminary report of the nature of lyoglycogen. Lyoglycogen was obtained from the liver of six guinea pigs, two of which had been starved for 72 hr. and others that had had a normal diet. Hydrolysis of the resultant samples of lyoglycogen was carried out by refluxing with $N/1$ sulphuric acid at 100° C. for 8 hr. The acid solution was neutralized with barium carbonate, filtered and concentrated *in vacuo*. The solutions were applied to Whatman 3 *MM* papers which were developed in *n*-propanol–ethylacetate–water for 15–18 hr. Locating-reagents used were aniline-diphenylamine and silver nitrate.

Glycogen will yield glucose and small quantities of isomaltose on hydrolysis; sometimes other oligosaccharides are present due to acid reversion. The presence of isomaltose in hydrolysates of glycogen is taken to indicate 1–6 linkages in that molecule.

Fig. 1 shows a typical chromatogram. Samples of glycogen from the animals that were well fed yielded glucose and isomaltose in the hydrolysate. Although no quantitative estimates of the amount of isomaltose present in the glycogen hydrolysates from the well-fed animals were made, some indication of the amount of isomaltose present compared with the amount of glucose present may be given by the depth of staining on the chromatogram. The yield of isomaltose from these samples of glycogen seems to be far in excess of the expected quantity or of any that could be produced by acid reversion, consequently the number of 1–6 linkages in the parent glycogen would be greatly increased and may be identifiable with that part of the glycogen molecule or separate polysaccharide that Rosenfeld[2] described as having 33–47 per cent of 1–6 linkages.

The glycogen from the starved animals yielded only glucose. The absence of isomaltose in the hydrolysate obtained from the starved animals suggests a basic difference in the structure of the glycogen molecule in these conditions in that 1–6 linkages are absent.

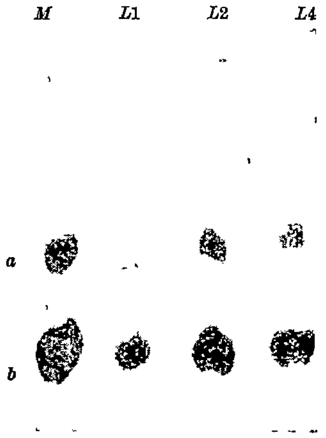

Fig. 1

Fig. 1. *M*, marker; *a*, isomaltose; *b*, glucose; *L1*, glycogen hydrolysate from starved animal (liver glycogen 242 μgm./100 mgm. liver wet weight). *L2*, *L4*, glycogen hydrolysate from fully fed animals (*L2*, liver glycogen 1,130 μgm.; *L4*, liver glycogen 1,012 μgm./100 mgm. liver wet weight). Note absence of isomaltose in *L1* and excessive amounts of isomaltose in *L2* and *L4*

From this preliminary work on glycogen that can be detected histochemically, it seems that the nutritional state of the animal has some influence on the structure of the glycogen molecule.

We thank Dr. W. J. Wheelan for his valuable suggestions and his gift of the disaccharide isomaltose.

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HÆMATOLOGY

Specific Antigens of Human Leucocytes and Thrombocytes

Attempts have been made to classify the specific antigens of human white blood cells and platelets, by using immune sera prepared in rabbits[1,2], sera from pregnant multiparous women[3,4] or from patients receiving multiple transfusions[5,6]. The different methods used by the various authors led often to inconsistent results.

In the present work immune sera from rabbits, monkeys and multiparous pregnant women have been used and compared using different serological methods.

Results obtained with rabbit anti-human leucocyte and thrombocyte sera showed the presence of common antigenic constituents of human erythrocytes, leucocytes and thrombocytes. These species specific antibodies of low titre (1/20–1/80) could easily be removed through appropriate absorptions. Common antigenic properties of thrombocytes and leucocytes of various species could also be observed. These antibodies, which were cell-specific for man, rabbit, mouse, sheep, guinea pig and rat, could be absorbed easily.

The titres obtained in rabbits against human leucocytes and thrombocytes were 1/640–1/1,280. The anti-leucocyte sera prepared against the leucocytes of a single donor reacted with leucocyte samples from every other human donor although with varying intensity, which may be due to differences in the agglutinability of the cells, with no correlation to their antigenic structure. The absorption of the serum with cells of any donor abolished completely the titre against all other donors. Similar results were obtained with thrombocytes.

The immune rabbit sera reacted strongly using the direct agglutination, agar-gel precipitation and tanned cell hæmagglutination tests. No correlation has been found between the intensity of agglutination of the leucocytes and the thrombocytes of the same donor.

The reactions obtained with the monkey anti-human leucocyte and anti-thrombocyte sera were similar to those obtained with the rabbit sera using the direct agglutination technique. The monkey antisera gave weaker reactions with the agar precipitation and the tanned cell hæmagglutination techniques.

The results obtained with sera of multiparous pregnant women differed significantly from those obtained with the animal anti-sera. Only direct agglutination could be used Agar-gel diffusion was negative and tanned-cell hæmagglutination weakly positive Sera of five hundred women during the second half of their pregnancy were examined All women had been pregnant between five and fifteen times Fifty sera (10 per cent) were found to contain leuco-agglutinins in a titre of at least 1/10 Two sera were found to contain thrombo-agglutinins of a similar titre These positive sera were examined each against leucocyte and thrombocyte samples obtained from thirty-six different blood donors[7] Only compatible blood donors were used, most of them being of group *O*

Table 1 summarizes the results obtained with fourteen different leucocyte samples when examined against ten different human anti-leucocyte sera It can be seen that some of the sera as well as some of the cell samples show antigenic similarity This similarity was especially well pronounced when whole families were investigated

Table 1 AGGLUTINATION OF LEUCOCYTE SAMPLES BY TEN DIFFERENT HUMAN ANTI-LEUCOCYTE SERA

Donors of leucocytes	Human anti-leucocyte serum No I	II	III	IV	V	VI	VII	VIII	IX	X
1	+	−	+	−	−	−	+	+	−	+
2	+	−	+	+	−	+	+	+	−	+
3	+	−	+	−	−	+	+	+	−	−
4	+	−	+	−	−	−	+	+	−	+
5	+	−	+	−	−	+	+	+	−	+
6	+	−	+	−	−	+	+	+	−	−
7	+	−	+	+	−	−	+	−	−	+
8	+	+	+	+	+	+	+	+	+	−
9	+	−	+	+	−	−	−	−	−	+
10	−	+	−	+	+	+	−	−	−	+
11	+	+	+	+	+	+	+	+	+	+
12	+	−	−	+	−	+	+	+	−	+
13	+	+	−	+	−	+	+	+	+	−
14	+	−	−	−	+	+	+	+	+	−

In Table 1 donors Nos 1, 2, 3, 4 and 5 are brothers No 6 is the father and No 7 is the mother Nos 13 and 14 are also brothers of another family

This communication describes part of the work for a Ph D thesis carried out by Mrs Gilboa-Garber at the Hebrew University, Jerusalem, and was supported in part by a grant from the Leukemia Research Foundation, Chicago

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PATHOLOGY

Cultivation of Monolayer Cultures of Frog Renal Cells

CONSIDERABLE evidence has been accumulated by Lucké[1], Duryee[2], Rose[3], Tweedell[4], and others for an agent which induces an organ-specific adenocarcinoma in the kidney of the leopard frog, *Rana pipiens* Lucké[1] has postulated that this agent is probably of a viral nature Although a percentage of the animals develop palpable renal tumours after a long and variable period of time following injection of tumour material, it is still desirable to devise other methods to propagate and possibly isolate the infective agent At the same time, the large dimensions of the frog cells make them an ideal system with which to study the initiation of any subcellular modifications following incubation of cells with the tumour-inducing agent

A procedure has been developed to provide an adequate *in vitro* host system Adult frog kidneys, normally relatively sparse, slow-growing tissues in culture[5], are treated with trypsin by a method modified from that of Youngner[6] and Bodian[7], both using mammalian cells The resulting cell suspension produces extensive cell sheets

The animals, after being pithed, are perfused with calcium-free amphibian Ringer's solution (sodium chloride, 0 66 gm , potassium chloride, 0 015 gm , dextrose, 0 02 gm , phenol red, 1 00 mgm , penicillin, about 500 units per ml , double glass-distilled water, 100 ml. and sufficient 0 15 *M* sodium bicarbonate to make a final *p*H of 7 6) through the still-beating hearts of the animals to rid the kidneys of a majority of the trapped red blood cells by the resulting dilution of the blood The organs are then dissected, minced, and washed twice in calcium-free amphibian Ringer's solution at room temperature To ensure proper digestion of the kidneys, the organs are kept for 24 hr , or overnight, at 2°–4° C in a fresh 0 25 per cent trypsin (Difco 1 250) solution in calcium-free Ringer s solution (*p*H 7 8) after 1 hr exposure to the trypsin solution at room temperature before cooling

The treated tissue, following the refrigeration period, is returned to room temperature and placed in a fresh trypsin solution (about 30 ml per kidney) and is stirred magnetically for 2–3 hr. The cell suspension thus obtained is centrifuged for 5 min at 1,000*g*, the supernatant trypsin medium discarded and replaced with calcium-free amphibian Ringer's solution, 1 ml. for each kidney. The suspension usually contains approximately 10^6 cells per ml per kidney Culture dishes are seeded with a final concentration of 500,000 cells per ml of culture medium The culture dishes then are sealed air-tight The composition of the culture medium is nutrient medium NCTC 109, diluted by adding 54 ml of double-distilled water with 100 ml of NCTC 109, 10 ml , calf serum, 2 0 ml , chick embryo extract, 2 0 ml , 5 per cent lactalbumin hydrolysate in amphibian Ringer's solution, 1 0 ml , glutamine (100 m*M*), 0 2 ml , penicillin, about 500 units per ml of medium

Extensive cell sheets form within two to three days when the cultures are kept at 25° C The cultures then are washed and fed with the above culture medium without lactalbumin hydrolysate The monolayer sheets have been maintained, by feeding every three or four days, in good condition for several months Attempts are now being made to derive a cell line from these frog kidney cultures

Successful eye implants have been obtained from the cultured cell sheets which previously had been incubated with a crude extract of frog renal tumour, with resulting growth of the implant in the intraocular region This preliminary evidence suggests that cellular transformations may have taken place, since normal kidney tissue implanted in the eyes of frogs seldom, if ever, exhibits any growth

This investigation was supported in part by a Public Health Service post-doctoral research fellow-

ship, *CF*–12,861, from the National Institutes of Health, U S Public Health Service.

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Acylase Activity of Ascites Tumour Cells on Dichloroacetyl Derivatives of Amino-acids

WHILE investigating the specificity of acylase activity of soil bacteria on acyl derivatives of amino-acids[1–3], we noted that the acetone powder of Ehrlich ascites carcinoma cells was able to hydrolyse dichloroacetyl-DL-aspartic acid but not (or only to a small extent) the dichloroacetyl derivatives of some amino-acids such as DL-glutamic acid, DL-serine. DL-methionine and DL-phenylalanine[4]

We have now investigated the acylase activity of lyophilized powder of ascites tumour cells such as Ehrlich ascites carcinoma, sarcoma 180, leukæmia *SN* 36, ascites hepatoma *MH* 134 and *MH* 129P in *C3H* mice, and *AH* 7974, *AH* 130, and Yoshida sarcoma in rats on dichloroacetyl derivatives of the following twelve amino-acids DL-aspartic and DL-glutamic acids, glycine, DL-alanine, DL-valine, DL-leucine, DL-phenylalanine, DL-tryptophan, DL-serine, DL-threonine, DL-methionine, and ε-*N*-benzoyl-DL-lysine

Lyophilized powder of ascites tumour cells was prepared as follows intraperitoneally transplanted ascites tumour cells were aspirated at the fifth day after transplantation, centrifuged at 1,000 r p m for 10 min washed three times with phosphate buffer solution, and then lyophilized It was confirmed that the cells contained almost 85–95 per cent tumour cells

Five mgm of lyophilized powder of ascites tumour cells were added to 1 c c of neutralized 0 1 *M* substrate and 1 c c of *tris* buffer at *p*H 8 2, and the mixture was incubated at 37° C for 2 or 18 hr in the presence of toluene, and then titrated by Grassmann–Heyde's method

The results obtained in the experiments carried out, under comparable conditions, on the acylase activity of lyophilized powder of eight ascites tumours are presented in Table 1 Ehrlich carcinoma, sarcoma 180 and leukæmia *SH* 36 were all able to hydrolyse dichloroacetyl-DL-aspartic acid but not (or only to a small extent) the dichloroacetyl derivatives of the other eleven amino-acids It is of interest that *MH* 134, *MH* 129*P*, *AH* 7974, *AH* 130, and Yoshida sarcoma differed from Ehrlich carcinoma, sarcoma 180, and leukæmia *SN* 36 in failing to hydrolyse dichloroacetyl-DL-aspartic acid Incidentally, *MH* 134, *MH* 129P, *AH* 7974 and *AH* 130 were hepatoma The low acylase activities of *AH* 7974 and *AH* 130 seem worth noting when considered together with the fact that the rat liver could hydrolyse dichloroacetyl-DL-aspartic acid[5]

Thus, our experiment showed that there is a significant difference among ascites tumours in their capability of hydrolysing dichloroacetyl derivatives of amino-acids, and that acylase activity may be available as a tool for studying biochemical specificity of living material[1–3]

We thank Dr S Tatsuoka and Dr T Kaziwara, of Takeda Research Laboratories, for kindly supplying lyophilized powder of ascites tumour cells

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BIOLOGY

Chloride Regulation in *Triops*

LITTLE physiological work on the branchiopods has been reported because of their relative scarcity and the unpredictability of supplies of animals Among the freshwater species Krogh[1] reports on some preliminary experiments with *Branchipus* and *Triops* (*Apus*), Panikkar[2] gives some figures for the blood concentration of *Chirocephalus*, and Fritzche[3] gives a blood concentration for *Daphnia*

An opportunity arose for me to use a few specimens of *Triops cancriformis* (Bosc) bred in laboratory conditions by Mr A R Longhurst The level of

Table 1 ACYLASE ACTIVITY OF ASCITES TUMOUR CELLS TOWARD *N*-DICHLOROACETYL AMINO-ACIDS

	Percentage of hydrolysed substrate for 2 and 18 hr at 37° C															
	Ehrlich carcinoma		Sarcoma 180		Leukæmia *SN* 36		*MH* 134		*MH* 129*P*		*AH* 7974		*AH* 130		Yoshida sarcoma	
Substrate	2	18	2	18	2	18	2	18	2	18	2	18	2	18	2	18
Dichloroacetyl-DL-aspartic acid	27	56	28	55	22	52	0	7	0	3	1	3	2	3	1	2
Dichloroacetyl-DL-glutamic acid	0	1	0	0	0	1	1	0	0	0	0	2	2	2	0	0
Dichloroacetylglycine	0	3	2	3	0	1	1	3	0	1	2	3	0	1	1	2
Dichloroacetyl-DL-alanine	1	4	1	4	0	2	1	5	0	1	2	2	2	2	1	4
Dichloroacetyl-DL-valine	0	2	0	3	0	0	0	2	1	3	1	2	1	2	1	1
Dichloroacetyl-DL-leucine	0	2	0	3	1	2	1	4	0	3	2	2	1	0	0	2
Dichloroacetyl-DL-phenylalanine	0	2	0	4	1	1	0	1	1	1	0	1	2	6	2	3
Dichloroacetyl-DL-tryptophan	0	1	0	1	0	0	1	0	0	2	0	1	1	5	2	3
Dichloroacetyl-DL-serine	0	2	1	2	0	1	0	2	0	1	2	3	1	3	0	2
Dichloroacetyl-DL-threonine	2	2	0	2	0	1	0	0	0	0	0	1	2	2	0	1
Dichloroacetyl-DL-methionine	0	3	1	2	0	3	0	4	0	0	2	1	1	2	1	2
α-*N*-Dichloroacetyl-ε-*N*-benzoyl-DL-lysine	0	2	0	1	1	1	0	0	0	0	1	2	1	0	0	1

The digests consist of 5 mgm of lyophilized sample, 1 c c of neutralized 0 1 *M* substrate, and 1 c c. of *tris* buffer at *p*H 8 2 at 37° C. Hydrolytic activity was measured by Grassmann–Heyde's alcoholic titration method.

blood concentration was measured and some simple experiments were made to determine the degree of osmoregulation which the animals could achieve Whole blood was withdrawn from the large dorsal sinus with soda-glass cannulæ and the chloride concentration of measured volumes determined by a Conway microdiffusion method[4] using Copenhagen sea-water as a standard.

The estimate for blood chloride concentration for six fed animals kept in filtered lake water (4 8 m moles chloride/kgm water) was 42·7 ± 5 4 m moles chloride/kgm water (range, 34 8–48 4) If chloride represents about 80 per cent of the anion concentration, the figure for total concentration would be about 55 m equiv /kgm water. It is clear from this estimate that *Triops* can maintain a blood concentration appreciably higher than the medium, and can be compared with some of the other entomostracan crustaceans in fresh water (Table 1)

Table 1

Animal	Blood conc m equiv /l	Method of estimation
Branchipus (ref 1)	30	Vapour pressure
Chirocephalus (ref 2)	77	Vapour pressure
Daphnia (ref 3)	68	Freezing-point
Triops	55	Chloride

In *Branchipus* and *Chirocephalus*, the ability to maintain this concentration against the osmotic gradient was dependent on salt uptake from the food, since starved animals quickly lost control To test the effects of starvation on osmoregulation, some specimens of *Triops* were put into a large volume of distilled water (40 l) The animals were sampled at intervals and the distilled water changed twice daily for 6 days The blood chloride concentration remained high even after 6 days in distilled water, except for two moulting animals (Fig 1) Within 4 days two other animals had moulted and died After 6 days, one of the two survivors was moulting and the other in a poor condition, although its blood concentration was still high

A further experiment was made in which some specimens were put into a sea-water dilution hyperosmotic (68 m eq /kgm water) to the blood It is clear that these animals have shown a very different reaction to this reversed osmotic gradient (Fig 1) After only 3 hr, the blood concentration rose to equivalence with the medium, and remained at this level for 3 days without any indication of a subsequent control At the end of this time the animals began to lose tone

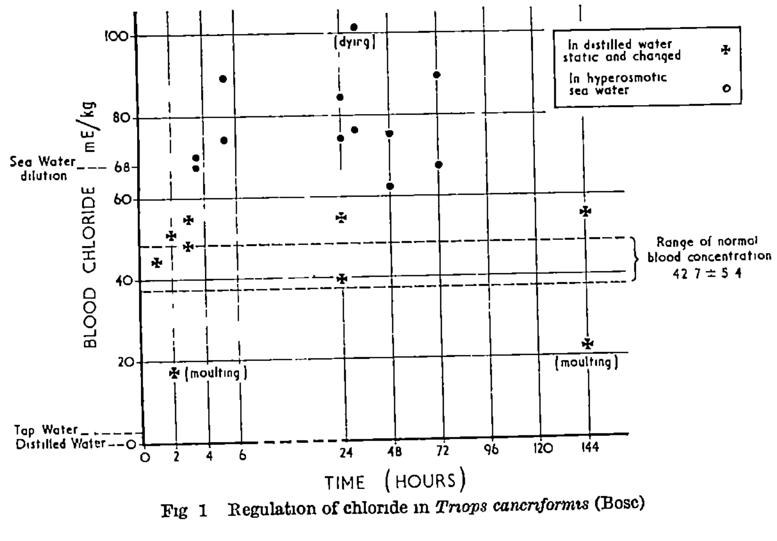

Fig 1 Regulation of chloride in *Triops cancriformis* (Bosc)

It can be concluded that in its normal environment, *Triops* maintains its blood more concentrated than the medium by excluding the osmotic inflow of water, that is. it is relatively impermeable, and that its salt requirements are met by food intake In a slightly hyperosmotic medium, however, it is unable to control either the influx of salt or the osmotic efflux of water

Moulting. and the associated breakdown of cuticle impermeability, seriously impair the mechanism of osmotic control so that animals in this condition quickly succumbed to an osmotic stress

It would be of interest to know more about the physiology of osmoregulation in this species especially in relation to the function of the renal organ This appears to be a large and complex structure, and perhaps one might predict that the animal has an efficient mechanism for salt resorption

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Effect of Feeding Griseofulvin on Rate of Growth of Chicks

GRISEOFULVIN, originally isolated from *Penicillium griseofulvum* by Oxford[1] *et al* in 1939, is produced commercially by the fermentation of three *Penicillium* species. *griseofulvum* Dierck X, *janczewskii* and *patulum*. Brian[2] *et al* first described its antifungal properties in 1946, but another twelve years elapsed before Gentles[3] demonstrated its efficacy against *Microsporum canis* and *Trichophyton mentagrophytes* when administered orally to infected guinea pigs

This discovery greatly stimulated interest in the clinical use of griseofulvin and many trials against a variety of dermatophytes have since been carried out[4]

Work on toxicity indicates that large doses injected intravenously in rats cause a transient depression of mitosis while intraperitoneal administration severely damages the seminal epithelium[5], but when taken orally, the toxicity of griseofulvin is low[6,7] In view of the low oral toxicity and in the absence of any published reports on this aspect, it was decided to investigate the influence of griseofulvin in the diet on the rate of growth of chicks

Four groups, each of 26 day-old broiler type cockerels, were used, two groups were fed commercial broiler crumbs, containing 5 mgm procaine penicillin/lb, and the other two received the same basal diet supplemented with 5 mgm griseofulvin/lb On arrival the newly hatched chicks were distributed among the groups according to weight so that the average initial weights were constant, 40 gm in this case. Rearing was carried out in wire-floored tier brooders and the chicks were weighed weekly over a period of four weeks. For the analysis of the results, regression coefficients of logarithm weight on time, based on the two weekly group means for each diet, were calculated and the significance of the difference between the regressions tested by means of a *t*-test in the usual way.

Table 1 AVERAGE WEIGHTS OF CHICKS (GM.)

Diets	Beginning of week					Regression coefficients, diets on weeks
	1	2	3	4	5	
Control + griseofulvin	40·0	86·2	175·7	305·3	467·7	0·2693
Control	40·0	85·3	169·8	291·4	447·9	0·2636
Difference	—	0·9	5·9	13·9	19·8	0·0057
						$t = 2·33$, $P < 0·05$

From the results shown in Table 1 it is apparent that griseofulvin stimulated growth significantly throughout the period of the trial. The amount of food consumed per lb. of live weight was 1·98 lb. for the griseofulvin supplemented group and 1·95 lb. for the control group. The difference between these figures is not significant.

The assistance of I.C.I., Ltd., in providing facilities for this experiment is gratefully acknowledged.

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Feeding of Bamboo Flowers to Laboratory Rats and Mice

ACCORDING to a popular myth there is a baffling connexion between the flowering of bamboos and the appearance of rat hordes which spread around destroying crops and eating chickens, puppies and even babies[1]. The present inquiry is an attempt to explain the mysterious phenomenon of an abnormal increase in field rats associated with the flowering of bamboos as recently witnessed in North-east Frontier and Tripura areas. The problem was approached from the point of view of evaluating the palatability, the œstrogenic activity and the reproduction stimulating values of bamboo flowers for rats.

Small quantities of 'mooli' bamboo flowers and fruits and 'pularwa' bamboo flowers from the affected area were received in a semi-dried condition. On dry-matter basis the percentages of crude protein in these samples were 11·39, 14·34 and 13·90 and of crude fibre 39·10, 19·42 and 31·71 respectively compared to the values of 12·81 crude protein and 3·58 crude fibre in the stock diet of rats. The laboratory rats did not relish these substances *per se*, but their inclusion in the normal diet of groups of rats at 10 and 20 per cent levels showed that the total dry matter ingestion may be slightly stimulated at the lower level of incorporation.

An evaluation of the œstrogenic activity in 'mooli' flowers was carried out according to Bickoff *et al.*[2]. Groups of six immature mice each were fed the normal and experimental diets for a period of one week before they were killed for determining the weights of the freshly excised uteri. The results in Table 1 show that the œstrogenic activity in the 'mooli' flowers was practically nil at the levels tested.

Table 1. UTERINE WEIGHT RESPONSE OF IMMATURE MICE ON DIETS CONTAINING THE EXTRACT FROM BAMBOO FLOWERS

Group No.	Addition to 100 gm. normal diet	Live weight (gm.)	Uterine weight (mgm.)	Difference compared to control
I	Nil	5·33 ± 0·61	3·01 ± 0·42	—
II	Stilbœstrol, 0·75 μgm.	5·50 ± 0·62	5·58 ± 0·63	Highly significant
III	Extract from 8 gm. of air-dried bamboo flowers	5·33 ± 0·61	3·20 ± 0·55	—
IV	Extract from 1·6 gm. of air-dried bamboo flowers	5·67 ± 0·76	3·30 ± 0·42	—

Direct evidence of the incorporation of bamboo flowers in the stock diet of rats on their reproductive performance was obtained with local bamboo (*Bambusa arundinaceae*) flowers. Six male and twelve female adult white rats were distributed in three identical groups. One group received the normal diet while the other two groups received diets in which shade-dried bamboo flowers were incorporated at 2 and 15 per cent levels, respectively. The male and female rats of each group were kept separately during the first 15 days of experimental feeding. They were then mated within the groups and continued on the scheduled diets until all the female rats brought forth litters. The average data are recorded in Table 2.

Table 2. OBSERVATIONS ON THE REPRODUCTIVE PERFORMANCE OF RATS ON BAMBOO FLOWER DIETS

Group No.	Level of bamboo flowers in normal diet (per cent)	Period from mating to littering (days)	Litter size	Weight per new-born rat (gm.)
I	Nil	30·00 ± 2·61	8·25 ± 1·44	4·88 ± 0·05
II	2	25·75 ± 1·25	8·00 ± 0·00	5·10 ± 0·11
III	15	36·75 ± 10·02	7·25 ± 0·85	4·80 ± 0·13

The differences between the control and experimental groups were statistically not significant in regard to the length of the period from date of mating to date of littering, litter size or the weight of the new-born rat. Evidently the incorporation of bamboo flowers in the stock diet under the conditions of the test did not have any stimulatory effect on the reproductive activity in rats.

The bamboo flowers were not palatable to rats even in the fresh state. The sugar content in the nectar of bamboo flowers is lower than in those of common honey plants like apple, dandelion and clovers[3]. Bamboo sprouts promote growth of pathogenic bacteria which barely grow on the basal medium[4], and the bamboo seed is superior to both rice and wheat in overall nutritive value[5]. Breeding disturbances in sheep in Australia have been related to the presence of œstrogenic substances in subterranean clover[6], but their high concentration in spring grasses in Great Britain has been without any reproductive disturbance in any species so far[7]. The search for œstrogenic substances in bamboo flowers was prompted by their well-established occurrence in plant materials and their value as growth promoters[8] The possibility that, as growth promoters, œstrogenic substances from diverse sources may have unrecognized useful potentialities may not be discounted

The limited evidence presented here does not support the myth of increase in rat population as a direct result of the ingestion of bamboo flowers

We are grateful to the Director, Indian Veterinary Research Institute, Izatnagar, for his interest in this work

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Classification of Fossil Microplankton

It is becoming increasingly desirable that a definite decision be made as to whether the fossil organic-shelled microplankton (dinoflagellates, hystrichospheres, and genera of presumed microplankton *incertae sedis*) should be classed, for nomenclatural purposes, in the animal or in the plant kingdom The dinoflagellates, a group of unicellular organisms some of which contain chlorophyll, are clearly algae, while others contain no chlorophyll and are vagile and predatory, and are at present alternatively regarded as the class Dinoflagellata or class Dinophyceae The hystrichospheres, typically marine in occurrence and defined by their morphology, are regarded as an order *incertae sedis*, the order Hystrichosphaeridia, and have been treated by most authors as animals the majority are now known to be dinoflagellate cysts, while others, less capable of definite attribution, may be zygospores of desmids, eggs of copepods or other planktonic organisms, or even plant spores

Much confusion about nomenclature must inevitably result when these organisms are indifferently classed in two kingdoms and may thus be dealt with under two different taxonomic codes The proposed third kingdom, the Protista, to comprise all unicellular organisms, has not secured general recognition it is highly unlikely that a third system of nomenclature will be set up A choice must thus be made as to which of the existing systems should be followed, and whichever is decided on must be applied consistently if further confusion is to be avoided

We consider that these groups should be classified as plants The dinoflagellates surviving as fossils are relatively thick-shelled forms, often having an armour of distinct plates Investigations of living plankton show that such forms are relatively sessile and contain chlorophyll the vagile, predatory forms have very thin shells and are probably not capable of fossilization. Evitt[1] has demonstrated that a large majority of the hystrichospheres and allied forms *incertae sedis* possess an opening in the shell (a pylome) through which escape can take place after encystment and are quite certainly the abandoned cysts of dinoflagellates, a conclusion suggested also in many genera by similarities in overall morphology. For these forms, use of the Botanical Rules[2] would be greatly advantageous in that there is provision for the creation of form genera and species the confusion that might result from application of the rule of priority, should the identity of a motile dinoflagellate with an encysted form be proved, may thus be avoided No such provision exists in the Zoological Rules, the comparable vehicle of parataxonomy[3] having been rejected by the International Committee in London, 1958

Classification under the Botanical Rules conforms to a large degree with present practice Deflandre, in his work on the fossil dinoflagellates, has classed them as Dinophyceae the Russian authorities, such as Naumova, regarded all hystrichospheres as plants for taxonomic purposes The majority of workers in this field are palynologists for whom the procedure here proposed would be considerably more acceptable

We therefore propose that the fossil microplankton be regarded as subject to the Rules of Botanical Nomenclature, as applied to fossil plants that, within the framework of this classification, fossil dinoflagellates in the motile stage be regarded as genera and species fossil cysts of dinoflagellates as form genera and species and hystrichospheres and other genera *incertae sedis*, for which a relation to the dinoflagellates has not been proved, as genera and species or form genera and species *incertae sedis*

Until a full re-definition of the order Hystrichosphaeridia, on the basis of a more complete knowledge of their affinities, has been undertaken, we do not feel it desirable to alter the present grouping into families of the hystrichospheres For the present, we consider that it would be adequate if these organisms were classed as plants and descriptions of them are formulated on this basis

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Alteration of Permeability of *Lactobacillus plantarum* caused by Chlorpromazine

The mechanism for penetration of oxalacetate into *Lactobacillus plantarum* 17–5 is inducible Unless *L. plantarum* is grown for a few hours in a medium which contains either oxalacetate, acetoacetate or α-ketoglutarate, there is poor penetration of oxalacetate into cell suspensions of this organism afterwards prepared Oxalacetate decarboxylase (which is also inducible) can be readily detected manometrically in whole organism suspensions of *L. plantarum* grown with any of the three compounds already mentioned, but can only be detected in disrupted (acetone-dried) organisms if the bacteria had been grown with L-malate, a compound which induces synthesis of oxalacetate decarboxylase but does not induce the mechanism for oxalacetate to penetrate the cell[1]. An agent which increases cellular permeability, at least with respect to oxalacetate, may be detected by increase of oxalacetate decarboxylase activity of undisrupted *L. plantarum* which had been grown in a medium containing L-malate.

For the experiments reported here, *L. plantarum* was grown at 35° C. in a medium composed of yeast extract, glucose, and L-malate (1.0 gm./100 ml. each). Chlorpromazine was added to the growth medium after the organisms began to increase exponentially. The amount of chlorpromazine added varied within the range of 0.5–5.0 mgm./100 ml. final medium. Growth was allowed to continue for 3–6 hr. after addition of chlorpromazine.

Samples of each culture were examined for gross morphological changes and for differences in density of final growth. Cultures which had grown with less than 3.0 mgm. chlorpromazine appeared like the untreated control. There were fewer cells produced in cultures which contained more than 3.0 mgm. chlorpromazine.

Cultures grown either without or with 1.0 mgm. chlorpromazine were collected by centrifugation, washed and assayed for both oxalacetate decarboxylase and malic decarboxylase (malic enzyme) using methods previously described[1,2]. Since malic decarboxylase is also inducible, but can be readily detected in suspensions of undisrupted cells, changes in the activity of this enzyme served as a control that chlorpromazine inclusion in the growth medium did not quantitatively alter enzyme synthesis[2].

Only cell suspensions of bacteria which had been grown with chlorpromazine showed appreciable increase in oxalacetate decarboxylase activity (Table 1). There was no effect on malic decarboxylase activity of cell suspensions or of either oxalacetate decarboxylase or malic decarboxylase activity of acetone-dried cells.

Table 1. EFFECT OF CHLORPROMAZINE (CPZ) DURING GROWTH ON SUBSEQUENT ENZYME ACTIVITY OF *L. plantarum*

Source of enzyme	Treatment during growth	Decarboxylase measured	Per cent activity
Cell suspension	None	oxalacetate	10
,, ,,	CPZ	,,	100
,, ,,	None	malate	100
,, ,,	CPZ	,,	100
Acetone-dried cells	None	oxalacetate	100
,, ,,	CPZ	,,	100
,, ,,	None	malate	100
,, ,,	CPZ	,,	100

It has already been shown that one site of activity of chlorpromazine in *L. plantarum* involves diphosphophyridine nucleotide and is related to the ability of chlorpromazine to serve an electron donor[2,3]. Quite possibly, the alteration in membrane permeability caused by chlorpromazine in the experiments reported here also involves a site dependent on diphosphopyridine nucleotide. In other recent experiments we have shown that chlorpromazine increases membrane permeability of animal-type cells[4], and plant-type cells (Nathan and Zahalsky, in preparation). A preliminary survey of phenothiazines indicates that ability to alter membrane permeability is linked to ease with which free radicals are formed.

Chlorpromazine promises to be a useful tool for making materials in the surrounding milieu accessible to the inside of cells without injuring the cell to the extent that it cannot grow and reproduce.

This work was supported by a grant from the Peninsula Health Fund.

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A Chamber for culturing and collecting the Nematode *Panagrellus redivivus*

Panagrellus redivivus L., Goodey, 1945, is frequently the nematode chosen for laboratory work because of its size (maximum length of approximately 1.5 mm.) and the ease with which it can be cultivated[1]. This free-living worm has been employed for the evaluation of nematocides[2] and repellants[3], in metabolic studies[4], and to induce morphogenesis in predaceous fungi[5].

A constant supply of *P. redivivus* was necessary for work in this laboratory on the activities of fungi that capture and kill nematodes. To meet this requirement a chamber that served for both the culture and collection of *P. redivivus* was designed and employed. The apparatus (see Fig. 1) consisted of a funnel (15 cm. diameter, 60° angle) with a ground lip and attached standard-taper stopcock. A saddle (*A*) constructed of glass rod was placed in the funnel as support for a heavy Petri dish bottom (*B*) and approximately 25 gm. of oatmeal and 45 ml. of water

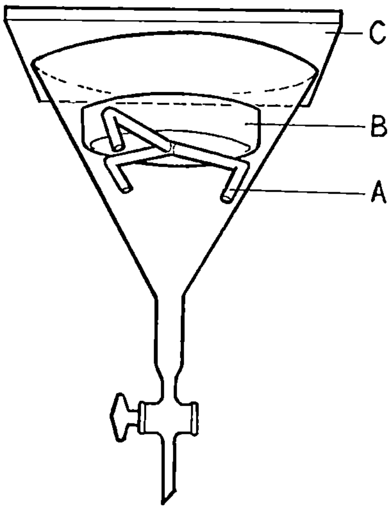

Fig. 1. Chamber for the culture and collecting of *P. redivivus*

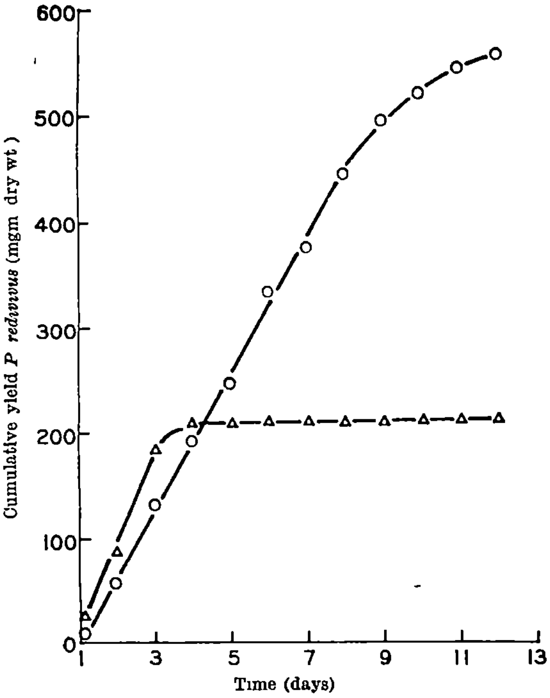

Fig 2 Influence of temperature on the yield of *P redivivus* O, 20° C , Δ, 28° C

were added to the dish (2 × 9 cm) The stopcock was closed and water was then added to the funnel until the liquid-level was 3–4 mm below the open end of the dish The moist oatmeal was inoculated with *P. redivivus* and the unit was closed using a ground-glass plate (*C*) as lid The dish was arranged in a manner such that it did not touch the wall of the funnel and was approximately 1 0 cm below the lid.

P. redivivus multiplied rapidly and after 3–5 days at 28° C or 7–8 days at 20° C numerous adults and larvæ moved out of the oatmeal, over the edge of the dish and into the water contained in the funnel. Since the density of nematodes exceeds that of water, the worms were concentrated above the stopcock by sedimentation The dry weight of nematode tissue yielded by chambers incubated at 20° and 28° C. was determined at daily intervals from the time of first harvest The results, illustrated in Fig 2, showed that 20° C. was the more suitable storage temperature Although development of *P. redivivus* was more rapid at 28° C , worm production at that temperature ceased after 4 days At 20° C nematodes were produced for 12 days and the total yield exceeded 550 mgm. Since a dry weight of 1 0 mgm. was equivalent to approximately 30,000 individuals, more than 16×10^6 worms were collected in 12 days from each chamber incubated at 20° C No attempt was made to collect or enumerate the many nematodes that remained in the oatmeal, but on prolonged incubation their numbers were depleted by sporadic migrations from the dish into the funnel

The chamber as described is efficient and convenient and serves simultaneously for the culture and collection of *P. redivivus* However, it can be sterilized or modified to meet specific requirements. The stem of the funnel can be refrigerated to maintain worms in a state of relative inactivity until collected Moreover, the stem can be elongated and filled with glass beads. Worms collected after entering the funnel and working their way down the bead column to the stopcock are relatively free of contaminating micro-organisms.

We thank J Postman and the New Brunswick Scientific Co (New Brunswick, New Jersey) for construction of the chambers This investigation was supported in part by National Science Foundation grants *G*5949 and *G*9749

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Periodic Acid–Schiff Technique for the Demonstration of Extra- and Intra-cellular Stages of Gregarine and Coccidian Protozoa

DURING a histological investigation of gut structure in the rhynchocœlan *Lineus ruber*[1] it was noted that both extra- and intra-cellular stages of the gregarine *Urospora nemertes*, which infected approximately 75 per cent of the specimens examined, stained intensely with the periodic acid–Schiff technique[2]. The intra-cellular stages, in particular, are difficult to demonstrate by routine staining methods, but after periodic acid–Schiff treatment they are strikingly prominent and may be readily located in sections with the low-power objective

In view of the potential value to the protozoologist of this use of the periodic acid–Schiff technique its effectiveness with other gregarine species and the related group of parasitic protozoa, the Coccidia, was investigated In all cases every stage in the life-history gave a strong positive reaction and the four species of coccidians examined (*Eimeria tenella*, *E maxima*, *E acervulina* and *E. necatrex*, from the gut of the domestic fowl) were readily located even when only small numbers were present in the infected tissue. Particularly striking results were obtained with the gametocyte stage (Fig 1)

The choice of fixative does not appear to influence the reaction to periodic acid–Schiff to any significant extent and comparable results were obtained after

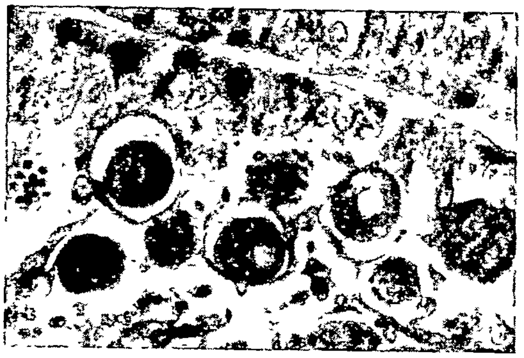

Fig 1 Transverse section of fowl cæcum showing macro- and micro-gametocytes of *Eimeria maxima* Trichrome periodic acid–Schiff (× 650)

fixation in Susa, 5 or 10 per cent neutral formalin or 90 per cent alcohol If details of host tissue are required, the periodic acid–Schiff may be followed by the celestin blue/hæmalum and orange *G* method[2] to give a full trichrome stain

I wish to thank Dr. Horton-Smith, Houghton Poultry Research Station, Houghton, Huntingdonshire, for supplying material infected with the four species of *Eimeria*

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ENTOMOLOGY

Evaluation of the Activity of Some Diphenyl Compounds on Winter Eggs of the Fruit Tree Red Spider

For several years some red spider ovicides have appeared on the market Most of these compounds are active chiefly on the summer eggs, and far less active on the winter eggs of the fruit tree red spider *Metatetranychus ulmi* Koch. One of these compounds, 2,4,5,4′-tetrachlorodiphenyl sulphone (tetradifon), shows extremely high ovicidal and larvicidal activity on Tetranychids, but is not active on the winter eggs of *Metatetranychus ulmi* In the course of investigation of the past five years a large number of diphenyl compounds has been tested on tetranychids[1] Some of them have been examined for activity on the winter eggs and compared with some other compounds The latter experiments are reported here

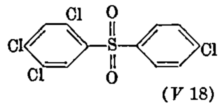
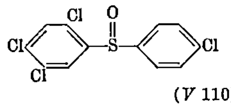
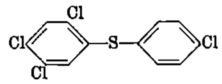
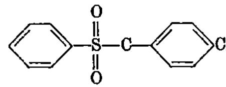
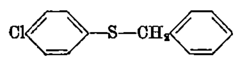
Apple twigs bearing winter eggs of the fruit tree red spider were collected during winter and kept outdoors Before treatment the twigs were cut into pieces of about 4 in and then dipped into miscible oil–water emulsions The treated pieces were put into water-filled vials The whole was kept in climatically conditioned racks under continuous illumination at 20–22° C and 80–90 per cent relative humidity

A serious difficulty of these laboratory experiments is the high percentage of untreated eggs that do not hatch This percentage may vary between 30 and 80 after one month of incubation Hatching is especially reduced if heavily infested twigs are used, probably as a result of overpopulation Nevertheless, the differences between the various substances seem justified by their consistency under various levels of hatching in the batches

The reduction of hatching shown in the tables refers to the percentages calculated after Abbott's formula Table 1 shows the results of trials with tetrachlorodiphenyl compounds as compared with the known acaricides CPBS and 'Chlorbenside' Tetrachlorodiphenyl sulphone is not active at all, whereas the sulphide homologue is highly active The latter appears to be the best of the compounds examined

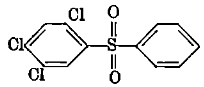
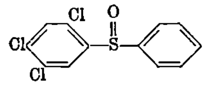
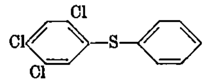
Table 2 summarizes the results obtained with the trichloro-diphenyl compounds and tetrachlorodiphenyl sulphide.

Table 1 Reduction of Hatching of Winter Eggs of *Metatetranychus ulmi* Koch after dipping of Egg-bearing Apple Twigs in Emulsions Mean Percentages of Three Experiments in 1957 and 1958

Compound	Concentration (p p m)	Percentage reduction
 (*V* 18)	500 250 125	7 10 5
 * (*V* 110)	500 250 125	7 14 0
 (V 101)	500 250 125	100 90 87
 (CPBS)	500 250 125	71 61 29
 * ('Chlorbenside')	500 250 125	64 66 79

* One experiment

Table 2 Reduction of Hatching of Winter Eggs of *Metatetranychus ulmi* Koch after dipping of Egg-bearing Apple Twigs in Emulsions Mean Percentages of Three Experiments (1959)

Compound	Concentration (p p m)	Percentage reduction
 (*V* 17)	1,000 300 100	0 0 0
 (*V* 114)	1,000 300 100	92 0 0
 (*V* 113)	1,000 300 100	98 61 0
V 101	300 100 30	100 98 97
50/50 mixture *V* 18/*V* 101	150/150 50/50 15/15	99 92 56

In a previous paper[2] it was shown that *V* 18, *V* 110 and *V* 101 were equally effective on eggs and larvæ of *Tetranychus urticae* Koch on bean plants When dipping eggs of *Tetranychus urticae* Koch in emulsions of these compounds, however, *V* 18 shows the highest ovicidal activity After hatching of the surviving eggs, the larvæ are killed by the deposit of all three compounds, so that the overall mortality is the same But if leaves bearing eggs of *Tetranychus* at the under side are treated on the upper side only, the highest egg-kill is obtained by *V* 101, and the least kill by *V* 18, whereas *V* 110 takes an intermediate position This result is evidently a consequence of the different penetration properties of the compounds

In this respect it is interesting that the highest activity on winter eggs of *Metatetranychus* is shown by *V* 101, which also has the best leaf-penetration In laboratory trials *V* 101 appears also more active than CPBS, which has been in use for the control of winter eggs of the fruit tree red spider for some years The addition of *V* 18 does not seem to influence the action of *V* 101

The same sequence of activity on the winter eggs is seen with the trichloro-compounds *V* 17, *V* 114 and *V* 113 (Table 2) Also in this case the sulphide homologue is more active than the sulphone, presumably as a result of the better penetration through the egg shell The activity, however, is far less than with *V* 101

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A Technique for exposing Individual Flies on Insecticidal Deposits

A NUMBER of methods have been described for testing residual insecticides against various insects, and for the bio-assay of insecticide deposits on treated surfaces In general these entail confining the insects for testing under a transparent container on the treated surface for a certain time of exposure, and measuring the mortality after holding in clean cages for a further period Wiesmann[1] used halves of Petri dishes, while Hadaway and Barlow[2] used glass tubes, inverted funnels and 'Perspex' cylinders

One of the difficulties common to these methods, particularly when testing flying insects, has been to ensure that the insect remains in contact with the treated surface for the whole of the time of exposure According to the type of apparatus, one is advised to blow down the cylinder, or to rotate the tube in order to induce the insect to settle on the insecticide deposit

The method described below was evolved for the bioassay of treated leaf surfaces, using *Stomoxys calcitrans* as the test insect It has the advantages that the flies do not have to be anæsthetized or handled, and are forced to walk on the treated surface throughout the period of exposure, irrespective of the duration of this period

The apparatus used is shown in Fig 1 It consists of two squares of 'Perspex' (*A* and *B*) measuring 2 × 2 × 3/16 in, each having a central circular hole 1 in in diameter The two squares are held together by thin strips of 'Perspex' cemented along opposite edges, but leaving a space to allow a thin 'Perspex' shutter (*C*), 3 × 2 in, to slide between the squares At one end of the shutter is a hole 1 in in diameter, so that in one position of the shutter the holes in the three pieces of 'Perspex' are concentric A 3 × 1-in glass cylinder (*D*) is cemented into the hole in the 'Perspex' square (*A*)

A 3/16 in 'Perspex' disk (*E*) cemented to the end of a 4-in glass rod (*F*) slides smoothly inside the cylinder, through a cork-guide (*G*) The glass rod is fitted with an adjustable rubber ring (*H*) which determines the downward travel of the 'Perspex' disk

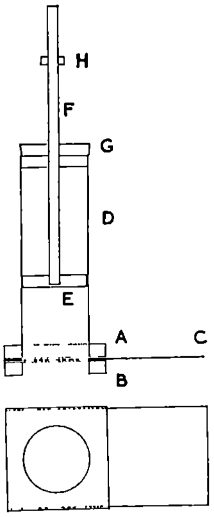

Fig 1

To use the apparatus, individual flies in 3 × 1-in. tubes are used A 3 × 1-in tube containing a single fly is fitted into the hole in 'Perspex' square *B* The tube containing the fly is darkened by holding in the hand, and the shutter opened, to allow the fly to pass into the apparatus, and the shutter again closed. This operation is performed with the circular disk (*E*) in the fully raised position

The apparatus is placed on the surface to be treated, the shutter opened to allow access to the treated surface, and the disk depressed to a pre-set position depending on the height of the test insect. Care must be taken to ensure that the fly is in a walking position on the treated surface In this final position it is impossible for the fly to turn over or walk on the wall of the cylinder

At the end of the period of exposure, the disk is raised to allow the fly to move off the treated surface, and the shutter then closed The fly can now easily be transferred to a holding cage

Other advantages of this method are (1) With practice, exposures can be performed very quickly, for example, with *Stomoxys calcitrans* it is in the order of the exposure period plus 15 sec (2) The smooth undersurface of the 'Perspex' disk causes no damage to the dorsal surface of the insect (3) The apparatus can be dismantled and cleaned by rinsing and swabbing with alcohol Acetone, benzene and similar solvents cannot be used as they cause crazing of the 'Perspex', and loosening of the cemented joints This disadvantage would be overcome if the apparatus were made entirely of glass (4) The apparatus can be made easily and cheaply in any laboratory (During the preparation of this communication for publication, it was learnt that a similar apparatus had been described by Kernaghan of Northern Nigeria, at a recent meeting of the International Scientific Committee for Trypanosomiasis Research at Jos)

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GENETICS

Primary Embryonal Sex Ratio in Normal Pregnancies determined by the Nuclear Chromatin

MANY attempts have been made to establish a sex ratio for embryos of a younger gestational age Unfortunately, external genitals are an unreliable index for this purpose before the 5-cm stage Former investigations were based on histological examination of gonadal tissue[1] Recently, Tricomi *et al* [2] determined the ratio of male to female embryos by examining the placental tissue obtained by curettage for the treatment of incomplete abortions Their investigation was based on the observations of Graham[3] and Glenister[4], who noted the presence of the sex-chromatin in the trophoblastic cells before gonadal differentiation Park[5] was able to demonstrate that a morphological sex difference is present in the trophoblastic cells of 10–12-day-old embryos

The accepted secondary sex ratio of man the world over is 106 males to 100 females All data available now indicate that more males than females are lost during the early months of pregnancy Tricomi *et al* [2] examined 242 abortion specimens and found a ratio of 160 100

The primary sex ratio, that is the ratio which prevails at the time of fertilization, is not yet known The investigators mentioned accept an incidence of abortions of 10 per cent and by using a weighted mean they assume that the primary sex ratio may approximate 111 100, male to female

Since all investigations until now were carried out on placental tissue obtained from curette material of incomplete abortions, it seemed to us worth while to examine the distribution of sexes in normal early pregnancies

Fresh placental tissue obtained from 300 medical abortions in apparently normal early pregnancy was investigated Identification of the sex-chromatin mass was carried out according to the usual histological method. One hundred cells from each specimen were examined at an approximate magnification of 1,000 (oil immersion) The percentage of the sex-chromatin positive cells for female tissue ranged from 30 to 80, that for male from 1 to 8 Preliminary investigations established the reliability of the procedure[6]

165 of our specimens have been classified as male and 135 as female This result gives a ratio of 122 100, male to female Though the material is too small a series to be conclusive, the results of our investigations indicate a greater incidence of the male sex in the primary ratio than that calculated by Tricomi *et al* [2]

Since theoretically there should be as many Y-bearing as X-bearing sperm, the ratio at the time of conception should be 100 100 Why an egg should be fertilized more frequently by a sperm with Y-chromosome would then have to be explained

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A Rare Phenotype within the *Gm* System

IN 1956 Grubb[1] published the discovery of a serologically demonstrable γ-globulin factor, which was found to be inherited as a Mendelian dominant Since then several related factors apparently belonging to a common hereditary system, the *Gm* system, have been demonstrated At present the system comprises the following factors· $Gm(a)$ (ref 1), $Gm(x)$ (ref 2), $Gm(b)$ (refs 3,4) and *Gm*-like (ref 5)

Disregarding *Gm*-like, which has not been found in Caucasians, the following phenotypes have been reported $Gm(a+x+b+)$, $Gm(a+x-b+)$, $Gm(a+x+b-)$, $Gm(a+x-b-)$ and $Gm(a-x-b+)$ The phenotypes $Gm(a-x+b+)$, $Gm(a-x+b-)$ and $Gm(a-x-b-)$ have not been encountered in spite of rather extensive investigations

According to the study material factors $Gm(a)$ and $Gm(b)$ appear to be Mendelian dominants, being controlled by allelic genes $Gm(x)$ also behaves as a Mendelian dominant, but until now has only been demonstrated in sera exhibiting the $Gm(a)$ factor also In work involving $Gm(a)$ and $Gm(x)$ typing in adults from a series of Danish paternity cases one example of the phenotype $Gm(a-x+)$ was encountered The serum sample belonged to a healthy woman, A P , and the result was checked by examining a new blood sample at a later date The distribution of *Gm*-phenotypes in the series is shown in Table 1

In order to clarify the significance of the unusual phenotype an investigation of the kindred of A P was undertaken The result of the *Gm* typing is shown in the pedigree (Fig 1)

It is seen that the quite common phenotypes in the P-generation give rise to the rare phenotype

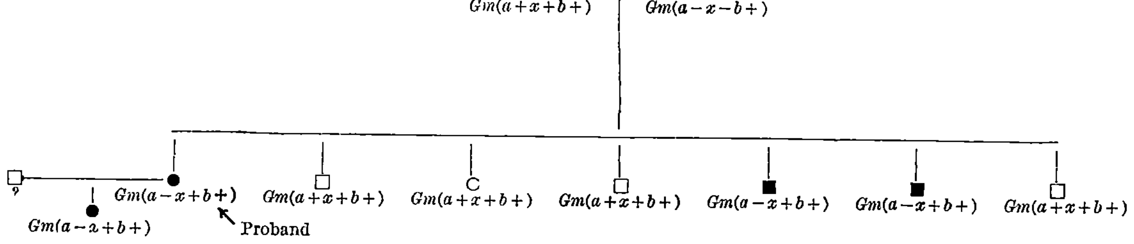

Fig 1

Table 1

Phenotype	No	Percentage
$Gm(a+x+)$	201	24 2
$Gm(a+x-)$	272	32 7
$Gm(a-x-)$	358	43 0
$Gm(a-x+)$	1	0 1
Total	832	100 0

$Gm(a - x + b +)$ in 3 out of 7 sibs in the F_1 generation and in the serum of the single representative of the F_2 generation This may well be explained by the assumption of a rare gene—or closely linked gene combination—*bx*, beside the frequent gene *ax*, the genotype of the father of the proband being *ax*/*bx*

Our thanks are due to Dr M Harboe, Rikshospitalet, Oslo, who checked the results of our *Gm* typing

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Glucose-6-Phosphate Dehydrogenase Activity in Papuans

During a recent investigation into hæmolytic and other anæmias in Papuans the glucose-6-phosphate dehydrogenase (G-6-P D) activity of erythrocytes was estimated in 45 cases Blood was collected into heparin, and on several occasions into acid–citrate dextrose solution, and dispatched by air to Melbourne, arriving the same day The G-6-P D activity was estimated in the Biochemistry Department of the Peter MacCallum Clinic, Melbourne, by the method described by Hsia[1] By this method normal readings are 150–210 units/100 ml The enzyme appears to be relatively stable for at least a week, provided the cells remain intact The highest values were obtained in cases of thalassæmia major, acute hæmolytic anæmia of undetermined cause, and congenital hæmolytic anæmia of unknown cause There was no significant difference in the results obtained, whether A C D solution or heparin was used as an anti-coagulant The twelve subjects used as controls all gave readings within the accepted range of normality

Three examples of deficient activity were found The first was a Papuan adult male, who denied any possibility of racial admixture and appeared to be a typical Papuan from the Rigo district, a few miles from Port Moresby He was suffering from an attack of hæmolytic anæmia of undetermined cause when first seen on June 12, 1960 Electrophoresis of the hæmoglobin showed no abnormality and the alkaline-resistant hæmoglobin test and the direct Coombs test were both negative No G-6-P D activity was found at this time This result was not due to inhibitors, because the addition of blood from a case of thalassæmia major (518 units/100 ml) gave the expected values About two months later 8 units/100 ml of enzyme activity were found and in a further six months the result was 11 units/100 ml Although the patient was given 30 mgm of 'Primaquine' daily for 10 days no evidence of hæmolysis was obtained He stated that his brother had died one year previously from an attack of what seemed to be hæmolytic anæmia He denied that either of them had eaten beans or taken any drug apart from 'Camoquin' at any time

The two other patients with low values of G-6-P D were children One was suffering from thalassæmia major, for which his spleen had been removed His G-6-P D values on three occasions were 56, 46 and 36 units/100 ml The other child was suffering from anæmia apparently due to tuberculosis infection and the G-6-P D value was found to be 66 units/100 ml It has not been possible to confirm this result

It is probable that anæmia which is determined by genetical factors is not nearly as common in New Guinea as is anæmia due to parasitic and nutritional factors However, the finding of thalassæmia[2] and deficiency in G-6-P D in the Territory suggests that further attention should be given to investigating genetical factors

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VIROLOGY

Cyclic Events in the Viral Cycle

The polio virus is able to develop only within given limits of temperature The simplest reason for this seems to be that low and high temperatures block viral development by altering the metabolism of the host cell Yet mutants of the polio virus can be obtained which thrive at these low or high temperatures at which the original type is unable to multiply the sensitivity of viral development to temperature is thus controlled by the genetic constitution of the virus Moreover, it was discovered that one mutational event shifts the whole curve expressing the sensitivity of viral development to temperature towards the left or the right psychrosensitivity decreases when thermosensitivity increases and vice versa[1]

The polio virus is able to develop normally if submitted to supra-optimal temperatures during the first part of the cycle only or to infra-optimal temperatures during the second part only Infra-optimal temperatures, which do not block the second part, interfere with the primary phase whereas supra-optimal temperatures, which do not block the first part, interfere with the secondary phase The polio virus is in fact, able to complete its development at high and low temperatures both incompatible with viral multiplication, provided they alternate in the right sequence during one growth-cycle

The effects of infra-optimal temperatures are increased by heavy water, the effects of supra-optimal temperatures decreased In the presence of heavy water the whole curve expressing the sensitivity of viral development to temperature is shifted heavy water decreases the apparent temperature[2]

The fact that a single mutation modifies at the same time in opposite directions the psychro- and the thermo-sensitivity and affects the events taking place during the early phase as well as during the late one, leads to the hypothesis that both phases are controlled by one viral molecule Things happen as if this molecule would undergo reversible structural alterations, the equilibrium of the reaction being shifted by temperature changes

$$A \underset{\text{Supra-optimal temperatures}}{\overset{\text{Infra-optimal temperatures heavy water}}{\rightleftharpoons}} B$$

The state A would allow the completion of the primary phase only, the state B the completion of the secondary phase only Infra-optimal temperatures, by shifting the probability of the molecular structure towards B, prevent the completion of the early phase Supra-optimal temperatures, by shifting the probability of the molecular state towards A, prevent the completion of the secondary phase The effect of deuteration shows that hydrogen bonds are involved in the reaction $A \rightleftharpoons B$

The optimal temperature for viral development probably corresponds to the transition temperature of the reaction at which both states of the alleged viral molecule are equally represented Infra- and supra-optimal temperatures decrease or block viral development by shifting the equilibrium, thus favouring one of the states at the expense of the other The completion of the viral cycle would thus imply a reversible cyclic alteration of a metastable molecule[3]

During the life-cycle of a cell a certain number of molecules, or molecular complexes such as mitotic fibres, genes, chromosomes, undergo reversible alterations of structure On the other hand, vegetative functions, that is, the activity and the biosynthesis of enzymes, are regulated by feed-back mechanisms depending in the last analysis on structural molecular changes It is felt that reproductive and 'cyclic' functions must also be under the control of some feed-back regulating mechanisms Metastable molecules able to undergo reversible structural alterations could be responsible for these mechanisms[4]

This work was supported by a grant from the U S National Foundation

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Multiplication of Mouse-adapted Influenza Virus in Mouse Lungs after Infection with Very Low Doses

WE studied the dynamics of the infection with influenza virus in mice after intra-nasal administration of extremely low doses White mice were infected with the mouse-adapted Em_2 strain of the $A1$ type influenza virus The virus, used in the form of infected allantoic fluid, showed LD_{50} titres of $10^{5\ 1}$ and $10^{5\ 45}$ 0 1 ml , respectively, as estimated by intra-nasal inoculation of white mice, and a $TCID_{50}$ titre of $10^{4\ 88}$ and $10^{5\ 52}$ 0 1 ml , respectively, as determined in suspended chlorio-allantoic membrane tissue cultures[1]—herein designated as Horváth's *TC* Mice weighing 16–18 gm were inoculated under light ether anæsthesia intra-nasally in individual experiments (altogether 6 experiments in 3 independent series) with 0 05 ml of infected allantoic fluid, containing $10^{-2\ 12}$–$10^{0\ 88}$ $TCID_{50}$ 0 1 ml , in other words, in each case with a lower dose than 1 mouse LD_{50} Starting with the first day after inoculation, 3 mice were killed daily and from their lungs 10 per cent suspensions in Horváth's[1] nutrient medium were prepared. The suspensions were clarified by centrifugation at 2,500 r p m for 10 min and serial 10-fold dilutions of them inoculated into Horváth's *TC* The cultures were incubated at 35° C for 72 hr and the reproduced virus detected by hæmagglutination test on plastic plates[2] The results are shown in Fig 1 It can be seen that the multiplication of the virus in the lungs depends on the dose of the virus administered and shows a cyclic character After administration of the lowest dose (0 0038 $TCID_{50}$ in 0 05 ml), virus was first detected three days after infection It is still present on the fourth day, but thereafter disappears and is not detectable up to day 12 After administration of a 10-times higher dose the virus appears in the lung also on day 3, it is not detectable for the next five days and appears repeatedly on day 9 or 10 after infection The higher the dose administered, the shorter the time intervals

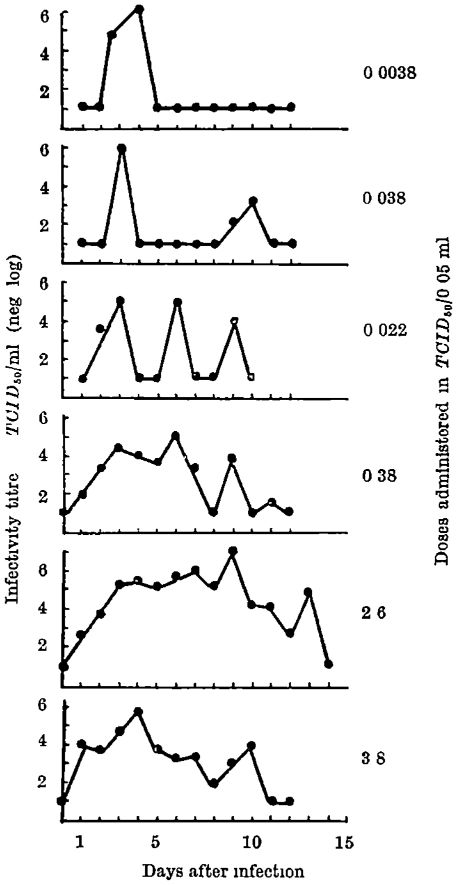

Fig 1

of the cyclic appearance and disappearance of virus in the lungs. After infection with 2·6 $TCID_{50}$ and 3·8 $TCID_{50}$ in 0·05 ml., respectively, a complete disappearance of virus from lungs was not detected during the whole observation period, although even in these cases an appreciable variation in infectivity titres was seen. It is to be noted that the first disappearance of virus and/or a marked lowering of its titre occurred in all experiments 4–5 days after infection. Virus-neutralizing antibodies were not detected in sera of mice for 10–14 days after infection.

We supposed that the disappearance or the marked drop in the infectivity titre of the virus in mouse lungs was due to an interferon-like substance, formed as a consequence of the virus infection. Therefore, 0·1 ml. of undiluted suspensions, in which no virus could be detected, were inoculated into Horváth's *TC* and incubated at 35° C for 24 hr. Thereafter the cultures were treated with a heterologous influenza virus (*A*, *PR*8). We found that after 72 hr incubation the infectivity titre of the added virus was lowered—in comparison with controls—by 90 per cent. Virus-containing lung suspensions were tested for the presence of an interferon-like substance. The suspensions were kept at 4° C for six weeks. During storage the virus was inactivated, for no virus reproduction could be detected after inoculation of such suspensions into Horváth's *TC*. Then Horváth's *TC* was inoculated with these suspensions and treated with homologous (Em_2) and heterologous (*PR*8) virus, respectively, in the same way as mentioned above. In these cases also 90–100 per cent decrease in infectivity titres—in comparison with the controls—were observed. Lung suspensions of non-infected mice, to which Em_2 virus was added *in vitro* and stored also for six weeks at 4° C., did not inhibit the reproduction of the added virus at all. It must be noted that also in these control lung suspensions the virus was inactivated completely during storage. We designate the substance formed in mouse lungs on infection with influenza virus and inhibiting the multiplication of added virus as an 'interferon-like substance', although it is very probable that the interference phenomenon observed in our experiments was caused by Isaacs's[3] interferon, taking into account the results published by Isaacs and Hitchcock[4].

The observed cyclic reproduction of influenza virus in mouse lungs is in agreement with Wagner's[5] hypothesis concerning the interaction of virus and interferon in permanently infected tissues. We suggest that when non-lethal doses are used on mice, the interferon produced prevents the aggressive spread of infection by occupying most of the susceptible cells in the vicinity of the primary focus of the infection. The possibility of a similar interaction of virus and interferon should be assumed after infection of human beings with influenza virus. Interferon could prevent, in some cases, the manifestation of the infection until specific antibodies are formed.

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AGRICULTURE

Nitrogen-fixing Blue-Green Algæ in Australian Rice Soils

A FEATURE of myxophycean algæ is the capacity of certain members of the class to fix elemental nitrogen. Blue-green algæ are widely distributed in Nature. They are known to be particularly abundant in regions devoted to the cultivation of rice where the activities of nitrogen-fixing species probably make significant contributions to the maintenance of soil fertility[1,2]. Australia offers excellent opportunities for research. Previously, these have not been exploited even though 46,000 acres are sown to rice annually in the temperate Murrumbidgee Irrigation Area and extensive tracts of country are being developed for rice production in tropical Northern Australia.

As a first step, I have undertaken a survey to determine the distribution of nitrogen-fixing blue-green algæ in the rice areas of Australia and to build up a culture collection for use in laboratory work.

During a visit to Darwin in February 1960, a number of samples was collected from the developing commercial rice crop at Humpty Doo, from the experimental plots of the Commonwealth Scientific and Industrial Research Organization in the same area, and from the two Northern Territory Administration experimental rice stations at Beatrice Hill and Marrakai. Humpty Doo and Beatrice Hill are on the heavy alluvial clays of the Adelaide River and Marrakai is on the somewhat lighter soils of the 'bull dust' plains[3].

The samples were collected in sterile bottles in various situations including supply ditches, at the water surface in the rice crops, from the rice plants and from the submerged soil surface. Every effort was made to ensure that the material was representative of the areas surveyed. No more than five days elapsed between collection of the samples and their return to the University of Sydney.

It is of interest to note that, while blue-green algæ apparently capable of nitrogen-fixation were isolated from almost every sample, their presence in the field was not always obvious. Although typically gelatinous colonies of *Nostoc* were found sometimes to be abundant in the rice fields, they were not common. At present, no simple explanation can be offered for their uneven distribution. In general, algal development was most marked where the movement of the irrigation water was reduced to a minimum and its turbidity was low. Nitrogen-fixing species were not found in the experimental plots possibly because the level of soil nitrogen had been raised considerably by fertilizer applications.

Uni-algal cultures were obtained by routine culture techniques using a range of media both in the liquid form and solidified with agar. Those capable of continued growth on a nitrogen-deficient medium were retained. The composition of this medium, a Winogradsky nutrient solution modified by Acierto (personal communication) and known as No. 5, is KH_2PO_4, 0·25 gm., $MgSO_4.7H_2O$, 0·125 gm., $NaCl$, 0·125 gm., $FeSO_4.7H_2O$, 2·5 mgm., $MnSO_4.4H_2O$, 2·5 mgm., $NaMoO_4.2H_2O$, 0·25 mgm., $CaCO_3$, 5·5 gm.; double glass-distilled water, 1 l., pH 7·2–7·4.

The Northern Territory collection comprises some 36 cultures, none of which has been identified fully

Only two genera are represented, *Nostoc* and *Anabaena*, although one isolate may be a species of *Cylindrospermum* Four isolates have been obtained in a bacteria-free state In December 1960, a survey was conducted in the Murrumbidgee Irrigation Area of New South Wales The samples collected are being treated in the same manner as those from the Northern Territory Already one culture, a species of *Anabaena*, has been obtained free of contaminants

Algæ with probable nitrogen-fixing capacities were found to be a minor element of the algal flora under these conditions However, species of *Anabaena* were almost ubiquitous *Nostoc* was not widespread and representatives of other genera, reported to be nitrogen-fixing, were not found It should be noted that heavy dressings of ammonium sulphate are applied to the soils and that copper sulphate is introduced commonly into the irrigation water in concentrations up to 5 p p m This practice has been adopted, in fact, to control the growth of algal 'scum', largely *Spirogyra*, *Lyngbya* and *Phormidium*, which interferes significantly with the early growth of the rice seedlings

Gelatinous colonies, closely resembling those of a *Nostoc* found in the Northern Territory, were found only occasionally floating in supply ditches and in the rice fields Subsequent examination has shown these algæ to be a species of *Gloeotrichia* It will be of interest to determine whether this organism is capable of nitrogen fixation The Kimberley region of Western Australia, where rice is also being grown, has not yet been surveyed However, several soil samples from that area have been examined It is not regarded as significant that nitrogen-fixing algæ could not be found in this material Attempts are at present being made here to study some of the factors affecting the growth and fixation of nitrogen by several of the bacteria-free isolates in the collection

Financial support for this work is being provided by a research grant from the University of Sydney During the survey, assistance was given by the personnel and officers of both private and government bodies I am grateful to them

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Control of the Army Worm on Grazing in Kenya

Infestations of *Laphygma exempta* Wlk (Lep - Noctuidae) on natural grazing present a problem in control because conventional tractor or aerial spraying is uneconomical, and aerial drift spraying is impractical on many farms due to their small area and irregular shape, and to the localization of cleared paddocks between strips of uncleared vegetation Such infestations have therefore been considered uncontrollable and have been left to complete their cycle with a consequent severe loss of grazing The development of the exhaust sprayer for the control of *Schistocerca gregaria* (Forsk)[1,2] offered a cheap and speedy method of control and six tests with this appliance were performed by us on an outbreak in 1959

The sprayer was mounted on a four-wheel-drive vehicle fitted with a governor control which enabled it to be driven at a speed of 5 m p h , and to give an emission-rate of 3–4 gall of spray material a mile The spray nozzle was mounted 6 ft above the ground and the spray plume was emitted vertically upwards so that, with the vehicle travelling across wind, a drift of 100–150 yd was obtained in quite light breezes A standard formulation containing 20 per cent dieldrin in heavy oil was used in the first test, but thereafter crude formulations of DDT in light oil mixed 1 1 with engine oil were used in order to reduce toxicity hazards to operators and to stock

The results of the tests are summarized in Table 1 together with data on air movement and vegetation. Assessments have been recorded visually because this was the only method used in tests 1 and 2, while in 4 a post spraying count was obviously unnecessary, and in 5 and 6 reductions of a fairly high order had occurred on the controls In test 3, a pre-spraying count gave an estimate of four larvæ/ft ^{2} while 72 hr after spraying the estimated populations (based on sixty samples of one foot square from each plot in an area between 7 and 40 yd downwind of the spray track) were

Treatment	Larvæ/ft ^{2}
Control	3 78 ± 0 58
Single application	1 37 ± 0 20
Double application	0 87 ± 0 20

The post spraying counts were intentionally biased on the treated plots, samples being taken in the thickest grass cover where initially more larvæ would have congregated, but even ignoring this bias, the reduction is very satisfactory

The failure in test 4 may be attributed to a combination of factors The very slight air movement was

Table 1

No	Insecticide (per cent)	Wind	Vegetation	Result after 72 hr	
				Sprayed area	Control
1	Dieldrin 20	Light	Grass, occasional trees	Heavy reduction	Slight reduction
2	DDT 12 5	*c* 5 m p h	Good grass cover, no bushes	Heavy reduction	No change
3	DDT 12 5 DDT 12 5 × 2*	*c* 5 m p h *c* 5 m p h	Good grass cover, no bushes Good grass cover, no bushes	Heavy reduction Very heavy reduction	No change No change
4	DDT 12 5	Negligible	Very thick grass cover, numerous tall trees	No change	No change
5	DDT 15 0	*c* 5 m p h	Sparse grass, some bushes	Very heavy reduction	Rather heavy reduction
6	DDT 15 0	Light	Sparse grass, no bushes	Heavy reduction with numerous dead larvæ	Heavy reduction, no dead larvæ

* Two runs in opposite directions were made along the same track in order to double the rate of application

severely interrupted by the numerous trees so that dispersion of the spray was unsatisfactory. Secondly, the heavy grass cover, in places 9 in. deep, caused dilution of the spray in terms of quantity per unit volume of vegetation.

In test 5, the results were confused by a nuclear polyhedrosis which caused a pronounced reduction on the controls. Although the decrease was greater on the treated area, the spray was obviously applied to a debilitated population and had only enhanced the action of the disease.

Test 6 was difficult to assess, as heavy reductions had occurred on both treated and untreated areas. However, on the control, the reduction was due entirely to pupation and no dead larvæ could be found on the surface; on the treated area, although pupation may have had some effect, many dead larvæ were found on the surface. The treatment may therefore be regarded as having caused a diminution in numbers.

The distribution of live larvæ after treatment accorded well with the observed distribution of spray recorded by Courshee[3]. In tests 2, 3 and 5, no deaths due to spraying occurred within 5 yd. of the spray track; mortality then increased to a peak at 30–50 yd. where very few live larvæ could be found, and beyond this limit decreased more slowly to become negligible 100–110 yd. from the spray track.

Should further tests fulfil the promise of those conducted, we believe the exhaust sprayer will have great value in the control of *L. exempta* and other 'army worms' on grazing. Its use is limited by the nature of the vegetation only in that the presence of too many trees interrupts air movement and too thick a grass cover causes excessive dilution. Its value lies in its low capital cost, low running costs, speed, and manœuvrability.

We envisage two uses for the sprayer: (1) it can be used to provide a barrier between infested and uninfested grazing along a wide front; (2) it can be used to eliminate infestations from paddocks by parallel spray runs at intervals of 75–100 yd. In this latter case, using a cost of 20*s.*/gall., a rate of application of 4 gall./mile, and a distance between runs of 75 yd., the spray would cost 3*s.*/acre plus the running costs of the vehicle.

We are grateful to the Director of the Desert Locust Survey, Nairobi, for the loan of the exhaust sprayer, and for the advice given by him and his staff on its use.

J. A. BULLOCK
D. C. BOND

Entomology Section,
Department of Agriculture,
Kenya.

[1] Sayers, J. H., and Rainey, R. C., Desert Locust Survey (1958).
[2] Sayers, J. H., *Bull. Ent. Res.*, **50**, 371 (1959).
[3] Courshee, R. J., *Bull. Ent. Res.*, **50**, 355 (1959).

SOIL SCIENCE

Influence of Some Organic Salts on the Absorption of Potassium by Vermiculite

A MARKED effect of the ammonium ion on release and fixation of potassium by vermiculite has been shown by many workers. The presence of ammonium ions in an extracting solution inhibits the removal of potassium which has been absorbed or fixed in vermiculite. The fixation of potassium by vermiculite is also reduced if the solution contains ammonium ions. The effect of the ammonium ion on potassium reactions is presumably due to the similarity of the two ions with respect to their size and chemical reactivity. Both ions are known to have an optimum ionic diameter for fixation within the vermiculite lattice. In the presence of ammonium ions the vermiculite lattice apparently is contracted so that potassium ions can neither enter nor leave the internal vermiculite surfaces.

The influence of other nitrogen compounds on potassium absorption by vermiculite was considered. The term 'absorption' is used here in preference to 'adsorption' since most of the effects noted here are associated with the internal surfaces of the vermiculite mineral. The compounds used in this work were amines of various kinds which may be considered in a sense as analogues of the ammonium ion in that one or more of the hydrogen atoms around the nitrogen are replaced with organic groups of various kinds. The substitution of organic groups for the hydrogens of the ammonium ion of course results in great changes in the properties of the ion.

Two types of investigations were made. One was concerned with the effect of the organic salts on the rate of potassium absorption by vermiculite, the other with the effect of organic salts on the amount of absorption under conditions where equilibrium could be approached. The vermiculite used in this work had a cation exchange capacity of 160 m.equiv./100 gm.

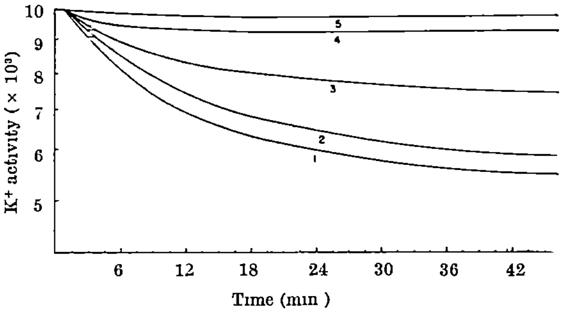

Fig. 1. Potassium activity in the presence of 0·25 gm. of 150–250μ diameter vermiculite as a function of time at 20° C. 1, No other salt; 2, 1 m.equiv. of *n*-butylamine hydrochloride; 3, 0·1 m.equiv. 2,4-diaminophenol dihydrochloride; 4, 1 m.equiv. aniline hydrochloride; 5, 1 m.equiv. 2,4-diaminophenol dihydrochloride

Fig. 1 shows the effect of some organic salts on the activity of potassium in the presence of vermiculite as a function of time. The activity measurements were made continuously with the 78137 *V* cationic electrode produced by Beckman Instruments, Inc., in combination with a Beckman Zeromatic *p*H meter and a Sargent recorder. The potassium chloride solution was stirred continuously with a magnetic stirrer. After the recorder deflexion was static, the organic salt was added. There was only a small effect of the organic salts on the potassium ion activity since the amount added was not enough to affect the activity coefficient of the potassium ion a great deal. Also the electrode was insensitive to the organic ions used although it was found to be sensitive to the presence of ammonium ions. At zero time 0·125 gm. of vermiculite was added. A short lag time of about 1 min. is apparent in Fig. 1 before the potassium started disappearing from solution. This lag time was noted

Table 1 001 Spacings of Vermiculite after Contact with Potassium Chloride in the Presence of Various Salts

Compounds	M equiv of compound present	Vermiculite (gm)	M equiv of KCl present	Hr contact	001 spacing (Å)
None	—	0 25	0 5	2	12 1 and 10
2,4-Diaminophenol dihydrochloride	1 0	0 25	0 5	2	14 5
2,4-Diaminophenol dihydrochloride	0 1	0 25	0 5	2	14 3 and 12 6
Aniline hydrochloride	1 0	0 25	0 5	2	14 5
n-Butylamine hydrochloride	2 0	0 25	0 5	2	12 1 and 10
n-Butylamine hydrochloride	1 0	0 25	0 5	2	12 1 and 10
n-Butylamine hydrochloride	0 1	0 25	0 5	2	12 1 and 10
p-Phenylene diamine dihydrochloride	0 25	0 125	0 25	48	14 5–13 0
Histidine hydrochloride	0 25	0 125	0 25	48	13 8–12 3
Ammonium chloride	0 25	0 125	0 25	48	11 3 and 10
Lysine hydrochloride	0 25	0 125	0 25	48	11 3 and 10

in all cases and is probably the time required for the complete wetting of the mineral The magnesium ion was initially the saturating cation on the exchange complex of the vermiculite, the electrode being insensitive to this ion

The main observation to be made is that *n*-butylamine hydrochloride (curve 2) had little effect on the rate of absorption of potassium as compared with the rate when no organic salt was present (curve 1) On the other hand, 2,4-diaminophenol dihydrochloride (curves 3 and 5) and aniline hydrochloride (curve 4) greatly affected potassium absorption The 2,4-diaminophenol dihydrochloride added at the 0 1 m equiv -level (curve 3) reduced absorption of potassium vermiculite much more than did 1 0 m equiv of *n*-butylamine hydrochloride (curve 2) The 001 spacings of vermiculite obtained by X-ray diffraction 2 hr after the absorption reactions began are given in Table 1

Table 2 shows the amounts of potassium absorbed from solution by sodium-saturated vermiculite in the presence of various organic salts, ammonium chloride, and in the instance when no foreign salts were present These analyses were made on the solution phase with a Coleman flame photometer after 48 hr were allowed for absorption Large differences are apparent in the effects of organic salts on potassium absorption Particularly noteworthy is the fact that the *n*-butylamine hydrochloride had no effect whatsoever on potassium absorption at either of the two concentrations Also, as noted in Table 1, the 001 spacing of the lattice has contracted in exactly the same manner as where only potassium chloride was present On the other hand, some of the amines. particularly those with a cyclic molecular structure, greatly inhibited the absorption of potassium. Apparently the number of electrical charges per molecule was not too important since the aniline hydrochloride was about as effective as 2,4-diaminophenol dihydrochloride and para-phenylenediamine dihydrochloride in preventing potassium absorption Of the three amino-acid salts examined, histidine monohydrochloride was the most effective in inhibiting absorption Histidine possesses a ring structure while the other two amino-acids used are linear in form Histidine was almost as effective as the ammonium ion in preventing potassium absorption.

Table 2 Absorption of Potassium by Vermiculite in the Presence of Some Salts*

Salt	M equiv K absorbed/100 gm Salt/KCl ratio 0 1	 1 0
n-Butylamine hydrochloride	120	120
2,4-Diaminophenol dihydrochloride	108	36
Aniline hydrochloride	106	32
p-Phenylenediamine dihydrochloride	108	28
Tetramethylammonium chloride	118	100
Histidine monohydrochloride	112	58
Arginine monohydrochloride	108	96
Lysine monohydrochloride	120	88
Ammonium chloride	106	52
None	120 m equiv K^+/100 gm absorbed when no other salt was present	

* Absorption by 0 125 gm vermiculite in 200 ml of 1 25 × 10^{-3} *N* KCl after 48 hr at 20° C Each figure is the average of analyses of duplicate samples with extremely low variation between duplicates

Apparently the organic molecules which affect potassium absorption do so in a manner different from that of the ammonium ion Table 1 shows that the 001 spacing of the vermiculite collapsed to spacings of 11·3 and 10 Å in the presence of ammonium chloride However, the lattice remained pretty well expanded in the presence of the organic molecules which effectively prevented absorption Those organic molecules which did not greatly affect potassium absorption allowed a greater amount of potassium to enter the internal surfaces This resulted in a greater degree of contraction of the lattice as shown by the smaller values of the 001 spacings The potassium thus entrapped in the internal surfaces of the contracted lattice of the vermiculite was fixed and was not exchangeable under ordinary conditions of cation exchange

Since the vermiculite lattice was fully expanded in the presence of those organic cations which most effectively prevented potassium absorption, these ions undoubtedly prevent the entrance of potassium ions into the internal surfaces of the vermiculite structure This could be accomplished if the energy of adsorption of the organic ion were so large that the potassium from the solution phase could not exchange with the organic cation at the edges of the mineral. The potassium thus would not be able to move into the interlayer spaces Undoubtedly the mechanism of cation movement into the lattice is diffusion along cation exchange sites[1] In order for this to occur, the potassium must be able to exchange with other ions on the cation exchange complex If the cation exchange complex were 'clogged' with ions which potassium could not displace, the potassium would be unable to enter the internal vermiculite surfaces

In soils, a large number of different kinds of organic molecules appear as the result of the decomposition of organic matter Some of the organic molecules used in this work were more effective than the ammonium ion in preventing potassium absorption, while histidine, a possible product of decomposition of organic matter, was almost as effective as the ammonium ion Organic molecules may play an important part in the release and fixation of potassium by vermiculitic clays in soils The complete lack of effect of the *n*-butylamine ion on potassium absorption was anomalous since all the other compounds used in these experiments had some effect This amine apparently did not compete with the potassium for cation exchange sites

The apparent interaction of organic compounds in potassium reactions with vermiculite may be of importance in soils containing this mineral Fixation. and possibly release of potassium, may be modified by the presence of certain organic compounds

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[1] Walker, G F , *Nature*, 184, 1392 (1959)

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 6

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W 12), at 4 p m —Dr M. A Floyer "Possible Factors in the Mechanism of Hypertension" *

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W C 1), at 5 30 p m —Dr W H Thorpe "The Idea of Emergence in Biology"

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATION SECTION (at Savoy Place, London, W C 2), at 5 30 p m —Mr R G Medhurst "The General Problems of F M Multi-Channel Communications"

UNIVERSITY OF LONDON (at King's College, Strand, London, W C 2), at 5 30 p m —Dr F A E Pirani and Dr G F Elliott "Ballistic Missiles and Nuclear Weapons—Nuclear Physics" * (Fourth of seven lectures in War Studies Further lectures on November 13, 20 and 27)

UNIVERSITY OF LONDON (at King's College, Strand, London, W C 2), at 5 30 p m —Prof J Meixner (Aachen) "On the Theory of Linear Passive Systems" * (Further lectures on November 7 and 9)

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S W 1), at 6 30 p m.—Dr N Goodway "The U K A E A and its Relations with Industry"

SOCIETY OF INSTRUMENT TECHNOLOGY, CONTROL SECTION (at Manson House, 26 Portland Place, London, W 1), at 7 p m —Mr P R Wyman "Semiconductor Diodes and Rectifiers in Control Engineering", Dr G D Bergman "The Transistor in Control Engineering".

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the WOOLWICH POLYTECHNIC SCIENTIFIC SOCIETY, at the Woolwich Polytechnic, Thomas Street, London, S E 18), at 7 30 p m —Prof C W Davies "Ion Exchange Resins"

Tuesday, November 7

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1 15 p m —Prof G C Allen "The Impediments to Britain's Industrial Progress" *

SULGRAVE MANOR BOARD (in the Joint Staff Common Room, University College, Gower Street, London, W C 1), at 5 p m —Mr Stephen Toulmin "Science and the National Mind". (Further lectures on November 9, 14 and 16)

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Dr H E Huxley "Structural Aspects of Muscular Contraction" * (Seventh of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

INSTITUTION OF MECHANICAL ENGINEERS, LUBRICATION AND WEAR GROUP (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "Refrigeration Lubrication"

UNIVERSITY OF LONDON (in the Library of the Institute of Bankers, 10 Lombard Street, London, E C), at 6 p m —Prof D T Jack "Industrial Relations" * (Second of five lectures on "Britain's Economic Future" organized by the Department of Extra-Mural Studies and the Institute of Bankers)

SCIENCE FORUM (at Friends House, Euston Road, London, N W 1), at 7 30 p m —Prof L S Penrose, F R S "Genetics, Radiation and the Future".

Wednesday, November 8

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W 12), at 2 p m —Dr C P Stewart "Acid-base Metabolism in Modern Terms" *

BRITISH INSTITUTION OF RADIO ENGINEERS, MEDICAL ELECTRONICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Mr E L Gregory and Mr E A Piper "Electronics in Chromatography"

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Dr H E Merritt "Gear-Tooth Contact Phenomena"

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S W 1), at 6 15 p m.—Dr H E C Powers "Crystallization of Sucrose"

BRITISH INSTITUTE OF MANAGEMENT (at the Connaught Rooms, London, W C 2), at 6 30 p m —Field-Marshal the Viscount Slim, K G "Leadership" (Seventh Elbourne Memorial Lecture)

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMISTRY GROUP (at "The Feathers", Tudor Street, London, E C 4), at 6 30 p m —Discussion Meeting on "Derivatography as a New Thermoanalytical Method" introduced by Prof L Erdey (Budapest University).

WOMEN'S ENGINEERING SOCIETY (at "Hope House", 45 Great Peter Street, Westminster, London, S W 1), at 7 p m —Mr R Wailes "Windmills—Their Rise and Decline".

Wednesday, November 8—Thursday, November 9

ZOOLOGICAL SOCIETY OF LONDON, and the ASSOCIATION FOR THE STUDY OF ANIMAL BEHAVIOUR (in the Meeting Room of the Zoological Society of London, Regent's Park, London, N W 1), at 10 30 a m daily—Eighth Symposium

Wednesday, November 8

"Evolutionary Aspects of Animal Communication".

Thursday, November 9

"Imprinting and Early Learning"

Wednesday, November 8—Friday, November 10

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W C 2)—Conference on "Non-destructive Testing in Electrical Engineering"

Thursday, November 9

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1 15 p m —Dr H Judith Milledge "Form and Colour in Nature" *

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W 1), at 4 30 p m —Mr M J Lighthill, F R S "Sound Generated Aerodynamically" (the Bakerian Lecture)

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W 1), at 5 p m.—Dr Wm T Stearn "Some Puzzling Linnean Abbreviations", Mr P. R O Bally "The Retreat of Nature in Modern East Africa"

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W C 2), at 5 15 p m —Dr Harold Heywood "New Developments in the Use of Solar Energy"

UNIVERSITY OF LONDON (at King's College, Strand, London, W C 2), at 5 30 p m —Prof J Meixner "On the Theory of Linear Passive Systems".* (Last of three Special University Lectures)

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMATIC CONTROL GROUP (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Brains Trust on "Automatic Control" (Inaugural Meeting)

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W 1), at 7 p m —Dr R S Teeble "Applications of Microwave Spectroscopy"

RADAR AND ELECTRONICS ASSOCIATION (in the Lecture Hall of the Royal Society of Arts, John Adam Street, Adelphi, London, W C 2), at 7 p m.—Dr W. F Hilton "Space Communications Part 2 Orbits and their Economics".

ROYAL INSTITUTE OF CHEMISTRY (at Croydon Technical College, Fairfield, Croydon, Surrey), at 7 30 p m.—Mr R B Collins "Modern Colour Photographic Processes".

Friday, November 10

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Prof F Fenner (Australian National University) "Myxomatosis—a Study of Evolutionary Changes in an Infectious Disease" *

UNIVERSITY OF LONDON (at the Royal Holloway College, Englefield Green, Surrey), at 8 15 p m —Mr William Cooper "Science and Literature"

ROYAL INSTITUTION (at 21 Albemarle Street, London, W 1), at 9 p m —Prof W S Bullough "Modern Research on the Skin of Mice and Men"

Saturday, November 11

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 3 30 p m —Dr E J Lindgren "Reindeer Farming in Europe, Asia, and North America" *

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned

ASSISTANT LIBRARIAN (graduate with some library experience)—The Secretary and Registrar, The University, Highfield, Southampton (November 11)

LECTURER or ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, The University, Sheffield (November 11)

LECTURER or ASSISTANT LECTURER (preferably a specialist in the field of human geography with first-hand experience of a major overseas area outside Europe) IN GEOGRAPHY—The Registrar, The University, Leeds 2 (November 13)

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (honours graduate with appropriate training and research experience), to work as a member of a team undertaking research on hill sheep nutrition—The Secretary, Hill Farming Research Organisation, 48 Palmerston Place, Edinburgh 12 (November 13)

LECTURER in EXPERIMENTAL PHARMACOLOGY—The Secretary of University Court, The University, Glasgow (November 15)

LECTURER IN PSYCHOLOGY at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (South Africa and London, November 15)

ASSISTANT LECTURER or LECTURER IN ANIMAL HUSBANDRY—The Registrar, The University, Liverpool, quoting Ref CV/T (November 18)

LECTURER IN CYTOLOGY IN THE DEPARTMENTS OF BOTANY AND ZOOLOGY in the Faculty of Science—The Registrar, The University, Manchester 13 (November 18)

LECTURER or ASSISTANT LECTURER IN STATISTICS—The Registrar, The University, Sheffield (November 18)

SCIENTIFIC ASSISTANT (with at least G C E at "O" level in four subjects, including geography, with an interest in biology and a capacity for neatness and precision), for recording and mapping reported movements of locust swarms, copying-maps, and generally assisting in the geographical work of a small team—The Director, Anti-Locust Research Centre, 1 Princes Gate, London, S.W 7 (November 18)

LECTURER (with an interest in electronics or telecommunications) IN ELECTRICAL ENGINEERING at the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (November 29)

CHAIR OF PHILOSOPHY IN THE SCHOOL OF GENERAL STUDIES, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, November 30)

LECTURERS IN THE DEPARTMENT OF PHYSICS, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W.1 (South Africa and London, November 30)

PROFESSOR OF EXPERIMENTAL PHYSICS—The Registrar, The University, Nottingham (November 30)

READER or SENIOR LECTURER IN ARCHÆOLOGY at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (November 30)

LECTURER IN SEDIMENTARY PETROLOGY, to initiate and direct research on sedimentation in Cardigan Bay and St George's Channel, as part of an investigation into the origin and geological history of this region—The Registrar, University College of Wales, Aberystwyth (December 15)

LECTURER or ASSISTANT LECTURER IN THE HISTORY OF SCIENCE at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (December 31)

AGRICULTURAL OFFICERS (male, with a degree in agriculture or horticulture and at least two years postgraduate experience) IN THE DEPARTMENT OF AGRICULTURE, Tanganyika, for extension work among both African and non-African farmers and experimental work on agricultural stations—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W 1, quoting RC 213/145/01/C1

ASSISTANT CHEMIST (male or female, with H N C in chemistry or biochemistry or an equivalent qualification), for routine and development work—The Secretary, Wright–Fleming Institute of Microbiology, St Mary's Hospital Medical School, London, W 2

BIOCHEMIST (Basic grade) IN THE DEPARTMENT OF ENDOCRINOLOGY—The Hospital Secretary, New End Hospital, London, N W 3

COCOA OFFICER (male, under 45, with a degree in agriculture, preferably in cocoa extension or development work) IN THE DEPARTMENT OF AGRICULTURE, British Solomon Islands Protectorate, to assist in cocoa development and extension of cocoa planting and processing—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S W 1, quoting RC 213/18/02/C1

FISHERIES OFFICER (male, qualified biologist with an honours degree in a natural science, preferably zoology, and experience in research work) in Tanganyika, to investigate the ecology of inland waters in relation to fish production—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S W 1, quoting BCD 63/8/012/C1

HEAD (man or woman, honours graduate, preferably in physics) OF THE SCIENCE DEPARTMENT—The Headmistress, Sherborne School for Girls, Dorset

RESEARCH ASSISTANT (British Empire Cancer Campaign) (biochemist, preferably holding a Ph D , with experience in enzymology), to work on the effects of steroids on tissue metabolism—The Department of Chemical Pathology, Royal Free Hospital, London, W C 1

RESEARCH OFFICERS (FISHERIES) (male, under 40, with an honours degree in natural sciences or equivalent qualification, together with at least one year's postgraduate experience in appropriate research work) IN THE CO-OPERATIVE DEVELOPMENT AND FISHERIES DEPARTMENT, Hong Kong, for duty with a research team investigating the fisheries of the South China Sea, and to supervise the work of Assistant Research Officers—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S W 1, quoting RC 289/68/01/C1

RESEARCH OFFICERS (with a first- or second-class honours degree in chemistry, biochemistry or chemical technology, or equivalent qualification, plus at least two years postgraduate experience) with the Federal Government of Nigeria, to carry out laboratory research and development work on Nigerian raw materials—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W C 2, quoting Ref E 14/1

SENIOR LECTURER (man or woman) IN EDUCATION—The Principal, Radbrook College, Shrewsbury

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Other Countries

Department of Scientific and Industrial Research Road Research 1960 The Report of the Road Research Board with the Report of the Director of Road Research Pp iv+138+8 plates (London H M Stationery Office, 1961) 7*s* net [258

Frank Mason and Co , Ltd Director's Report and Statement of Accounts for the year ended 31st March 1961 Pp 8 (London Frank Mason and Co , Ltd , 1961) [258

Soil Mechanics Limited Geotechnical Pamphlet No 7 Chemical Consolidation in Civil Engineering Practice Pp xii (London Soil Mechanics, Ltd , 1961) [258

Privy Council Medical Research Council Special Report Series, No 300 Cerebral Infarction The Role of Stenosis of the Extracranial Cerebral Arteries By Peter O Yates and Edward C Hutchinson Pp viii+ 95+20 plates. (London H M Stationery Office, 1961) 14*s* net. [258

Department of Scientific and Industrial Research Geological Survey and Museum Geology and Ceramics a Brief Review of the Nature, Geological Occurrence, Processing, and Principal Industrial Applications of the Rocks and Minerals used in British Ceramic Manufacture By Dr P J Adams Pp ii+28 (London H M Stationery Office, 1961) 3*s* 6*d* net [258

The Edinburgh School of Agriculture , The Edinburgh and East of Scotland College of Agriculture Calendar for 1961-62 Pp 113 (Edinburgh The Edinburgh School of Agriculture , The Edinburgh and East of Scotland College of Agriculture, 1961) 1*s* [258

The North of Scotland College of Agriculture Calendar, 1961-62 Pp 146 (Aberdeen The North of Scotland College of Agriculture, 1961) [258

Other Countries

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Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO , LTD
ST. MARTIN'S STREET, LONDON, W C 2
Telephone Number Whitehall 8831 Telegrams Phusis Lesquare London

Advertisements only should be addressed to
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CONTROL OF GOVERNMENT EXPENDITURE ON RESEARCH

THE Council for Scientific and Industrial Research, since its constitution some four years ago, has been concerned with the most effective means of budgetary control of scientific research in Britain In its 1958 report the Council pointed out that, while it was possible to estimate research needs fairly accurately two years ahead, this could only be done provisionally five years in advance, and, accordingly, the Council sought as much flexibility as possible in allocating the funds at its disposal A year later the Council stated that the Treasury had agreed that, without prejudice to the rest of the five-year plan to which the Council seeks to work, the Council might enter into commitments for research grants at a substantially higher level than was possible with the sums provided in the plan and that this level should be reviewed year by year

In the Council's latest report, this policy was taken a stage further and the introduction was announced of an experimental scheme which should be more adaptable to rapid and unpredictable change Under it the Council proposes each year to try to forecast its needs for five years in advance and to agree with the Treasury, subject to approval by Parliament, first the expenditure for the following year, secondly, reasonably firm estimates for the two succeeding years, and thirdly, less-definite estimates for the fourth and fifth years This means that each year the estimates will be raised and brought forward one year, so that expenditure of each year will be planned against a fairly clear indication of probable expenditure in the years immediately following and with some regard to longer-term plans Major departures from the estimates for the second and third years would only be made if there were important unforeseen changes in the scientific or financial situation

This scheme seems to meet one comment made by the Select Committee on Estimates in its Fifth Report for the Session 1957–58, reviewing the work of the Department, when the Committee expressed the view that the timing of the termination of a piece of research could be as important as the timing of its initiation, and welcomed the Department's practice of regular reviews, not tied to the approval of the annual programmes, working independently of the advisory boards of the establishments Strong support for this more flexible policy is now forthcoming from the Committee which, under the chairmanship of Lord Plowden, reported last June on the *Control of Public Expenditure** This support is perhaps the more significant, first because although the Plowden Committee was not appointed to deal specifically with scientific or technical expenditure, it quickly distinguished the difficulties which characterize the control of such expenditure to-day, and secondly, because the difficulties encountered by the Council for Scientific and Industrial Research arise not simply from the mounting cost of scientific work but also from the effect of interlocking decisions taken elsewhere, as in educational policy, or in the fields of fuel, power, communications, space research, and the like

Under the traditional system in Great Britain, as it developed in the nineteenth century and has continued throughout the lifetime of successive Governments to the present day, the tendency is for decisions on expenditure to be taken piecemeal It is increasingly apparent that, where science and technology are concerned especially, this system is wasteful and liable to cause frustration and waste of resources of finance and material as well as of man-power This is fully recognized by the Plowden Committee, and the core of its proposals is the development and use by Government of long-term surveys of expenditure and resources.

The report is, in fact, a State paper of outstanding significance which deserves—and indeed demands—close study by all who are concerned with the public finance of research and development and the most effective allocation of available resources It is a sequel to an earlier report on Treasury Control of Expenditure from the Select Committee on Estimates in 1958, and the Committee was appointed in the summer of 1959 Several confidential reports have already been submitted to the Chancellor of the Exchequer Mr Selwyn Lloyd, and his predecessor, and the present report is a consolidation of those suitable for publication and discusses more especially the principles and practice of the control of public expenditure It should stimulate exactly that constructive thinking and foresight which are imperative in dealing with many of the proposals for research and development projects to-day. In the judgment of the Committee, the crucial questions are not those of detail—the precise nature of the organization and chains of command within and between Departments, important though these are The real problems are the wider issues of the purpose which the Government organization is attempting, its attitudes, what it regards as important, and its approach to its work on all matters involving public expenditure Such issues are not always clear-cut, nor do they lend themselves readily to specific recommendations, but the scientist or administrator who reads this report side by side, for example, with the concluding passages of Sir Charles Snow's *Science and Government*, may find it a suggestive experience, for the whole report is designed to indicate fruitful lines of development in the future

At the outset the Committee pays a handsome and deserved tribute to the work of the Civil Service which scientists should note. Changes, both in scope and character, in the activities of Government during the past two decades have produced strains in traditional structures and practices, and Lord Plowden and his colleagues have been impressed by the way the Civil Service has adapted itself to deal with these changes In particular, notable progress has been made in inter-departmental co-operation there is a growing realization that in place of single departments dealing with individual problems in comparative isolation, the public interest requires a team pursuing its common objectives with a single purpose. This attitude of mind, and the readiness to initiate changes to improve control of expenditure, were manifested throughout the discussions between the Committee and the permanent secretaries and other officials of the major Departments Furthermore, members of the Committee with experience of industry and commerce, and the specialist advisers who joined them for the purpose, consider that the scale and quality of effort directed to the provision of management services in Whitehall compare favourably with standards outside Departments with nation-wide organizations appear to be conducted with real appreciation of the scope for application of management services

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* *Control of Public Expenditure* Pp 33 (Cmnd 1432) (London H M S O, 1961) 1*s* 9*d* net

The report deals, first, with the system of making decisions about Government expenditure, next, with the responsibilities of the Departments and of the Treasury in handling expenditure, and thirdly, with the question of Parliamentary control; while a final section contains some concluding observations on the future management of public expenditure The central problem is that of how to bring the growth of public expenditure under better control and contain it within such limits as the Government may think desirable This is the matter to which the Plowden Committee devoted most attention, and it is important that it should be clearly understood that its report is concerned with the control of expenditure within the limits laid down by policy, and not with the use of finance to concert policy In practice, the piecemeal decisions which the Plowden Committee criticizes can have the effect of confining or impeding policy, and the definite conclusion is reached that decisions involving substantial future expenditure should always be taken in the light of surveys of public expenditure as a whole, over a period of years, and in relation to the prospective resources Admittedly, judgments of the relative importance of one kind of expenditure or another may differ, but much of the criticism of Government expenditure in the scientific and technological field would lose its force if more care were taken to provide public opinion with the means for sound, informed judgments

The new situation is attributed to the social, political and economic changes of the past two decades. Supply expenditure is now 22 per cent of the gross national product, compared with 12 per cent in 1930 and 6 per cent in 1910, while the public sector of the economy as a whole now employs about one-quarter of the total labour force and public expenditure of all kinds represents about 42 per cent of the gross national product Moreover, its increasing complexity, including the cost of advanced technological projects and scientific research, the financing of commercial risks that the private sector cannot take, aid of many kinds to under-developed countries and social insurance schemes of unprecedented scope, involve commitments several years ahead Further, the Budget is now seen as a sophisticated process in which the instruments of taxation and expenditure are used to influence the course of the economy, and the main weight of Parliamentary and public pressure is for innovations and improvements which cannot be effected without increases in public expenditure

To meet this situation, the Plowden Committee favours a reconstruction based on four elements. regular surveys of public expenditure as a whole, over a period of years ahead, and in relation to prospective resources, decisions involving substantial future expenditure being taken in the light of these surveys, the greatest practicable stability of decisions on public expenditure, so that long-term economy and efficiency throughout the public sector have the best possible opportunity to develop, improvements in the tools for measuring and handling public expenditure problems, including in particular major simplification of the form of estimates, modernization and clarification of the Exchequer accounts, and more widespread use of quantitative methods in dealing with these problems throughout the public service; and more effective machinery for taking collective decisions and the bearing of collective responsibility by Ministers on matters of public expenditure

One advantage of stability in expenditure policy noted in the report is regarded as the most important of all, although often overlooked. Such stability encourages sound planning by the Departments before the Government decision is sought. There is less danger of projects being rushed to decision to take advantage of a favourable event without being first subjected to adequate financial examination or measurement against other projects or means of securing the same purpose That, and failure to fit the project into a rational expenditure pattern, can lead to great waste, and the major proposals in the first two parts of the report are intended to strengthen the organization against faulty decisions of all these types On that ground alone they deserve close study by the scientist, for it is frequently in situations that arise out of scientific advance or technological change that the Government is required to act quickly and effectively and in the face of what appears to be great urgency, fanned by Parliamentary and public opinion Apart from improving the control of public expenditure, they could provide a real safeguard against such pressure leading to undue support in one sector of science or technology rather than another

The Committee's proposals are also designed to lead gradually to greater public interest in the priorities of expenditure, to stimulate academic thought about it, and to greater awareness of the implications and choices involved in policies as they come forward. The Committee emphasizes, in conclusion, the importance of building up an informed public opinion on the whole subject Public opinion has probably now come to accept that public expenditure and private expenditure both have their place in the national life, and that what is required is a balance between them There are differences of opinion as to where that balance should be, nor is the balance easy to find, for insufficient is known as to the relation of public expenditure to the issues of growth and solvency of the national economy Any Government, however, may find it difficult to maintain whatever balance it believes to be right in the face of the pressures, always powerful, to increase public spending in the modern community, and the best system and most up-to-date techniques of control will only succeed if public opinion is actively stimulated and enabled to take a balanced view of the alternative uses of national resources that are put forward, and to bring to the consideration of problems of public expenditure and resources the same qualities of common sense and realism that the average household shows all the time in handling its own affairs

As regards the tools of control of expenditure, proposals for a new lay-out for the Estimates have already been made by the Committee and these were published in a Treasury Memorandum included in the Third Report from the Estimates Committee for the Session 1960–61* The Government has already accepted the Plowden Committee recommendation for these changes, and the present report merely directs attention to one feature of the new lay-out, which includes for the first time for each Vote a statement showing how the total is divided between the categories used in the analysis in the National Income and Expenditure Blue Book The Treasury has also begun a review of the form of Exchequer accounts, but generally the Plowden Committee received the impression that insufficient study had been given both inside and outside Whitehall to the effect of the size and composition of public expenditure on the growth and solvency of the national economy, nor have clear criteria been established to permit a rational choice to be made between different kinds of expenditure

Developments envisaged in this report will call for a wider application of mathematical techniques, statistics and accountancy to problems of public expenditure, and there may also be scope for the use of the techniques generally described as operational research Stress is laid on the advantage of seizing every opportunity for interchange of ideas and experience in management with people in industry Further, since Parliament and public opinion no longer apply as systematic a restraint to public expenditure as they did in the past, the Plowden Committee suggests that some improvement is required in the arrangements to enable Ministers to discharge their collective responsibility for the review of public expenditure as a whole. Apart, however, from its stress on the importance of efficient management, the report makes some comment on Parliamentary control, though it does not go deeply into the relations between Parliament and the Executive in the control of expenditure Here its main purpose is to show how Parliament's influence and authority can best be brought to bear constructively and in this respect it is the complement of Basil Chubb's book under the same title, just as the major part of the report is a natural counterpart of Prof S H Beer's study, *Treasury Control*

There is thus much in this report that needs careful study by those concerned with the organization of Britain's research effort, whether in the purely scientific or the technological field The report supports some of the proposals of the Select Committee on Estimates in its Sixth Report for the Session 1957–58, but it would be interesting to see the report of Sir Claude Gibbs's committee on managing and controlling research projects The emphasis on the interlocking of public expenditure, for example, should be particularly noted, while the affirmation that in terms of administration it is unwise ever to attempt to make reductions or expansions in public expenditure by a process of equal percentage subtractions or additions all round will be warmly welcomed Departments cannot sensibly be treated equally, and steady or falling expenditures require just as careful examination as those which may be rising

In view of these pronouncements and the further emphasis on stability of expenditure, it is startling to find that the Council for Scientific and Industrial Research has suddenly, following the measures announced by the Chancellor of the Exchequer on July 25, suspended five major new grants that are imminent In the statement making this announcement, which concerns the grants for the nuclear reactors for Scottish universities, and for the Universities of Liverpool and Manchester jointly, and the University of London, respectively, and for two radio telescopes for the Universities of Manchester and Cambridge, the Council expresses concern that, in view of the exceptional rate of expansion in numerous fields, the demands on its funds for 1962–63 are likely to exceed the planned resources Until consideration of the detailed estimates for 1962–63 is complete, the Department is unable to enter into new major commitments

The manner in which the announcement was made, however, suggests that an element of high politics has prevailed, all the more in that, as noted already, the Department has hitherto been almost unique among Government departments in using the system of estimates of expenditure for five consecutive years in advance On the face of it, the Council, whether yielding to higher pressure or not, is departing from its own practice and flouting the unequivocal recommendation of the Plowden Committee Much, of course, depends on how long the suspension proves to be The projects are all the outcome of discussions between the universities and the Department, and it will not escape notice that meanwhile the radio-telescope for the Radar Research Establishment at Malvern is unaffected Long delay with the projects could warp development in two fields of science in which Britain has established a leading position It is, in any event, difficult to reconcile the announcement with the need to consider long-term requirements as well as the present situation which the Plowden Committee rightly stresses An early examination of the position by, and an authoritative statement from, the Minister for Science, who apart from this should be studying the Plowden Report with some care and urgency, seem imperative

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* Third Report from the Estimates Committee together with part of the Minutes of Evidence taken before Sub-Committee A, and Appendix Session 1960–61 Pp vi+58 (London H M S O, 1961) 4s 6d net

That is the more important if the informed public opinion on which the Plowden Committee rightly insists is to be built up, for full and accurate information is an essential basis for public confidence and sound judgment The point is the more significant in that in what is probably the weakest passage of the report, the Plowden Committee swings right against its concluding emphasis on informed public opinion and appears to endorse a policy of withholding from publication the long-term surveys of expenditure, prospective development of income or economic resources Unless the maximum information is supplied, informed discussion of the issues is impossible neither the perspective nor the basis for sound judgment is available, and Lord Hailsham might well reflect as regards the recent announcement by the Council for Scientific and Industrial Research, that in the case of departures from accepted policy, frankness and full publicity are the more essential Secrecy is no basis either for confidence or sound judgment

PARASITES OF MAN

Introduction to Parasitology, with special reference to the Parasites of Man.

By the late Prof Asa C Chandler and Prof Clark P Read Tenth edition Pp xii+822 (New York and London John Wiley and Sons, Inc , 1961) 78*s*

THE appearance of yet another edition of this well-known book will be welcomed by all parasitologists, but their welcome will be greatly saddened by the knowledge that Asa Chandler, whose long life was devoted to the study of parasites and to the alleviation of the suffering caused by them to man and other animals, died while he was in the act of preparing this new edition His great achievements in research and the devoted service he gave to human and veterinary medicine, and also to biology in general, will always be remembered by workers in these fields all over the world, for Chandler laboured, and was well known, in many countries, and the long list of his publications proves not only the width of his biological interests but also the practical value of the detailed work he accomplished

In 1918 he embodied his knowledge and experience in a book entitled *Animal Parasites and Human Disease*, the fourth edition of which was, in 1930, rewritten to provide a general introduction to parasitology , and the value of this second work was proved by the demand for further editions of it, its title being changed, in 1940, to its present form Now, in this tenth edition, we are given the revision prepared by Prof Clark Read, who has succeeded Prof Chandler in the chair of biology at the Rice Institute, Houston, Texas Prof Clark has, he tells us in his preface, taken over full responsibility for the chapters on the Protozoa and the helminths, that is to say, for 512 of the 796 pages of text, and he has rewritten the chapters on the arthropods with the aid of notes left by Prof Chandler

The result is a volume which fully maintains the high standard of the earlier editions It deals primarily with the parasites of man, but references are also made to parasites of domesticated and other animals, especially to those which are important in one way or another to human life and affairs A notable change, which some readers will perhaps regret, is the deletion of the chapter on spirochætes, these organisms being now treated in a chapter on arthropod-borne organisms other than Protozoa, which also deals with the viruses, bacteria, *Rickettsiae* and the group of organisms of uncertain systematic position, such as *Anaplasma, Eperythrozoon, Grahamella, Bartonella and Haemobartonella* Systematics have never been emphasized in this book, though necessary classifications, not always those favoured by English workers, are given, together with keys for the identification of species The book has, in fact, always been remarkable for its exposition, in lively but not abstruse language, of the principles of parasitology, though it has always contained at the same time a great deal of detail cunningly woven into the text Prof Clark has maintained this combination of attractive style and detail and the text is now as up to date as any book, in these days of very rapid advances in knowledge, can hope to be. The references appended to each chapter and the list given of journals in which advances of knowledge are recorded, add, as the good index and the abundant illustrations also do, to the value of the book Prof Clark is to be congratulated on his careful and competent completion of what must have been a difficult job G LAPAGE

TREES OF CONSEQUENCE

The Eucalypts

Botany, Cultivation, Chemistry, and Utilization By A R Penfold and J L Willis (World Crop Books) Pp xx+551+61 plates (London Leonard Hill (Books), Ltd ; New York Interscience Publishers, Inc , 1961) 80*s* net

SOMEONE reviewing a recent world conference in Brazil on eucalypt-growing made the rather sombre observation that the Earth is becoming covered with eucalyptus trees The first specimen known to science was collected less than 200 years ago at Botany Bay on Captain Cook's first voyage, and now plantations throughout the world are estimated to cover 3½ million acres The award of a volume in the series of World Crop Books is a mark of recognition of this success-story The genus owes its progress equally to its versatility and its utility One species or another can be provided to suit a wide range of habitats, often growing with remarkable vigour and sometimes in places which other trees find discouraging

The genus *Eucalyptus* is reckoned as having more than 500 recognizable species, of which many are of economic value In discussing eucalypt as a crop-plant, it is essential that we be clear what species we are concerned with, and inevitably much of this book is taken up with botanical descriptions and means of distinguishing one species from another The first few chapters contain an account of the genus, the characters of stem, flower and fruit. This leads up to sub-division of the genus and classification of the species, in which the system devised by Blakely is adopted.

The central part of the book deals with the eucalypts as crop-plants, their cultivation, their introduction as a forest-crop in many countries, the diseases and pests to which they are subject In discussing its uses, the authors acclaim the genus as the most valuable source of hardwood in the world, good for structural work, for furniture, for sporting goods (Can those forceful Australian cricket-bats be made of eucalyptus?), for plywood and for fuel No less remarkable in the genus is the wide range of essential oils, on which the authors write with particular knowledge, and from which are derived such varied products as antiseptics, inhalants, perfumes, sheep-dips and stain removers

In the last, and by far the longest, chapter an attempt is made briefly to describe each species, what it looks like, where found, what conditions it needs, and what it is good for The book includes some maps to show where eucalypts grow, an extensive bibliography, a good subject index, and some plates of uneven merit The authors write clearly and competently, handling in orderly fashion a great mass of information This book will prove a valuable guide to all who have to do with the production or products of this great genus, the stature of which as a world crop seems certain to grow mightier yet

T A Russell

COMPUTER DESIGN

Computer Logic

The Functional Design of Digital Computers By Dr Ivan Flores (Prentice-Hall Electrical Engineering Series) Pp xii+458 (London Prentice-Hall International, Inc, 1960) 45*s*

Design Fundamentals of Analog Computer Components

By Prof R M Howe Pp x+268 (Princeton, N J D Van Nostrand Company, Inc, London D Van Nostrand Company, Ltd, 1961) 7 50 dollars, 56*s* 6*d*

THE legend on the dust cover of the first of these books reads "How computers are put together—how they work—how to use them" This describes the book better than the title It is not a text-book on computer design, but is addressed to the general scientific reader of whom a minimum is required in the way of engineering and mathematical background Nevertheless, it contains a very solid treatment of logical design, the binary system, adders, multipliers, control units, input and output devices, magnetic tape, and so on, all will be found described here, together with the usual ration of Boolean algebra and formal logic More attention is paid than in similar books to the mechanical details of input and output equipment

Only a small part of the book is concerned with programming, but this part I found unsatisfactory It seems a mistake, now that modifier registers are the order of the day, to introduce the reader first to the old-time procedure for modifying instructions in the arithmetic unit The last chapter, on programming a particular scientific problem, is a veritable museum piece, and leads one to think that the author must have been entirely insulated from the development of programming techniques It exhibits the most primitive form of machine language coding possible, in which even the conversion of relative addresses in sub-routines to absolute addresses must be done by the programmer himself before his programme is punched In these days, even if a programmer does not use some form of automatic programming, he may expect to have the assistance of a more or less elaborate input programme which provides, among other facilities, automatic relative addressing The book would have been better without the last chapter

The purpose of the second book is to give users of present-day analogue computers some idea of how these machines came to be designed as they were The author believes, quite rightly, that a complete understanding of the capabilities of an electronic analogue computer cannot be obtained without a considerable knowledge of the component details and how they affect the computer solutions The book contains chapters on systems design, component accuracy, d c amplifiers, multipliers, function generators, recorders and miscellaneous equipment An important and valuable feature of the book is the large amount of detailed and comparative design information given about some of the better-known commercially available analogue computers The book is very well written, and those not themselves working in the analogue field may find it interesting as showing the remarkable way in which analogue computers have developed from the primitive devices that they were ten years ago to their modern form

M V Wilkes

THE BACTERIAL CELL

Synthesis and Organisation in the Bacterial Cell

By E F Gale (Ciba Lectures in Microbial Biochemistry, 1959). Pp vii+110 (New York and London. John Wiley and Sons, Inc, 1959) 3 50 dollars, 28*s*

ACCORDING to *The Times* of August 14, the National Union of Students reported in a memorandum to the Committee on University Teaching Methods that they have received complaints of lectures in universities being ill-prepared, badly presented and giving only what is already available in books One wonders to what extent these criticisms apply to teachers of biochemistry Biochemical research is so prolific and occasionally so disturbing even to basic concepts that an unrevised text-book more than ten years old is scarcely more useful to a final honours student than last term's cell-free bacterial extract It is possible that there exist lecturers who fear that "to be intelligible is to be found out"[1], but the same writer also affirmed that "the truth is never pure and rarely simple"[2]

These three eminently intelligible lectures by Dr Ernest F Gale are concerned with synthesis and organization in the bacterial cell, the truth about which grows increasingly complicated, but he selects, discusses and condenses into a small volume those findings from a vast accumulation of recent research publications which are of real significance The style is lucid, the topics stimulating and the book beautifully presented thus, all readers will admire the electron micrograph of Plate I and the fluorescent micrograph of Plate III. These lectures are a model of how to tackle one difficult task of a lecturer to final-year students, although no doubt such students made up only a very small proportion of his American audience.

This task was to re-assess the findings of ten years ago in the light of those made in the past two. For example, Dr. Gale shows that although many experiments concerned with amino-acid incorporation into disrupted *Staphylococci* contributed to our present knowledge of protein biosynthesis, some of the results were bewildering. Now, they may be interpreted readily since knowledge of the chemical nature of the cell wall has advanced and it is realized that certain amino-acids may be incorporated into substances other than true protein. The lecture reported in Chapter 1 deals with the structure of the bacterial cell, with an account of the cell wall and membrane, and Chapter 2 deals with amino-acid incorporation. Chapter 3, on nucleic acid and protein synthesis, is stimulating but the data are already in need of a second reappraisal. Since the lecture was delivered, the ribosomes in which bacteria are so rich and which excited so much interest a few years ago seem no longer to hold their central place in metabolism as templates for protein synthesis. "Ribosomes are non-specialized structures which synthesize, at a given time, the protein dictated by the message they happen to contain"[3]. As a consequence of the discovery of this type of RNA called 'messenger', Dr. Gale's convenient nomenclature of rna and RNA, according to the size of the molecule, will need revision. It is certain that present discoveries will lead to a tremendous spate of new publications, some of which will no doubt appear to be contradictory, and it is to be hoped that Dr. Gale will be persuaded to survey the field of protein biosynthesis again in about a year's time if only to lighten the burden of the harassed lecturer and his critical, if not angry, young audience.

The book contains few errors. A glucosyl residue in Fig. 2 lacks a hydroxymethyl group, while the apt quotation from Sir Thomas Browne omits whales from the list of prodigious pieces of Nature given in the first authentic edition of *Religio Medici* (1643) and states that the civility of the little citizens more nearly ('more neatly' is correct) "sets forth the wisdome of their Maker".

S. DAGLEY

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[1] Wilde, O. O'F., *Lady Windermere's Fan*, Act 1 (1892).

[2] Wilde, O. O'F., *The Importance of Being Earnest*, Act 1 (1895).

[3] Brenner, S., Jacob, F., and Meselson, M., *Nature*, 190, 576 (1961).

POLYMER CHEMISTRY

Preparative Methods of Polymer Chemistry

By Wayne R. Sorenson and Tod W. Campbell. Pp. viii + 337. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1961.) 79*s*.

THE polymer chemist in search of a good method for the laboratory preparation of a particular polymer has in the past been obliged in many cases to carry out a tedious and time-consuming search through *Chemical Abstracts*, all too often with the frustrating result that the desired information is contained in an inaccessible journal. The appearance of a laboratory handbook which collects details of the great majority of polymer-forming reactions and preparative data for a very wide range of polymers will therefore be noted with interest and probably also relief by workers in all branches of this subject.

This cannot have been an easy book to write. The field to be covered is very extensive indeed, it is expanding rapidly, and its sub-division into types of polymer or reaction is not always straightforward. Experimental methods in the literature range from the most meticulous high-vacuum techniques with highly purified materials down to the crudest 'cooking together' in air of commercial-purity reagents, so that the authors must have devoted a lot of thought to selecting the most suitable standards of procedure to adopt in a book of this type. There exists also the difficult problem of the reliability of various published preparations—How is this to be judged? It is therefore greatly to the authors' credit that satisfactory solutions to all these problems have been found.

After a short introductory chapter, some fifty pages are devoted to experimental techniques for the preparation, fabrication and characterization of polymers. The remainder of the book consists of detailed methods of preparation, divided into chapters headed "Polycondensation and Hydrogen Transfer Reactions", "Addition Polymers from Unsaturated Monomers", "Ring Opening Polymerization", "Non-classical Routes to Polymers", and "Synthetic Resins". Each sub-section, containing a number of representative preparations, is preceded by a brief theoretical introduction outlining the principles and mechanisms involved, and giving numerous references to sources of more detailed theoretical treatment. The experimental procedures, although mainly directed to polymer preparation, include quite a few well-chosen examples of reactions on polymers.

The book has many features which deserve favourable comment. The importance of purity of materials is stressed in Chapter 2 and again on numerous occasions throughout the book. The potential danger in many polymerizations is emphasized repeatedly, and where any particular preparation involves special hazard from the type of reaction or the poisonous nature of the materials, this is clearly stated in bold type. The book is very comprehensive, but on those occasions where topics are considered outside its scope, references to other sources of information are given. The lists of references at the ends of the chapters are most extensive, totalling more than five hundred. The methods included are of a high enough standard (with few exceptions) to satisfy everyone but the most demanding research worker, who can easily modify the procedures to suit himself. The details given in the preparations are not the over-abridged accounts which appear in some of our more space-conscious journals, but include numerous helpful comments. The information is thoroughly up to date, few of the latest types of polymer-forming reaction are omitted, except (understandably) in the field of inorganic polymers. In addition to a very good general index, there are indexes by initiator system, reaction system and functional groups. I was impressed also by the clear type, attractive layout and good binding. There are very few misprints.

This is a book which most polymer chemists would find useful. It would also be helpful in connexion with undergraduate courses on practical methods of polymer chemistry. It is an important addition to polymer literature which I recommend highly.

I. C. MCNEILL

Proceedings of the Natural Rubber Research Conference

Kuala Lumpur, 1960 (Sponsored by the Rubber Research Institute of Malaya, with the support of the Government of the Federation of Malaya and the Rubber Producers' Council of the Federation of Malaya) Pp x+986+xix (Kuala Lumpur Rubber Research Institute of Malaya, 1961) 53*s* 8*d*

THIS volume constitutes a comprehensive review of present-day research relating to natural rubber From the opening address the conference faced up to progress in competitive synthetic rubbers and showed from the papers collected in this volume the impressive and many-sided research activities designed to maintain and expand natural rubber production

The longest section, 436 pages, deals with scientific study of *Hevea brasiliensis* with the view of encouraging it to ever greater production of latex Several papers in each sub-section deal with nutrition of the tree, stimulation of latex yield on tapping, ground cover and weed control on the plantation, breeding and selection, pests and diseases A shorter section follows on the production of dry rubber with more stable properties and on modifying its properties at the latex stage or in the manufacturer's factory , a paper herein on factory processing of synthetic *cis*-polyisoprene indicates an absence now of an ostrich-like attitude towards such a synthetic competitor Chemistry and technology of latex occupy most of the remainder of the book

The book is likely to be found by botanists and biochemists to have interest in the individual reports themselves, and as an object lesson in the concerted effort on the one plant both from many disciplines and from many countries Larger libraries collecting the literature of rubber will no doubt obtain it and many rubber technologists will find it interesting to browse through W F WATSON

Elements of Functional Analysis

By L A Liusternik and V J Sobolev Translated from the Russian by Anthony E Labarre, Jr , Herbert Izbicki, and H Ward Crowley Pp ix+227 (New York Frederick Ungar Publishing Company, London Constable and Co , Ltd , 1961) 55*s* net

IN 1936, Liusternik wrote a report on fundamental concepts in functional analysis His summary of methods and results was expanded by Sobolev into a course of lectures, and the resulting account of the elements of functional analysis grew into the Russian edition of this book The date of the Russian edition is not stated, most of it has the air of having been written fifteen or twenty years ago, and the general style and outlook is similar to that of Gelfand, Raikov and Shilov in their work on normed rings (about 1946) which at the time led many mathematicians to appreciate the power of the new methods There is an isolated reference to a paper published in 1958 and a few others to publications of the late 1940's

The first four chapters are devoted to spaces and operators They are clear and concise, and as useful an account as can be found anywhere of what every young functional analyst should know The leading ideas are necessary not only to the functional analyst but also for research in almost any field Chapter 5 introduces the reader to the methods of spectral theory by bounded self-adjoint operators, this will form a good background to the large amount of recent work The mention of non-linear analysis in the title of Chapter 6 leads the reader to expect interesting and important developments, but the chapter is mainly concerned with rather heavy theorems on implicit functions and the calculus of variations J C BURKILL

Theoretical and Experimental Physics

By Dr H G Jerrard and Dr D B McNeill Pp xiv+624+6 plates (London Chapman and Hall, Ltd , 1960) 75*s* net

THIS book is primarily a manual of laboratory work in physics, but has the advantage that it also contains a considerable amount of the relevant theory and about fourteen hundred useful references as well It should be of great value to university students who are studying physics to the pass-degree level and also, in parts, to those taking an honours course in physics

Chapters 1 and 2 are essentially an introduction to the remainder of the book The former has three sections dealing with the representation of experimental results by means of graphs, experimental errors and units (including rationalized M K S units) Chapter 2 is a very useful one and is devoted to a general description of some of the standard apparatus and equipment used in a modern physics laboratory, it is divided into three parts, dealing with optical, electrical and more general apparatus

The remaining chapters, 3–20, then describe experiments in mechanics, properties of matter, heat, acoustics, geometrical optics, physical optics, electricity, electronics and magnetism The presentation in these chapters is a very novel one The right-hand pages contain a description of the experiments while the left-hand pages contain, in smaller type, material of a more advanced nature meant to amplify the work described on the right-hand pages The material on the left-hand pages may be omitted if the reader so desires

The appearance of the book is to be welcomed and it should prove stimulating to both students and to those who teach them D H TREVENA

Bibliography and Index of Geology and Allied Sciences for Wales and the Welsh Borders

1897–1958 By D A Bassett Pp 376 (Cardiff National Museum of Wales, 1961) n p

THIS bibliography covers works on geology and allied sciences that either deal specifically with Wales and the Welsh Border country, or contain references to the geology of these regions The period covered is 1897–1958 Earlier works are listed in several published bibliographies the titles of which are given by the compiler

The book is divided into a number of sections, the principal ones being a list of books, papers, etc , an author-index, and a subject-index There is also a useful section listing unpublished higher degree theses on the geology of Wales The first of these sections is further subdivided chronologically into sections for each year This does not make for ease of reference if, for example, one wishes to consult all the works of a single author, Again, the compilation of a subject-index is a task which must inevitably be affected by the personal equation This one is no exception, and it would be easy to fault it As a whole this bibliography seems somewhat over-elaborate, but nevertheless as a work of reference it will undoubtedly prove valuable to all workers in the field of Welsh geology V A EYLES

A SURVEY OF NEW PENICILLINS

By Prof. ROBERT KNOX
Guy's Hospital Medical School, London, S.E.1

SINCE Fleming's discovery of penicillin in 1929[1] and its later development by Florey and Chain and their collaborators[2] many new antibiotics have been discovered, some of them active against organisms not affected by penicillin, others with a broader spectrum of antibacterial activity. But penicillin (benzyl penicillin or penicillin G) is still in many ways the best. It is effective in minute doses against sensitive organisms, is often bactericidal, almost completely non-toxic, and does not show the rapid development of resistant mutants often seen with other antibiotics such as streptomycin. However, penicillin G is rapidly excreted and comparatively ineffective by mouth, it is liable to give rise to hypersensitivity reactions, sometimes fatal, and the range of its activity is limited—for example, many Gram-negative organisms are insensitive to it. Although most species of organisms do not become rapidly resistant to penicillin G either *in vitro* or *in vivo*, penicillinase-producing staphylococci all over the world have become an increasingly serious menace, especially in hospitals.

For many years it has been known that different types of penicillin were produced by *Penicillium chrysogenum* when cultural conditions were varied. When appropriate precursors were added to the fermentation liquor, as in the production of benzyl penicillin, chemical and microbiological assays of the penicillin formed were in agreement, but when the precursor was omitted from the fermentation liquor a substance was formed which gave the chemical reactions of a penicillin but had much less antibacterial activity. Kato[3] suggested that this was the penicillin nucleus which Sakaguchi and Murao[4] had claimed could be obtained by splitting benzyl penicillin with an amidase. Sheehan[5] reported that he had synthesized the nucleus, 6-aminopenicillanic acid, but the chemical synthesis was difficult and the yields were low. In 1959, Batchelor, Doyle, Nayler and Rolinson[6], at the Beecham Research Laboratories, isolated pure 6-aminopenicillanic acid from fermentation liquors to which no precursor had been added[7–12]. This was a discovery of the first importance and has deservedly been recognized as a major step forward in chemotherapy, both for its practical results and for its theoretical interest. 6-Aminopenicillanic acid could now be obtained in high yield from fermentation liquors with no added precursor. As a result of this work new penicillins are being produced at bewildering speed. Some of these represent important advances in chemotherapy, others are not quite in that class but may have real advantages in special conditions, while others are interesting more because of their theoretical possibilities or because they may serve as prototypes for better models in the future. But there has been a good deal of overlapping and confusion and in some cases the differences are only marginal. It is therefore worth while to survey the present situation and to consider what in fact has been achieved, what reasons there are for expecting further achievements in the future and what light these penicillins may throw on the mode of action of penicillin and the origins of penicillin resistance.

The New Penicillins

It was early realized that there were many penicillins which showed differences in antibacterial activity, for example, penicillin X (*p*-hydroxybenzyl) or K (*n*-heptyl penicillin). But it was soon accepted that the most active for general purposes was benzyl penicillin or penicillin G. At that time when we referred to the 'penicillin' type of antibacterial spectrum we meant the spectrum of penicillin G.

From 1953 onwards penicillin V or phenoxymethyl penicillin came into use[13–15]. It is acid-stable and effective by mouth. It has much the same spectrum of antibiotic activity as penicillin G, but there are differences, some of which may be important.

The first 'synthetic' penicillin was phenoxyethyl penicillin or phenethecillin ('Broxil', 'Syncillin')[16–18]. Like penicillin V it is effective by mouth. The relative merits of phenethecillin and penicillin V have been disputed. Phenethecillin gives higher blood levels than penicillin V, but against most sensitive species of organisms its *in vitro* activity is less. However, phenethecillin is more active *in vitro* than either G or V against some strains of penicillinase-producing staphylococci[19–24].

Shortly after this the discovery of methicillin ('Celbenin')[25–34] was announced. Methicillin was the first penicillin with considerable activity against large inocula of both penicillin G-sensitive and resistant staphylococci. The value of this discovery can scarcely be exaggerated. Methicillin has found an accepted place in the treatment of resistant staphylococcal infections[35]. It has also great theoretical interest. Besides being highly resistant to the action of staphylococcal penicillinase, it is a powerful inducer of the enzyme. Staphylococci are thus stimulated to form the enzyme by an inducer which they cannot use as a substrate and therefore cannot destroy. Methicillin, however, has its defects. Although adequate blood levels can be obtained its antibacterial activity against staphylococci is of the order of 1–4 μgm./ml. as compared with about 0.02 μgm./ml. for the activity of penicillin G against sensitive staphylococci. Methicillin is not effective by mouth and injections are sometimes very painful, though this difficulty can be overcome[35].

Phenoxypropyl penicillin (*PA* 248, Pfizer, Ltd.)[36] was reported to be slightly more active *in vitro* than phenethecillin against penicillinase-producing staphylococci. But it does not approach methicillin in activity against large inocula of these. Against other coccal infections it seems to have about the same activity as other oral penicillins such as penicillin V or phenethecillin.

Recently, new ground has been broken by the announcement that 6[D(–)α-aminophenylacetamido] penicillanic acid or α-aminobenzyl penicillin ('Penbritin')[37–40] has been synthesized at the Beecham Research Laboratories and tested experimentally

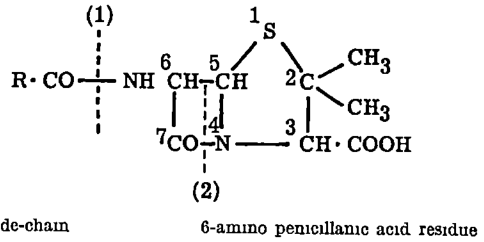


Fig 1 General formula of a penicillin (1) Site of action of penicillin amidase (splitting the amide linkage) (2) Site of action of penicillinase (giving rise to penicilloic acid)

and clinically It shows considerable activity against many organisms against which penicillin G is relatively ineffective, including many of the Gram-negative coliform organisms such as *E coli* itself, *Proteus*, *Salmonella*, *Shigella* and *H influenzae* It is thus the first broad-spectrum penicillin and is therefore of great practical and theoretical importance[41] Clinically it promises to be useful in the treatment of urinary infections and chronic bronchitis and in many gastro-intestinal infections, especially typhoid fever Its spectrum of activity is similar to that of the tetracyclines and chloramphenicol It has considerable activity against the cocci and other organisms naturally sensitive to penicillin G

The penicillins so far discussed have all shown sufficient promise to justify their commercial production for clinical use Two other substances should be mentioned here because of their great theoretical interest and importance 6-Aminopenicillanic acid itself, the penicillin nucleus, is the starting point for the synthesis of many new penicillins, either chemically or by means of amidases which occur in many species of micro-organisms Amidases can also hydrolyse penicillins into the free side-chain and 6-aminopenicillanic acid[42,43] This type of action is quite distinct from the opening of the β-lactam ring which occurs when 6-aminopenicillanic acid is acted on by penicillinase These two types of enzymic action are shown in Fig 1

Cephalosporin C [44] has a nucleus, 7-aminocephalosporanic acid, which consists of a β-lactam ring, fused, not with a thiazolidine ring as in 6-aminopenicillanic acid, but with a 6-membered sulphur-containing dihydrothiazine ring Cephalosporin C and related substances have some of the properties of penicillins Cephalosporin C itself like methicillin is very stable to staphylococcal penicillinase and is also a powerful inducer of the enzyme But its antibacterial activity is very low Although recently considerably more active derivatives of cephalosporin C have been obtained the activity is still less than that of penicillin G

Some of the main characteristics of these penicillins are summarized in Table 1

Different 'Functions' of Penicillin

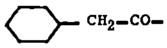
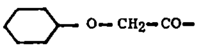
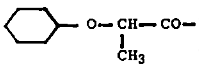
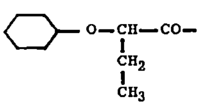
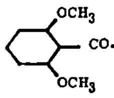
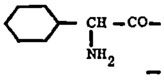
There are at least seven 'functions' or activities of a penicillin to be considered—toxicity, activity in the presence of protein, acid-stability, production of hypersensitivity, stability to penicillinase, ability to induce penicillinase and finally antibacterial activity. To be of real clinical value, a penicillin must if possible be non-toxic, active in the presence of protein, capable of producing adequate levels in blood in therapeutic doses, and, if it is to be given by mouth, acid-stable The new commercially produced penicillins have all these properties—except methicillin, which is ineffective by mouth So far as is known, patients hypersensitive to one penicillin are likely to be hypersensitive to another, but more clinical and experimental evidence is needed It is not even certain how frequent hypersensitivity to the older penicillins really is Some reports have suggested that allergic reactions, many of them serious, are becoming more frequent[45] A recent survey[46], however, shows that of 35,496 patients attending clinics for penicillin injections only 255 (7 2 per 1,000) showed reactions, and only 9 patients showed moderate or severe 'anaphylactic' reactions But the frequency of reactions increased up to 66 per 1,000 in patients receiving large doses for several days

Antibacterial Activity, Penicillinase Stability and Induction of Penicillinase in Different Penicillins

The three functions of antibacterial activity, of stability to penicillinase and of capacity to induce penicillinase can to some extent be segregated in different penicillins[47] This segregation gives the facts on which some useful working hypotheses may be based It will be useful to start by comparing the antimicrobial activity of these different penicillins in terms of their stability to penicillinase (Table 1) With staphylococci sensitive to penicillin G it seemed at first as though the more easily a penicillin was split by penicillinase the greater was its antibacterial activity But the correlation no longer holds with α-aminobenzyl penicillin, which is much more easily hydrolysed by penicillinase than penicillin V or G, yet rather less effective against sensitive staphylococci With penicillinase-producing staphylococci, at first sight it seems that the more resistant it is to penicillinase the more effective will a penicillin be With large inocula this is true, but not with small Small, and sometimes even moderate, inocula of these are

Table 1

MAIN FEATURES OF DIFFERENT PENICILLINS

Structural Formula R =	Name	Antibacterial Activity Gram+ cocci	Penase+ staph	Gram neg rods	Hydrolysis by staphylococcal penicillinase	Induction of staphylococcal penicillinase
	Benzyl, G	+++	–	(±)	++	+
	Phenoxymethyl, V	+++	–	–	++	+
	Phenoxyethyl (phenethecillin, broxil, syncillin)	+++ –	(±)	–	++ –	+±
	Phenoxypropyl (PA 248)	+++ –	(±)	–	++ –	+±
	2 6 dimethoxyphenyl (methicillin, celbenin, staphcillin) (BRL 1241)	+	+	–	–	++
	α-aminobenzyl (BRL 1341)	++	–	+	+++	+
–	6-aminopenicillanic acid	(–)	(–)	(–)	(±)	(+±)

more sensitive to phenethecillin or phenoxypropylpenicillin than to either penicillin G or methicillin, despite the fact that both are slightly more stable than penicillin G and very much less stable than methicillin to staphylococcal penicillinase. A penicillin stable to one penicillinase is not necessarily stable to others. Methicillin is only slowly attacked by staphylococcal penicillinase but is more vulnerable to *B. cereus* penicillinase and probably to penicillinases from some strains of *Proteus*[48] (though it has not yet been proved whether destruction of methicillin by *Proteus* is due to an amidase or a penicillinase). The only highly active 'Gram-negative' penicillin so far available, α-aminobenzylpenicillin, is the most susceptible of all to staphylococcal penicillinase[47].

There is a closer correlation between the stability of a penicillin to penicillinase and its capacity to induce the enzyme in that species (see Table 1). With 6-aminopenicillanic acid itself both the rate of hydrolysis by staphylococcal and *B. cereus* penicillinase and its ability to induce enzyme formation seem to vary considerably with the conditions used. For example, Steinman's[49] figures do not agree with those of Batchelor *et al.*[11], who found that the *p*H for maximal speed of hydrolysis was different for 6-aminopenicillanic acid and penicillin G, and that at their respective *p*H optima both substrates were split at much the same rate. As regards inducing ability, it is necessary to distinguish between the concentration required for maximal or half-maximal induction and the total amount of enzyme finally formed. But while the relative 'efficiency' of 6-aminopenicillanic acid and penicillin G as inducers of penicillins is likely to depend greatly on the conditions used, for example, *p*H, evidence so far available[47,49] suggests that neither is as good an inducer as methicillin.

Structural Basis for the Activity of Different Penicillins

With several enzymes, notably succinic dehydrogenase[50–52] and cholinesterase[53], a good deal is known about the relation of molecular structure to specificity of action. Similarly, the new penicillins are already beginning to give a clearer picture of the relation between the different aspects of penicillin activity. Considering first the enzyme-substrate aspect, we may picture a penicillin molecule diagrammatically (Fig. 2) as having a number of specific groups which become anchored to complementary groups on the 'active patch' of the enzyme penicillinase. If the fit between the penicillin and the enzyme is good enough then carbon atom (7) is pulled away from nitrogen atom (4) (Fig. 1), the β-lactam ring is opened, the penicillin is hydrolysed, and another penicillin molecule takes its place in apposition to the active patch of the enzyme, and so the process continues. A penicillin resistant to penicillinase is one in which sufficiently close contact cannot be achieved for the sequence of reactions which result in the splitting of the β-lactam ring. This might be because other groups such as the two methoxy groups in methicillin cause steric hindrance, or, as in cephalosporin C, because the molecule is not quite of the right shape for the necessary contacts to be made with receptors on the enzyme surface all along its length. For induction, however, there may not be the need for 'multi-point' contact but only for 'anchoring' at a small number of key-points (*I* in Fig. 2). The immediate precursor of the penicillinase molecule or indeed perhaps the molecule itself may be regarded as having active groups on its peptide backbone corresponding to complementary groups on the penicillin molecule. One of these might be the C=O group at C (7), others may be at the ends of the penicillin molecule. In the uninduced enzyme the apposition between these complementary pairs of groups is inaccurate. The actual process of induction may consist of 'straining' the molecule of penicillinase (or enzyme-forming site)[54] so that now the corresponding groups on the enzyme and the penicillin can be anchored, perhaps by hydrogen bonds[52], perhaps in other ways. An inducer which is stable to penicillinase then would be one which can strain the enzyme molecule so that it is now capable of hydrolysing any penicillin which has the necessary groups on its molecule. But the penicillinase-stable inducer itself will not be hydrolysed because it lacks these or because other groups on its molecule prevent contact with the corresponding receptors on the enzyme molecule. The enzyme once induced will maintain its activity so long as it is presented with a penicillin—either with one which it can hydrolyse or with one which continues to maintain the enzyme in the induced state. Pollock[55] studied the capacity of different penicillins and penicillin derivatives to induce the formation of penicillinase in *B. cereus* and established some of the structural requirements for induction. The value of such comparisons will be greatly increased as new penicillins become available.

The interactions occurring at the specific site may be regarded then as involving two main steps. In the first, the inducer exerts on the pre-enzyme, on the enzyme-forming system, or on the actual enzyme the same forces that the substrate exerts on the enzyme (or the enzyme on the substrate). Thus both a substrate and inducer are, as it were, 'conditioned' for the next stage—the splitting of the β-lactam ring. But with an inducer which is not a substrate the actual splitting does not occur for the reasons already suggested.

It is less easy to construct a convincing model of the antibacterial activity of different penicillins. Cooper[56] has suggested that the process of attachment of penicillin to the receptor site in the penicillin-sensitive cell is associated with the opening of the β-lactam ring, in other words with a penicillinase-like reaction. It is not known whether the receptor with which the penicillin reacts is a precursor of the enzyme penicillinase, or the enzyme itself, or a 'penicillin-binding component' or enzyme-forming system which reacts with the penicillin molecule in such a way that either the penicillin destroys the cell or the cell destroys the penicillin[55,56]. Whichever of these alternative theories we adopt it seems clear that similar if not identical receptors are involved in penicillinase induction and activity and in the antibacterial action of penicillin. I have already suggested that for efficient hydrolysis of a penicillin to occur by means of the enzyme penicillinase there must be 'multi-point' contact all along the molecule with the corresponding active centres on the enzyme surface. Similar, though not identical, closeness of fit may be necessary for antibacterial action, and the type of antibacterial action may be determined by the closeness of fit at different sites. Two of these seem to be especially important—the 'amide' carbon and the first carbon beyond it on the side-chain.

Fig. 2 represents diagrammatically the ways in which these different properties of a penicillin may be related and suggests the following hypothesis The capacity to induce is a property of all penicillins and it depends partly on the length of the molecule When induction occurs the surface of the enzyme or the enzyme-forming system is strained so that it is brought into intimate contact with the two ends of the molecule and with carbon (7) in the β-lactam ring The induced enzyme can be regarded as being under some sort of tension, and it is only when it is brought into intimate contact in this way with these key-points that the rest of the molecule is able to exert its hydrolytic effect Fig 2(1*a*) shows the situation that may exist with benzyl penicillin when it is brought into apposition with an active penicillinase For maximal Gram-positive activity the area marked in the diagram must be brought into apposition with a cell 'receptor', which may be the same as a receptor on the enzyme surface, for maximal Gram-negative activity the area marked Gram-negative must be brought into apposition with the corresponding 'Gram-negative' area The penicillinase-stable methicillin can still induce, but splitting of the β-lactam ring is impossible (Fig 2(2)) There is still room for some Gram-positive activity with penicillins of this kind but the Gram-negative activity must necessarily be lost, in other words, the very steric hindrance which makes a penicillin of this kind resistant to penicillinase will make it ineffective against the Gram-negative organisms which have a receptor surface which must be brought into close contact with one of the side-chains attached to the carbon next to the aromatic ring This activity is greater when one of the hydrogens in penicillin G is replaced by NH_2 (Fig 2(1*b*)), which brings about close contact with the 'Gram-negative' site

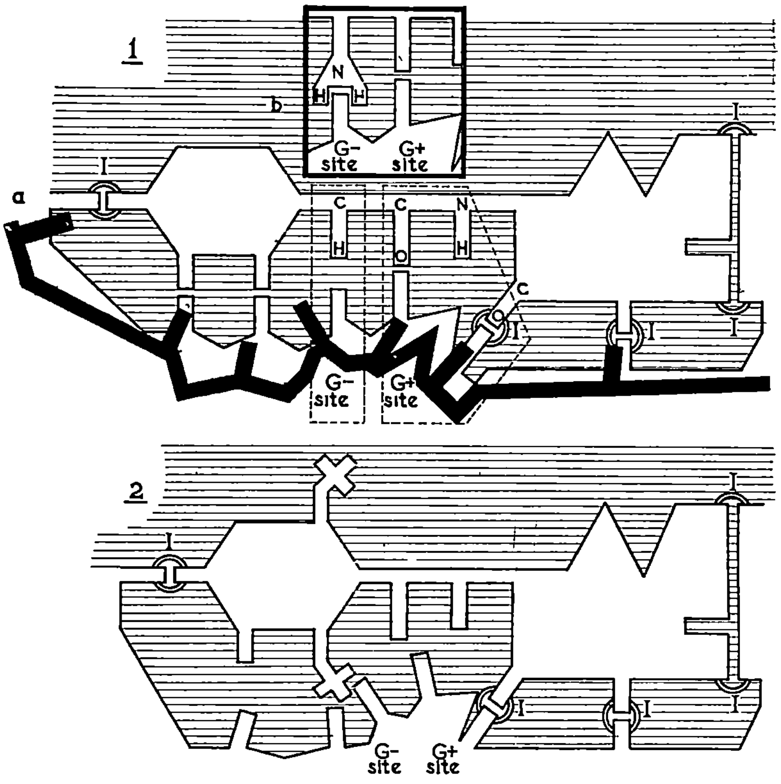


Fig 2

At the cellular level, there have been many theories about the site of the action of penicillin Many of the earlier observations can be explained in the light of recent evidence that penicillin interferes with the incorporation of amino-acids into the cell-wall and thus with the synthesis of cell-wall mucopeptide[57] Rogers and Jeljaszewicz[58] have shown that the order of effectiveness of different penicillins is the same for both antibacterial activity and interference with cell-wall mucopeptide synthesis

Resistance to Penicillin in Micro-organisms

With penicillin G clinically significant resistance is almost unknown except with the staphylococcus Other organisms such as hæmolytic streptococci, pneumococci, meningococci and others have remained fully sensitive to penicillin G In staphylococcal infections clinical failure is associated with the emergence of penicillinase-producing strains[59,60] These, though capable of destroying large quantities of penicillin when once the enzyme has been induced, are inherently sensitive to it and may be destroyed by it if they cannot produce penicillinase quickly enough On the other hand, in the laboratory it has proved easy to develop 'inherent' resistance of a non-penicillinase type in many different species of micro-organisms by serial sub-culture in gradually increasing concentrations of penicillin It is uncertain whether a similar non-penicillinase type of resistance is likely to be of clinical significance in any infections except perhaps for the gonococcus[61] It is also uncertain exactly how the penicillinase-producing types of staphylococci which are clinically so important actually arose It is doubtful whether there is any proved case of the emergence of penicillinase-producing staphylococci of the same phage type from an originally sensitive strain during the treatment of an individual patient with penicillin Thus penicillin shows the apparently paradoxical features of slow *development* of resistance and rapid *spread* of 'resistant', that is penicillinase-producing, strains by 'cross-infection'

It will therefore be particularly interesting to see what happens with new penicillins With methicillin a survey was carried out by Jevons at the Public Health Laboratory, Colindale[62] Of 4,430 strains from different regions only one showed increased resistance to methicillin So far it seems that methicillin-resistant mutants occur even more rarely than did penicillin G-resistant mutants in the early days of penicillin They have not so far been found to destroy methicillin In this respect they are rather more like the penicillin-resistant mutants produced by selection in the laboratory than naturally occurring penicillinase-producing strains Experiments we have carried out in our laboratory[63,64] have shown that staphylococci resistant to methicillin, whether naturally or by artificial selection in the laboratory, also show 'inherent' resistance to all other penicillins tested But with a mutant naturally resistant to about 25 µgm /ml of methicillin which showed 'inherent' resistance to penicillin G, further selection of mutants resistant to 1 000 µgm /ml of methicillin was not accompanied by any further increase in resistance to penicillin G More information is needed about the clinical importance and virulence of such mutants[65] and about cross-resistance in different penicillins

So far we have considered penicillin resistance almost entirely in staphylococci, but the new 'Gram-negative' penicillin, α-aminobenzyl penicillin, will raise some new problems Rolinson and Stevens[37] have pointed out that there is evidence for a penicillinase type of resistance in some strains of *E coli, Proteus vulgaris* and *A aerogenes* It is clear now that each new penicillin as it is produced creates special problems of its own Accurate information can be obtained only if careful records are collected in a number of different centres as a base-line for comparisons in the future

Conclusion

The isolation of 6-aminopenicillanic acid has already led to the discovery of penicillins with new properties and has given a new impetus to antibiotic therapy. The search for new antibiotics has become something more than an empirical screening of many groups of organisms for some new kind of antibiotic activity Deliberate and rational modifications to the penicillin molecule by the addition of specific side-chains have produced penicillins with different types of spectrum, and although the type of spectrum could not have been foretold from knowledge of molecular structure, once certain types of activity had been found, further modifications of the molecule could be made leading to improved activity of the kind desired There still remain groups of organisms untouched by any of the new penicillins, for example, mycobacteria, pseudomonads and viruses But the broad spectrum activity of a penicillin such as α-aminobenzyl penicillin raises some hopes that further modifications may produce other broad-spectrum penicillins, even perhaps some with antiviral activity. Quite apart from these practical results the isolation of 6-aminopenicillanic acid has opened up a fascinating field for research The specific activity of different penicillins has raised many new questions about the so-called penicillin type of antibacterial spectrum and about the nature of penicillin resistance, and at the same time it has helped to provide some of the answers, which may be of great value in relation not only to penicillin and other antibacterial drugs but also to the specificity of enzymes and their biosynthesis The diagram (Fig 2) represents an attempt to put into concrete form some of the conclusions which logically follow from the facts known at the present time It must inevitably be inaccurate in part and may even be regarded as a caricature of the facts If it does nothing more than arouse constructive criticism it will have served its purpose

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TRANSFER OF STRATOSPHERIC FISSION PRODUCTS INTO THE TROPOSPHERE

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MOST programmes of fall-out measurement are designed primarily to provide a regular inventory of the radioactivity from nuclear test explosions with the purpose of computing the radiological dose to human populations. In addition, the radioactive debris during its passage through the atmosphere serves as a valuable tracer of meteorological processes.

The significant parameters in this meteorological tracer experiment fall into two classes. Measurements are made of the concentration in air or in rain-water of (*a*) the long-lived fission products, as an index of the rate of fall-out, and of (*b*) the ratio of short-lived to long-lived fission products to give the 'age' of the debris, that is, the ratio of mixing between debris from recent and earlier test explosions. The mixing ratio is derived from comparison of the measured fission product ratio with that calculated for a fresh mixture. The data for several of these tracers is assembled in Table 1, using the yields from fission by 14-MeV neutrons of uranium-238 published by Cunninghame[1] and by Ford and Gilmore[2].

Table 1 FISSION PRODUCT DATA

Fission product	Half-life	Fission product ratio	Value of ratio at date of fission
Cæsium-137	30 years	Sr^{89}/Sr^{90}	185
Strontium-90	28 years	Zr^{95}/Cs^{137}	145
Cerium-144	285 days	Ce^{144}/Cs^{137}	~ 28
Zirconium-95	65 days		
Strontium-89	50 days		

The present discussion is concerned with the mixing between fission products from the 'polar' stratospheric injections at 75° N in October 1958 and those from earlier injections, in particular from 'equatorial' injections at 11° N about June 1958. Evidence relevant to the transfer of fission products from stratosphere to troposphere will be presented in three forms, describing the variation of the fall-out parameters with latitude, time and with altitude. This evidence is based on measurements made at Harwell, which are in substantial agreement with the results of work reported elsewhere, notably in the United States by Lockhart *et al*[3,4].

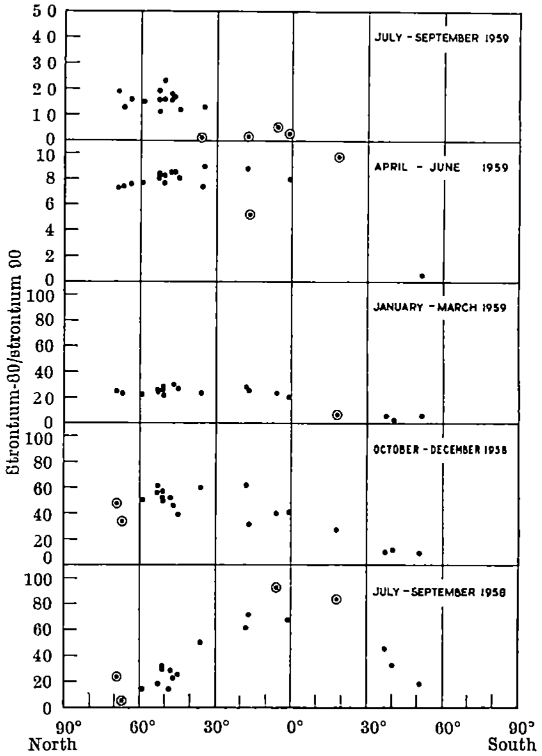

Fig 2 Ratio strontium-89/strontium-90 in rain-water v latitude, 1958–59
[O, Uncertain ratio due to low activities]

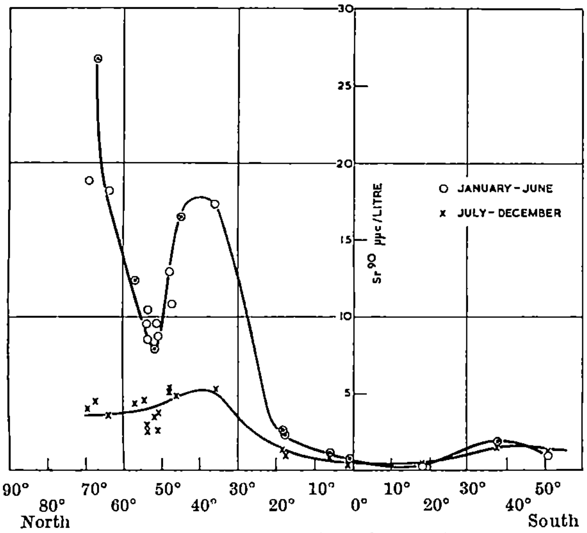

Fig 1 Mean strontium-90 content of rain-water, 1958–59

Variation with Latitude

The variation with latitude of the strontium-90 content of rain-water has been published by Crooks *et al*[5] for the period 1957–59. The main features of the variation, averaged over this period, is the rise to maximum values in the northern hemisphere in sub-tropical latitudes and above 60° N. The relative distribution varies with season as in Fig 1, which shows the mean concentrations of strontium-90 during the months January–June and July–December. Although the equatorial minimum is maintained throughout the year, the distribution above 30° N is comparatively uniform during the second half of the year.

A qualitative impression of the origin of the fission products during 1958–59 may be obtained from a study of the ratio strontium-89–strontium-90 in the same rain-water samples[6]. The variation of this ratio with latitude is shown in Fig. 2 for three-monthly periods from July 1958 to September 1959

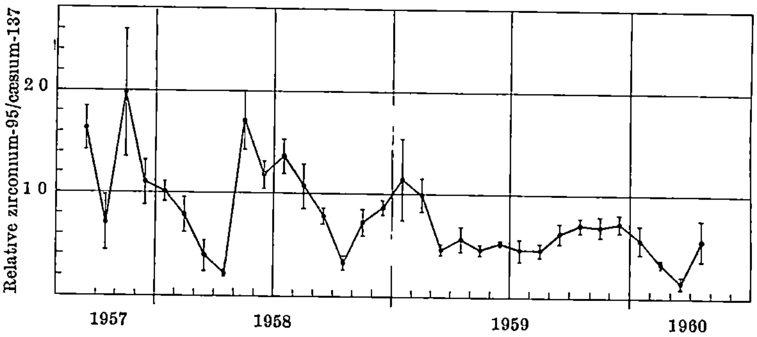

Fig 4 Ratio zirconium-95/cæsium-137 at 47,000 ft relative to 4,000 ft

From October 1958 the ratio strontium-89 – strontium-90, and hence the ratio of polar to equatorial debris, remained substantially independent of latitude in the northern hemisphere In the period July–September 1958, however, stratospheric fall-out from injections of fission products near the equator increased the ratio strontium-89–strontium-90 at low latitudes in both hemispheres

The content of strontium-89 in samples collected during this period is assigned to a stratospheric source because of the behaviour of the ratio[6] strontium-89–strontium-90 with time In the southern hemisphere, the ratio decreases from June 1958 according to radioactive decay The same observation can probably be applied to samples collected in the northern hemisphere, although in this case the record is confused by the arrival of fresh radioactivity from the test explosions of October 1958 If the strontium-89 were primarily tropospheric, it would disappear more rapidly, at a rate consistent with a tropospheric residence-time of 30 days[7]

Variation with Time

A periodic variation in fission product concentrations has been demonstrated[5,8] for samples taken from the troposphere and from rain-water in temperate latitudes of the northern hemisphere It was suggested by Stewart *et al* [9] that the observed periodicity is consistent with the meridional circulation in the stratosphere postulated by Brewer[10] to explain the low water content of the temperate stratosphere Recently, Goldsmith and Brown[11] have suggested that this ciculation is confined to the lower stratosphere. Martell[12] attributed the seasonal increase mainly to rapid fall-out from Russian stratospheric injections at high latitudes in the immediately preceding autumn and winter However, the spring maxima in 1955, 1960 and in 1961 followed autumns without stratospheric injection, and it can be shown that a peak remains in 1959 after the effect of stratospheric injections during the previous autumn has been subtracted[8] It is therefore probable that the seasonal cycle has a meteorological origin.

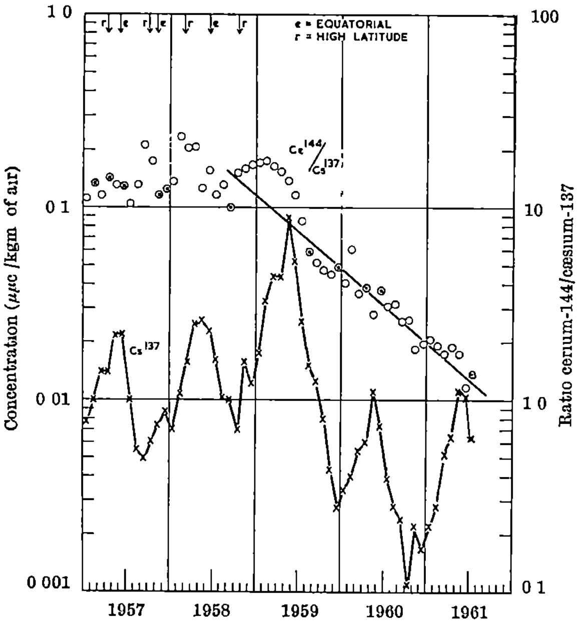

Fig. 3 Concentration of cæsium-137 and cerium-144 in air near ground-level at Chilton, Berkshire

In considering the variation with time of the isotope ratios, it has already been noted[8] that at ∼55° N the ratio zirconium-95–cæsium-137, in the case of tropospheric samples, and the ratio strontium-89–strontium-90 (rain-water samples) decreased according to radioactive decay, indicating uniform mixing of new and old debris, during the first six months of 1959. About June 1959 a discontinuity occurred in the isotope-ratio curves This effect is repeated in the record[6] of cerium-144–cæsium-137, shown in Fig 3, which extends the observations in air near ground-level at Chilton (Berkshire) until mid-1961. Owing to uncertainty in the fission yield data, there is not exact agreement in the proportions of polar and equatorial debris calculated from the various isotope ratios Nevertheless, using the cerium-144–cæsium-137 results, it is clear that whereas during January–June 1959 the proportion in these samples of long-lived debris produced by the Russian polar injections of October 1958 was about 70 per cent, for the period from mid-1959 until mid-1961 this proportion decreased to a value not greater than 20 per cent. This lower proportion was maintained through the subsequent spring peaks in 1960 and 1961, although the individual fission product activity varied by a factor of about five between spring and autumn

Variation with Altitude

This evidence is less general than the previous forms, in that the observations at the upper altitudes are restricted to the vicinity of the United Kingdom In January and February 1959 the ratio of polar to equatorial debris (measured by zirconium-95–cæsium-137) in the lower stratosphere at 47,000 ft and ∼55° N was similar[8] to that observed later in the troposphere. During the remainder of the year (Fig 4), the ratio zirconium-95–cæsium-137 was significantly higher in the troposphere than in the lower stratosphere[8], implying a higher proportion of polar debris in the troposphere

A Model of the Transfer Mechanism

The observations summarized above provide, in combination, a set of conditions that must be satisfied by any model of the transfer between stratosphere and troposphere One possible model will be described

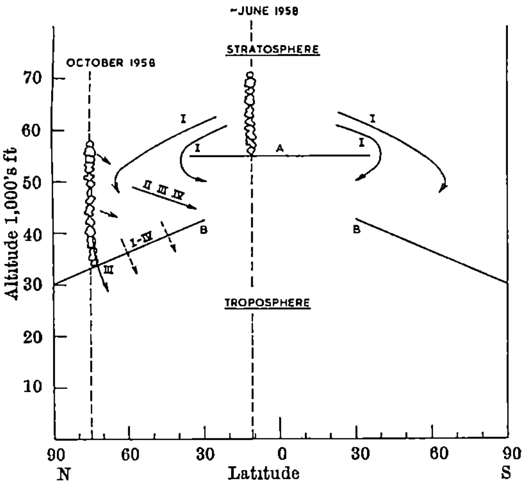

Fig 5 Transfer from stratosphere to troposphere

A, equatorial tropopause
B, temperate-polar tropopause
I, July–September 1958
II October–December 1958
III, January–March 1959
IV, April–June 1959

that attempts to explain the behaviour of the debris from the stratospheric injections in 1958 ('Hardtack') at 11° N about June and Russian at 75° N. during October Because the measurements of fission products are reduced to monthly average values, the model cannot account for short-term meteorological changes.

The movement of the debris is illustrated in Fig 5, which is a schematic diagram of the atmosphere showing stratosphere and troposphere separated by nominal tropopauses, equatorial and temperate-polar. Stratospheric fission products from the equatorial injection passed readily through the gap between equatorial and temperate tropopauses (Period I of Fig. 5) On the other hand, the polar debris appeared in the troposphere only after reasonably complete mixing with older debris the mode of entry during the autumn of 1958 (II) appears to be mainly through the same gap, but also possibly through the temperate-polar tropopause

It is only during the early months of 1959 (III) that a significant route appeared at high latitudes in the temperate-polar tropopause, in temporary competition with the gap between equatorial and temperate tropopauses Since the area of a band of latitude decreases with increasing latitude, it should be noted that the high concentrations occurring polewards of 60° N refer to only a small proportion of the total activity of the fission product The timing of this phenomenon is in accordance with the Brewer–Dobson circulation, in which the lower stratosphere is supplied in mid-winter by a subsidence of cold polar air. Despite the two possible paths of entry during this period, tropospheric samples were of such uniformity in the ratio of fission products (Fig 2) that the two streams must have arisen from a well-mixed source of equatorial and polar debris The mixing of debris must have occurred either in the lower stratosphere or in the troposphere If mixing occurred in the troposphere, under the influence of a tropospheric circulation system, then it is difficult to reconcile the uniform distribution of the ratio strontium-89–strontium-90 with the non-uniform distribution of strontium-90 (Figs 1 and 2) It is necessary, therefore, to postulate that mixing took place in the lower stratosphere to account for uniformity of the ratio of fission products, and that the distribution of individual fission products with latitude is probably controlled by the points of entry through the tropopauses, assisted by the tropospheric circulation system

In spring and early summer 1959 (IV), the exchange through the temperate-polar tropopause diminished, leaving the gap between equatorial and temperate tropopauses as the dominant path of entry. By this time the source of polar debris was largely exhausted and the supply to the troposphere contained a higher proportion of equatorial fission products

This new mixing ratio was maintained from mid-1959 through 1960 and into 1961 (Fig 3), apparently undisturbed by the Brewer–Dobson subsidence that would occur in the winters of these years This more recent evidence suggests that any residual polar debris (October 1958) was stored higher in the stratosphere with equatorial debris from 'Hardtack' (1958), and probably from earlier test explosions such as 'Redwing' (1956) and even 'Castle' (1954) The polar debris appeared as a small fraction of the well-mixed tropospheric samples collected during 1960 and 1961 This fraction, calculated from the ratio cerium-144–cæsium-137, is consistent with a date of origin lying between November 1957 and February 1958 A similar calculation made by Parker and Crookall[13] for samples collected during 1960 gave a mean date of origin in April–May 1958 The two sets of experimental ratios of fission products are in good agreement. the difference in estimated date is due to the assumption of different fission yields for the two isotopes

It was observed that during most of 1959 the proportion of polar debris sampled in the lower stratosphere over the United Kingdom was lower than in the troposphere It is difficult to fit this evidence into the scheme proposed in Fig. 5 Since it may represent a local anomaly, it is sufficient at present to note that in the latitudes of the United Kingdom the stratosphere and the troposphere maintained separate identities during this period This suggests that the two regions of the atmosphere are supplied from reservoirs of radioactive debris by separate routes and that there is little local mixing between them Alternatively, the difference in isotope ratio could be explained by a wash-out mechanism in the troposphere that favoured selective removal of older debris, or by selective filtering in the air-sampling apparatus From other evidence both these explanations are unlikely

Conclusion

The variations of certain fall-out parameters, namely, the concentration and ratio of fission products, have been summarized in relation to latitude, time and altitude These phenomena are influenced by the mechanism of transfer of fission products from the stratosphere into the troposphere

A speculative model of this mechanism has the main features that follow, based upon the behaviour of fission products injected into the stratosphere in the summer and autumn of 1958

Debris from the polar injection was well mixed with other debris before entry to the troposphere The tropospheric circulation system may contribute to the distribution pattern of the debris with latitude, although this appears to be controlled by the points of entry through the tropopauses

Well-mixed debris escaped in the early months of 1959 through the temperate-polar tropopause at latitudes greater than 60° N This escape was probably associated with subsidence of stratospheric air, according to Brewer and Dobson, and suggests a virtual disappearance of the troposphere at these latitudes

Most of the polar debris appeared in the troposphere during the first six months of 1959 A small residue from the polar injection may have lodged above the lower stratosphere This residue would have been brought down, together with older debris, during subsequent winter subsidences, and appeared as a well-mixed fraction of the fission product concentrations measured during 1960 and 1961

The proportion of polar debris in the lower stratosphere above the United Kingdom in 1959 was lower than in the troposphere This effect may be restricted in extent and have no great significance in a global model

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ELECTRICAL POWER DEVELOPMENT

THE annual meeting in June of the British Electrical Power Convention was held, as has been customary in recent years, at Eastbourne, under the presidency of Sir John Pickles, chairman of the South of Scotland Electricity Board In his presidential address he gave emphasis to the theme of the Convention—"Electricity in the Prosperity and Welfare of the Nation" The address was in the form of a review and prospect of the electricity supply industry in Britain from A D 1880 to 2000

Sir John considered that there would be a doubling of consumption in the ten years to 1970 and that this rate of increase would be maintained, with a further doubling by 1980, or 1985 at the latest This implies an increase in the country's installed generating plant capacity, at present 32,000 MW , up to 100,000 MW , the greater part of this probably being nuclear There would probably be 1000-MW single generating sets, and their operation, together with that of their boilers, would be controlled by computers In transmission a 750-kV system seems not unlikely Sir John concluded by referring to the possibilities of direct conversion of heat to electricity without the intervention of a steam cycle, attention being focused on thermionic, thermo-electric and magnetohydrodynamic generation The latter, comprising direct generation by the passage of hot gas through a magnetic field, is the most promising Nuclear fission may well prove highly suitable as a heat source

The second speaker, Sir Christopher Hinton, chairman of the Central Electricity Generating Board, described the British electricity distribution system from the inception of the 132-kV grid, which was intended for the transfer of energy between the large self-contained areas under conditions of difficulty or emergency Much expert opinion at that time held that it would be impossible to interconnect the complete system without leading, under conditions of rapidly changing demand, to major power flows which could not safely be handled Notwithstanding this, the London control engineers interconnected all the separate areas by means of the grid "to see what would happen" The interconnected system—the largest in the world controlled from one centre—has worked ever since Various extensions and reinforcing interconnexions have been added, giving additional security and flexibility

Work carried out at the formation of the British Electricity Authority and the area boards under the Electricity Act of 1947 showed a sound case for siting new generating plant near coalfields This implied the provision of a higher voltage transmission system superimposed on the existing grid A transmission voltage of 275 kV was selected, this being the next higher International Electrotechnical Commission standard It was considered that this voltage is not so high as to lead to possible insulation difficulties in the atmosphere of Great Britain Tower clearances, however, allow for up-rating in the future to 380 kV —the next higher level—when insulation problems have been solved So the 275-kV super grid system in Britain was formed, developing mainly into ring mains of double-circuit lines connecting large-capacity generating stations and substations with transformation, usually to 132 kV Transmission is by no means the junior partner of generation it is perhaps dominant, for without the present highly developed transmission system—planned with foresight and wisdom by the old Central Electricity Board and those associated with it—the modern 2000-MW generating station would be impracticable

Sir Christopher Hinton reported that the maximum demand on the system so far was 24,449 MW , on December 13, 1960, and he suggested that this might be trebled in fifteen to twenty years time The heaviest concentration of power stations would be in the central coalfields, with others in North Wales, the Severn Estuary, the Bristol Channel, the central south coast, the Thames Estuary, south-east Kent and the East Anglian coasts He showed a map giving the distribution of power expected in the mid-1970's, when the maximum demand is likely to be 70,000 MW and the projected 400-kV supergrid fully operative Nuclear generation should by then account for 30 per cent of the total consumption, indigenous fuel and oil consumption being roughly doubled The movement of coal long distances by rail should virtually have ceased by this time, and sea-borne supplies reduced to roughly half their present value As the load grows beyond 70,000 MW in the years after 1975, it is possible that a 700-kV. 'large mesh' network will be superimposed on the 400/132-kV system

Sir Christopher continued by explaining the many factors not susceptible of control by the area boards which make accurate forecasting of future needs very difficult. He referred to shifts of population, town planning legislation and Government decisions on residential and industrial development. Forecasting is simple on a national scale and difficult on a local scale.

About 40 per cent of the area of England and Wales is subject to restriction of some sort or other on the ground of amenity; but opposition to the construction of transmission lines and equipment is sometimes just as severe in industrial towns. Yet a transmission system of the present type is vital to British industry and essential if the very large stations (small in number and of high efficiency) are to be built. Although the public would not tolerate the ever-increasing extension of the network to a stage rightly described as a 'wirescape', underground cables for all additions to the network are out of the question on ground of cost. (A figure of about £100 million a year would be involved, representing 6*d*. on the standard rate of income tax, or 20 per cent on the bulk tariff.) The solution, perhaps a compromise, is to place underground the lower-cost distribution systems and leave the very high-voltage transmission system in the form of lines on towers. The latter must be accepted as canals, railways and motorways are, but the design of the structures must be the most graceful that can be devised and their routing must be most carefully planned. Sir William Holford set out in a paper to the Royal Society of Arts valuable rules relating to the siting of transmission lines. Switching stations, too, are now being most carefully considered. Sir Christopher Hinton concluded by emphasizing that the problem of visual amenity is one of the greatest facing the industry.

Mr. R. R. B. Brown, chairman of the Southern Electricity Board, presented a paper entitled "Some Aspects of Efficiency and Economy in Distribution". He reported that the national investment in distribution assets stands at more than £1,000 million, and additional expenditure at the rate of about £100 million annually is necessary to meet the expanding needs of the nation. Mr. Brown showed ways in which investment economies have been and will be achieved; and he dealt with tariff structures devised to encourage the full and efficient use of distribution systems. An interesting point made in connexion with standardization was that less than a hundred thousand consumers out of some 16 million now receive direct current supplies; consumers served by non-standard alternating current systems number about 1,500,000.

A paper by Dr. A. E. Grauer, chairman and president of the British Columbia Electric Co., Ltd., was on "The Electrical Power Industry in Canada". Canada, with a population of only 18 millions, is one of the world's largest producers (and consumers) of electric power. On a total output basis, she is behind the United States and the U.S.S.R., but on a par with the United Kingdom. Canada is second only to Norway on a *per capita* basis. Much of Canada's power is, of course, hydro-electric, and in Quebec power-rates are less than a third of the Canadian-American average. In Ontario and Manitoba they are about half the average in the United States. Canada's impressive power growth has been predominantly due to important power-consuming industries, smelting aluminium, refining copper, nickel and titanium and manufacturing inorganic chemicals. This growth has helped to open up new territory and expand Canada's world trade.

It is interesting that primary jurisdiction over natural resources, including water, was given to the Provincial Governments rather than to the Federal Government, and this, together with the huge areas of most of the provinces, has resulted in electrical utilities each essentially operating within one province. There are no electrical utilities which are national in scope.

Much of Canada's land mass (in area almost equal to China, which is itself second only to the U.S.S.R.) is virtually uninhabited, and population has tended to concentrate in a few areas strung mostly along the southern border and separated from each other by hundreds of miles. Almost a third of the population lives in the southern tip of Ontario. Nearly ten million Canadians occupy an area less than that of the United Kingdom.

The manner in which the electric power industry is organized varies a great deal from one province to another. In some provinces there is practically no private ownership, in others practically no public ownership; in others there is a mixture of both. The sizes of the utilities differ considerably, from Ontario Hydro, with a total capacity in excess of 6,000 MW, to quite small undertakings. Publicly owned utilities are usually run by a small commission reporting directly to the Cabinet. Investor-owned utilities operate under a Public Utilities Act administered by a Commission appointed by the Provincial Government. Fair rates are set by the Commission.

Canadians used, in 1959, 4,300 units of electrical energy per residential customer as compared with 3,700 units in the United States and about 2,000 units in the United Kingdom. One possible reason is that about 80 per cent of Canadians live in single or duplex dwellings. Also, the straight block-rate, with no demand charge, is almost universal, except for house heating. Off-peak tariffs are not used in Canada; industry works mainly on a three-shift system. It is interesting that Dr. Grauer's Company estimates each customer's bill for a ten-month period, sends him a bill each month for a tenth of this amount, and then balances the books by a payment of the difference either way at the end of the period.

Regarding rate of growth of electrical consumption, a relatively low figure might be expected from an already high consumption. Estimates point to an average figure of more than 6 per cent per annum and require the construction of more than 50,000 MW of new capacity by 1980. It is expected that most of the money to finance this will continue to be raised in Canada. This does not necessarily apply to the Peace River (British Columbia) and Hamilton Falls (Labrador) developments. It would appear that here are opportunities for British investment in Canada.

Canada's commercial water-power potential is considered by Dr. Grauer to be 70,000–80,000 MW; the present installed turbine capacity is about 20,000 MW. The largest undeveloped hydro resources are in Quebec and British Columbia, with Newfoundland and Manitoba following. Canada and the United States have, of course, signed a treaty respecting the Columbia River and construction is expected to start in 1962, with output of electrical energy by 1966.

Dr. Grauer made some interesting remarks concerning the development of thermal generation. The latter is necessary in some main industrial centres because of the existing full development of nearby water resources and because of the increasing costs of more remote hydro development. Steam-electric generation near the load centres is tending to cost less, improvements in design offsetting inflation. The margin of cost between hydro and thermal generation is thus narrowing steadily. A large coal-burning base load plant on the waterfront of Toronto can deliver electric power at a cost only about fifty per cent higher than can the new hydro-installations at Niagara and on the St. Lawrence Seaway. (About 1920 thermal electric power cost three to five times as much as hydro-electric power in southern Ontario.) Provinces using thermal generation in the future will be Alberta, Saskatchewan (with cheap fossil fuels and natural gas), Ontario (with almost fully developed hydro-resources she is importing coal from the United States) and British Columbia (with cheap residual oil and natural gas). Gas turbine plants are being built in sizes from 2,000 to 25 000 kW., but are unlikely to be used extensively because of their heavy fuel consumption compared with that of large steam turbines.

Canada has several large research reactors in operation, being particularly interested in heavy-water moderated systems fuelled by natural uranium. (Heavy water has been produced in Canada and could be again. Uranium is found in abundance.) The large conventional resources still available in Canada make it likely that nuclear power will first be commercially developed elsewhere, with southern Ontario as an exception to that statement in view of its present need to import coal from the United States.

Dr. Grauer made some useful comments on long-distance transmission and the possibilities of a national power grid. Most transmission in Canada is at 230 kV., with 360 kV. being introduced in recent years. Distances of transmission have not exceeded about 250 miles, but much longer distances are in prospect. Ontario Hydro is planning 500 miles of 460-kV. line. The Peace River proposal envisages 600 miles at 500-600 kV., and tests are under way, both in Canada and the United States. Present thinking in the U.S.S.R. is that distances less than 600-800 miles are more efficiently met with alternating current, while for distances greater than this, direct current is more efficient. Canada's distances are similar to those in the U.S.S.R. However, potential developments are so placed relative to population centres that few distances of greater than 600 miles are contemplated. Three large potential hydro-situations in the far north are about a thousand miles from the chief market and their development will presumably await the d.c. transmission of the future. Dr. Grauer believes that a national power grid, for a number of reasons (not the least being that natural gas in large pipe-lines can be transported for less than one-third the cost of transporting electrical energy over the highest voltage lines) is more likely to evolve over a period of time from regional systems stronger and more highly developed than exist to-day.

The final paper of the conference, presented by Mr. S. F. Steward, director of the British Electrical and Allied Manufacturers' Association, was entitled "British Electrical Manufacture in the National Economy". The British Electrical and Allied Manufacturers' Association, the trade association of the electrical industry in Britain, is this year celebrating the fiftieth anniversary of its foundation. The main aim in establishing the new trade association in 1911 was "to foster some order and regulation in the trading of its constituent firms". It is interesting to note that within a year or two of the Association's foundation the annual report invited "the earnest attention of members to industrial and scientific research as one of the bedrocks of commercial supremacy".

Early attention was given to the legal conditions applicable to contracts for heavy plant, and, in association with the Institution of Electrical Engineers, model general conditions were drawn up which, when published in 1914, became the first standard text of its kind in the world. Technical and commercial co-operation, publicity and export promotion were early recognized as main functions of a trade association. Standards were issued as early as 1913 for the use of member firms, priority being given to the standardization of electrical machinery.

Mr. Steward reviewed in considerable detail the development of the electrical industry throughout five decades, emphasizing the extent to which electrical manufacture has become a key industry. About ten per cent of the total United Kingdom export of manufactured goods represents electrical equipment. The total number of employees in the industry is nearly 800,000, 28 per cent of this number being engaged in the manufacture of electrical machinery and 38 per cent on electronics and telecommunications equipment. Electrical manufacture is the largest industrial employer of scientists and technicians. In 1959 the industry employed 13,380 qualified scientists and engineers.

The drawing up of conditions of contract and contributions towards the establishment and maintenance of standards continue to be important activities of the Association. Of increasing importance is the collection and dissemination of statistics and information, the study of trends and of overseas competition. "A trade association must be a realistic partnership of competing concerns, not with the purpose of restricting competition but to secure the best use of an industry's resources and to provide the basis for the full development of initiative and enterprise".

A. J. ELLISON

OBITUARIES

Prof. M. L. Anderson

THE death of Prof. M. L. Anderson, of the chair of forestry in the University of Edinburgh, occurred on September 6 at Bristol, while he and Mrs. Anderson were on a visit to their married daughter.

Mark Louden Anderson was born on April 16, 1895, at Kinneff in Kincardineshire. He had the misfortune to lose both parents when he was only eleven years old. He entered the forestry course at the University of Edinburgh in 1912, but saw service throughout the First World War, first in the Argyll and Sutherland Highlanders and the Black Watch, and then in the Machine Gun Corps, winning the Military Cross in 1918. Returning to Edinburgh, he graduated B.Sc. (Forestry) in 1919, and five years later was awarded the D Sc degree for a thesis on tree form and volume studies

Prof Anderson was appointed to the Forestry Commission and as a research officer he had much to do with early experiments in planting on peat, provenance of species trials and the selection and measurement of sample plots His work was conducted with that depth of vision so necessary in practical research It was unfortunate that he had to give up this particular work when he did, but Eire gained his services and there he eventually became director of forestry This was followed by five years on the staff of the Department of Forestry, University of Oxford It was then his intention to take up a post offered by the Australian Forest Service, but instead he returned to the University of Edinburgh to be professor of forestry, a post which he occupied with distinction for ten years

Prof Anderson was an enthusiastic forester who showed his keenness in a variety of ways He enjoyed being in the forest, and he regretted when ill-health prevented him from visiting again the Continental forests, where he admired the practice of good sylviculture and sound management At any time he would discuss forestry matters and even provoke controversy for the fun of enjoying the outcome As a teacher, Prof Anderson was never verbose His great knowledge was readily available to the keen forester, no matter what the latter's rank was He believed that a good forester should have a proper basic knowledge and know how to observe. He had a wonderful gift for languages, most of which were self-taught, and he tried by his numerous translations from French, German, Danish, Norwegian, Swedish, Finnish and Russian books and papers to bring important works of Continental foresters to the English-speaking world Knuchel's *Planning and Control in the Managed Forest*, Kostler's *Silviculture*, Larsen's *Tree Genetics*, Petrini's *Forest Economics* and Tamm's *Northern Coniferous Forest Soils* are some of the larger translations Prof Anderson's *The Selection of Tree Species* is a very valuable guide for the British forester and it is now in its second edition Not long before he died, Prof Anderson completed the manuscript of his monumental work, *A History of Scottish Forestry*—the product of some thirty years of painstaking research It is to be hoped that financial difficulties will be overcome to allow this encyclopædic work to be published

Another aspect of Prof Anderson's keenness to stimulate an interest in forestry and to try to raise its general standard was the zeal and time he gave to forestry societies He was an ardent member of the Royal Scottish Forestry Society and recently its honorary secretary and the creator of its Silvicultural Group, a founder member of the Society of Foresters of Great Britain and also of the Forestry Society in Eire He was also a regular contributor to *Nature*

But forestry did not occupy all Prof Anderson's very full life He found great delight in transcribing old Scots records, and in this he took infinite care to obtain accurate results from folios which, at times, were rather difficult to decipher As a result of this work he was a regular contributor to the *Dictionary of the Older Scottish Tongue* His *Proverbs in Scots* were published in 1957, another example of painstaking work on a manuscript dating from the first quarter of the seventeenth century

Prof Anderson will be remembered as a great forester, an ardent Scot, a man respected for his integrity, his capacity for hard work, his willingness to help others, his friendliness and his great determination

Prof Anderson leaves a wife, two sons and a daughter, to whom our sympathy is extended

C J TAYLOR

Dr. W. A. MacFarlane, C B E.

THE sudden death on August 19 of Dr W A MacFarlane, managing director of the National Industrial Fuel Efficiency Service and president of the Institute of Fuel, at the age of fifty-three, came as a severe shock to his many friends and is a grievous loss to fuel technology in Great Britain

Although Angus MacFarlane was by training, and in his early work, a 'pure' chemist, the major part of his varied life was concerned with the efficient use of fuel After a brilliant career at Oxford, where he was a student of Sir Harold Hartley at Balliol, he spent two years at the University of California as a Commonwealth Fund Fellow carrying out research in physical chemistry for which he was awarded the degree of Ph D

On his return to Britain in 1934, after eight years in academic life, he joined the staff of the Fuel Research Station of the Department of Scientific and Industrial Research and thus first came into contact with fuel technology During the four years at East Greenwich his main contribution was the initiation of the Department's work on the Fischer–Tropsch process, which was destined to become one of the major research programmes of the Fuel Research Board, and to continue for a total period of some twenty-five years In 1938 MacFarlane left the Fuel Research Station to become senior research chemist to the London, Midland and Scottish Railway Co at the invitation of Sir Harold Hartley, then vice-president of the Company This move naturally led him into wider fields, but he was soon to return to fuel, for in 1941, after the formation of the Ministry of Fuel and Power, he was appointed the first director of fuel efficiency In this position he made an important contribution to the War effort by ensuring that every ton of coal was directed where it was most needed and used to the best advantage

After seven years at the Ministry, MacFarlane was appointed to the British Embassy at Washington as director of the United Kingdom Scientific Mission and attaché for scientific questions During his six years in Washington he did a great deal to foster and cement Anglo-American friendship and co-operation in the scientific sphere and there must be very many who, like myself, remember with gratitude the efficient help and warm hospitality he always extended to British scientists visiting the United States

In view of his earlier work at the Ministry, the selection of MacFarlane as director of the National Industrial Fuel Efficiency Service on its formation in 1954 was an obvious one, and the undoubted success of this organization in conserving fuel and reducing costs to the fuel user offers testimony to his efficient management His services to the nation were recognized when he was made a C B E in 1958, and his election to the presidency of the Institute of Fuel in 1960 is evidence of the high esteem in which he was held by his fellow fuel technologists

MacFarlane combined a sound scientific knowledge with an ability to appreciate clearly and rapidly the practical and economic aspects of a problem He had the gift of inspiring loyalty and affection in those working under him, and his intellectual gifts and genial personality won for him a very wide circle of friends by whom he will be sadly missed

He leaves a widow and four children

C C Hall

Mr. H. V. Griffiths, M.B.E

The sudden death of H V Griffiths on June 28, at the age of fifty-nine, came as a shock to his many friends and colleagues

As engineer-in-charge of the British Broadcasting Corporation's Receiving and Measuring Station at Tatsfield, he was widely known both in Britain and overseas as an authority on radio reception, propagation and frequency measurement He represented the B B C on a number of technical committees concerned with these subjects, about which he has written from time to time in *Nature* and other journals

Like many of his colleagues in the early days of radio, Griffiths started his career as a ship's radio operator, but his interest was in the technical, rather than the operational, aspects of his work, and it was not long before he entered the engineering field He joined the B B C in 1924 as an assistant maintenance engineer and later became a member of a team of engineers to work on the first short-wave broadcast transmitter used at Chelmsford by the B B C He also co-operated with Marconi engineers in the study of diversity reception on short waves

He was appointed engineer-in-charge at the Tatsfield Receiving and Measuring Station in 1933 and over the years he built up for Tatsfield a reputation for accuracy and reliability which has made the Station a world-wide name in radio His work was recognized when he was made M B E in 1958

Among his many other interests was astronomy He was a Fellow of the Royal Astronomical Society and was keenly interested in man-made satellites and their future possibilities in the field of world-wide radio communication He took an active part in monitoring the transmissions from the first *Sputnik*

H. V. Griffiths will be greatly missed by his many friends and colleagues, who will remember him not only for his expert knowledge, which he was always ready to pass on to others, but also for his sincerity and kindly patience and, above all, his enthusiasm for his work

L W. Turner

NEWS and VIEWS

Iodine-131 in Milk in Britain

The first results of an investigation of iodine-131 in milk, which has been in progress at the Agricultural Research Council Radiobiological Laboratory since weapons tests were resumed by the U S S R , were released on October 24 simultaneously with a general statement by the Medical Research Council (see *Nature*, November 4, p 400) A further statement from the Agricultural Research Council (dated November 1) summarizes more recent measurements Milk from twenty depots in central and southern England, Wales, Scotland and Northern Ireland is now being collected daily and examined for levels of iodine-131. This represents a considerable expansion of the sampling which has been carried out during the past six weeks at eight sites in different regions (see Table 1) These measurements enable the general trend throughout the United Kingdom to be assessed

Table 1 Levels of Iodine-131 in Milk produced at Eight Sites in Different Parts of the United Kingdom

Week commencing	$\mu\mu c$ Iodine-131/litre
September 16, 1961	85
September 24, 1961	109
October 1, 1961	138
October 8, 1961	150
October 15, 1961	114
October 22, 1961	158
Mean for six weeks (September 16–October 28)	126

Information on regional differences of iodine-131 is now obtained each week by analysing samples collected from more than 200 depots which handle more than 40 per cent of the total milk produced throughout the United Kingdom The samples are combined in proportion to the quantity of milk handled by the depots from which they are obtained so that the average level throughout a zone can be ascertained The present results are summarized in Table 2

Table 2 Levels of Iodine-131 in Milk produced throughout the United Kingdom

Mean results for samples collected on October 2, 16 and 23, 1961

Zone	$\mu\mu c$ Iodine-131/litre
1 Northern England	111
2 North-West England	100
3 East and East Midland	82
4 West Midland	106
5. Wales	174
6 South and South-East England	99
7 Mid-Western England	96
8 Far Western England	143
9 Scotland	178
10 Northern Ireland	161
Country-wide mean weighted for production	121

The country-wide mean of 126 μμc per litre for the six weeks ending October 28, which is shown in Table 1, represents about one-eighth of the total quantity of iodine-131 which would give rise to the annual average of 130 μμc per litre specified by the Medical Research Council The results in Table 2 show that, on a regional as well as a national basis, the iodine-131 which has hitherto entered milk represents but a small fraction of that which would give rise to the level specified by the Medical Research Council Further statements will be released at intervals until the levels of iodine-131 in milk have decreased appreciably

Theoretical Physics in the University of Sussex : Prof R. J Blin-Stoyle

Dr R J Blin-Stoyle has been appointed to the chair of theoretical physics in the new University of Sussex He attended Alderman Newton's Boys' School in Leicester, and received a senior scholarship at Wadham College, Oxford, in 1943, but before taking this up in 1946 was commissioned into the Royal Signals and served as lecturer in the College of Radio Communications at Mhow, Central India He received a B A (1949), D Phil (1951) and, during 1951–53, held a Pressed Steel Fellowship at Oxford. After a year as lecturer in mathematical physics in the University of Birmingham he took up his present post at Oxford as senior research officer in physics Since 1956 he has also been a Fellow and lecturer at Wadham College During 1959–60 he served as visiting professor of physics at the Massachusetts Institute of Technology and the University of California

Prof Blin-Stoyle is widely recognized as one of the ablest young theoretical nuclear physicists He has made valuable contributions to the theory of nuclear structure, with special emphasis on nuclear moments and β-decay His main object in this work is to use the nuclear properties to learn about more fundamental things. such as the possible charge dependence or parity non-conservation of nuclear forces, and the nature of weak interactions He has also been concerned with the theory of quantum fields and reactions of polarized nuclei He is much appreciated as a tutor and lecturer as well as for his research work and, although his departure will be sadly felt in Oxford, he should have greater possibilities at Sussex for implementation of his progressive ideas on the teaching of physicists His appointment promises much for the future development of physics at the new university.

Engineering Group of the United Kingdom Atomic Energy Authority

Mr. J B W. Cunningham

Mr James Bernard William Cunningham, who resigns as managing director of the Engineering Group of the U K Atomic Energy Authority as from January 20, 1962, was born in Middlesbrough, Yorks, on December 9, 1911 During 1928–39 he served as an apprentice with Cochrane and Co, Ltd, Middlesbrough, and Stanton Ironworks Co, Ltd, Middlesbrough, rising to works manager. During 1939–44 he was assistant works manager at Dewrance and Co, Ltd, London, and during 1944–47 was works manager at Pegsons, Ltd., Coalville Mr Cunningham then joined the Department of Atomic Energy of the Ministry of Supply at Risley, Lancs, as senior engineer (supply) In 1948 he became assistant chief engineer (design) and then was promoted to deputy chief engineer (design) in 1950, and chief engineer (design) in 1953 He continued to hold this post on the formation of the U K Atomic Energy Authority a year later and in 1956 was appointed deputy director (reactors) and in 1957 deputy director (industrial power) In the following year he became director of industrial power, and in 1959, on the division of the Industrial Group, Mr Cunningham became deputy managing director (technical) of the Production Group He was appointed to his present post in April this year.

Mr H V. Disney, C.B E.

Mr Harold Vernon Disney, who is to succeed Mr J B W. Cunningham as managing director of the Engineering Group of the U K Atomic Energy Authority, was born in Ruddington, Notts, on July 2, 1907 He was educated at Ilkeston and at University College, Nottingham After apprenticeship with G R Turner, Langley Mill, Notts, Mr. Disney went as a designer to International Combustion, Derby, in 1931 He joined Imperial Chemical Industries (Alkali), Ltd, Northwich, in 1935 as a design engineer on chemical plant projects In 1940 he was seconded by Imperial Chemical Industries for special duties on the construction of Ministry of Supply propellent and filling factories, and in 1944 became deputy director (filling factories supply) Mr Disney was a member of the original team which started the Department of Atomic Energy at Risley in February 1946 and was appointed chief engineer, services and supply, in 1947. In 1950 he became chief design engineer for the diffusion plant at Capenhurst, and in 1957 was promoted to deputy director (defence plants) at the U K Atomic Energy Authority He was appointed director of engineering of the Industrial Group in May 1958, becoming director of engineering of the Development and Engineering Group in July 1959 and deputy managing director of the Engineering Group in 1961 As from November 1, and until he takes over full responsibilities, he will be managing director (designate) of the Group.

Royal Society of Edinburgh : Officers for 1961–62

At the annual statutory meeting of the Royal Society of Edinburgh, held on October 24, the following officers and members of Council were elected *President*, Prof E L Hirst, *Vice-Presidents*, Dr T R. Bolam, Dr. Douglas Guthrie, Dr. A W Greenwood, Prof R A Rankin, Dr C. E Lucas, Prof J A Macdonald, *General Secretary*, Prof Norman Feather; *Secretaries to Ordinary Meetings*, Dr Mowbray Ritchie, Prof A E Ritchie, *Treasurer*, Dr J. R Peddie, *Curator of Library and Museum*, Dr. R Schlapp, *Members of Council*, Prof H A Bruck, Dr. H R Fletcher, Prof. G L Montgomery, Prof. W. L Weipers, Prof. J H Burnett, Prof C F. Davidson, Prof D. C Pack, Dr A. B Stewart, Prof Jack Allen, Dr S. C Curran, Dr. G H Mitchell, Dr Magnus Pyke.

Jubilee of the Atomic Nucleus

There have been speculations on the nature of atoms since the days of the ancient Greeks, but the first firm foundation on which modern ideas are built was the nuclear theory put forward by Rutherford in Manchester in 1911. Jubilee celebrations and an international conference were held in Manchester recently, and now the Science Museum has set up a small group of exhibits to illustrate various theories of atomic structure and Rutherford's great contribution The exhibits are on view in the Atomic Physics Section of the Museum They include a group of models of atoms as visualized by J J Thomson before Rutherford's theory, a further group illustrating Rutherford–Bohr atoms; several historic photographs relating to Rutherford's great Manchester period of research, and a working mechanical model illustrating how the observed scattering of α-rays by heavy atoms led him to the inescapable conclusion that the atom must have a small 'hard core' (which is now known as the nucleus) This nucleus itself is now being investigated with all the powerful and elaborate apparatus of modern physics and engineering The Science Museum exhibit makes no attempt to illustrate the many practical applications of our knowledge of the structure of the atom, as these will be dealt with in a full-scale special exhibition, "Atoms at Work," which will be staged in the Museum by the United Kingdom Atomic Energy Authority in March 1962

Centenary of Plastics 1862–1962

A joint committee of the Plastics Institute and the British Plastics Federation has been set up to organize a number of events in connexion with a

Centenary of Plastics 1862–1962 It was at the International Exhibition in London in 1862 that Alexander Parkes showed for the first time to the public "a new material and manufacture which from its valuable properties had induced the inventor to patent the discovery in England and France and to devote his attention for the last 10 years to the development of the capabilities and application of this beautiful substance to the Arts" In the case shown at the Exhibition were a few illustrations of the numerous purposes for which, according to Parkes, 'Parkesine' might be applied These included medallions, salvers, hollow ware, tubes, buttons, combs, knife handles, pierced and fret work, inlaid work, bookbinding, card cases, boxes, pens, penholders, etc. Parkes received a prize medal for his exhibit 'Parkesine' contained no camphor, an indispensable component of the later material celluloid, and while Parkes mentioned camphor in his patent of 1865 he did not appreciate its unique properties There can be no doubt, however, that 1862 saw for the first time the material which led directly to the modern form of celluloid The programme to be organized by the Joint Committee will include open days in firms in the plastics industry and special meetings and lectures throughout the centenary year (1962–63), culminating in the International Plastics Exhibition to be held at Olympia during June 12–22. 1963

Personnel Management Salaries

DURING the summer of 1956 the Institute of Personnel Management carried out a survey of salaries paid to members. This was undertaken because the Institute had been frequently asked by individual members, and by companies filling vacancies, about salaries paid to personnel officers and other members of personnel departments To collect this information, questionnaires were sent to companies throughout Britain and senior personnel officers were asked to complete these on behalf of their personnel staff. A report based on this survey was issued at the beginning of 1957. This report proved to be of great value both to individual members and also to companies filling vacancies or setting up personnel departments The Institute has now taken a further and more extensive survey to obtain an up-to-date review of members' salaries in Britain (Personnel Management Salaries Report of a Survey undertaken in February/March 1961 Pp 32. London Institute of Personnel Management, 1961. 21*s*) Of the 2,900 questionnaires sent out to practising personnel officers, approximately 1,250 questionnaires were returned, of which 1,222 were included in the final analysis The personnel officers completing the questionnaire can be regarded as representative of the Institute members as a whole The report consists of eighteen tables which give salaries paid under a number of different headings, including salaries according to positions held, age group, type of company, industry, area, length of time with the firm, and number of employees

The National Mineral Collection, Ottawa

A NATIONAL Mineral Collection, containing specimens from Canada and all over the world, has been established at Ottawa through the co-operation of two Federal Government departments The Collection will consist of two series a systematic reference series to be maintained by the Geological Survey of Canada, within the Department of Mines and Technical Surveys, with H R Steacy as curator, and a display series, in the National Museum, under the curatorship of Colonel E Taylor The Museum is within the Department of Northern Affairs and National Resources A single catalogue, to be prepared and maintained by the Geological Survey, will cover the whole Collection Both Departments already possess substantial collections of minerals, accumulated since the Geological Survey was formed in 1842 The number of specimens in the keeping of the Survey is estimated at 50,000 Among them are rare gold nuggets and precious stones, as well as large pieces of silver—one of them 3½ ft long—found by geologists, prospectors, and mining crews in many parts of Canada The curators of both series will endeavour to expand and to improve their series year by year The Collection now has specimens representing about 30 per cent of the world's known mineral types—a valuable aid to training and research in geological science and exploration

Recent Developments in Medical Treatment

A WELCOME sign of how clinicians are adapting themselves to the changing outlook in medicine is shown in the current issue of the *Practitioner* (187, No 1120, October 1961) This is a special issue dealing with advances in treatment, and three of the contributors do not concern themselves with 'treatment' in the narrow sense of the term The contributors in question deal with advances in pædiatrics, general practice, and public health, the inclusion of the latter subject indicating that preventive medicine is an integral part of the practice of medicine The reasons why the other two subjects have been dealt with in a particular way are given by the contributors themselves "The Holistic conception of child life within the family circle", according to Prof W S Craig, "is fundamental to pædiatric thought, and a knowledge of human relations, and of the art of consultation and physical examination, must continue to provide the foundations of pædiatric teaching". "Too often", according to Dr Richard Scott, "advance is halted by our failure to apply existing knowledge It would appear that, as in many other spheres, so also in medicine are we more skilful in advancing knowledge than in applying it" That 'treatment', in the narrower sense of the term, is not overlooked is well exemplified by the other fifteen articles in the symposium

The Perkin Centenary Trust: Awards

APPLICATIONS for the awards of the Perkin Centenary Fellowship and Scholarships are invited by the Trustees. *The Perkin Centenary Fellowship* is offered for one or two years for the purpose of higher study of any subject approved by the Trustees Candidates will be required to show either that they have had experience in an industrial firm or other institution concerned in the manufacture or the application of colouring matters, or that their intended field of study has a direct bearing on these subjects The value of the Fellowship is £750 per annum with an additional grant of up to £100 per annum towards certain designated expenses It is tenable, from October 1962, at any university, technical college or other institution approved by the Trustees *The Perkin Centenary Scholarship* two such awards are offered, each for two years, to enable candidates employed in an industrial firm or other institution concerned with the manufacture or the application of colouring matters to receive an education at a university or technical college Each award will have a value of £350 per annum This may be increased, at the discretion of the Trustees, to £400 per annum, if the candidate appointed is required to live away from his normal place of residence Applications and inquiries should be addressed to the Secretary, Perkin Centenary Trust, c/o Chemical Society, Burlington House, London, W 1 Applications must be made by May 1, 1962

The U S Building Research Institute

THE Building Research Institute, a constituent part of the Division of Engineering and Industrial Research of the U S National Academy of Sciences–National Research Council, will become an independent technical society for the building sciences next year As Dr Detlev W. Bronk, president of the Academy–Research Council, remarked· "The Buckley report, which recommended in 1951 the establishment of the Building Research Institute within the Academy–Research Council, recommended also that the Institute be encouraged to work toward independent status It is truly gratifying that, within the span of a decade, the Institute has established itself as a uniquely effective instrument for the stimulation and correlation of interdisciplinary research in the building industry, the extraordinarily diverse interests of which otherwise depend on the attention of nearly 500 separate associations in the field"

Acoustical Society of America

DR LEO L BERANEK, president of Bolt Beranek and Newman Inc , of Cambridge, Mass , has been given the Wallace Clement Sabine Award of the Acoustical Society of America for his contributions to architectural acoustics The Pioneers of Underwater Sound Award has been conferred on Dr A B Wood, of the Admiralty Research Laboratory, Teddington. The officers of the Society are as follows *President,* Dr Laurence Batchelder, Raytheon Co , Burlington, Mass., *Vice-president,* Prof Robert T Beyer, Brown University , *President-elect,* Dr Robert W Leonard, University of California at Los Angeles, *Treasurer,* Herbert A Erf, Cleveland, Ohio, *Secretary,* Wallace Waterfall, American Institute of Physics, New York; *Editor-in-Chief,* Dean R Bruce Lindsay, Brown University.

Biological Engineering Society

AT the annual general meeting of the Biological Engineering Society, Prof. R F. Woolmer was re-elected president for the coming year, and Dr A. Nightingale, St Thomas's Hospital, London, S E 1, as honorary secretary. The following members were elected as members of council P E. K Donaldson, D Gordon, A Nightingale, G Pampiglione, W J. Perkins, J T Scales, B Shackel, A. M. Uttley, H S Wolff, B M Wright.

University News : Birmingham

THE following appointments have been made Dr J S Brimacombe, to be lecturer in the Department of Chemistry, Dr F. Rieband, to be lecturer in the Department of Mechanical Engineering, E D R Shearman, to be senior lecturer in electromagnetism in the Department of Electrical Engineering, Dr E C Grant, to be Research Fellow in the Department of Medical Biochemistry and Pharmacology

Edinburgh

THE following appointments to lectureships have been announced I W Sutherland (bacteriology); J Shepherd (electrical engineering), W. Dudgeon (senior lecturer in engineering), J A Linnet (engineering), Dr S Soundranayagan (engineering), H Montgomery (pathology), M B Smith (pathology), Dr. P D Mitchell (senior lecturer in zoology)

London

DR J. CLUNIE, lecturer at the Imperial College of Science and Technology, has been appointed to a readership in mathematical analysis tenable at that College

New York

NEW YORK UNIVERSITY has combined its two physics departments into a single all-University department and has appointed Prof Bruno Zumino, professor of physics, as its head The re-organization is one of the final steps toward unifying the departments at the several centres of this University. The two units involved in the change are the University Heights Physics Department, formerly in the College of Engineering, and the Washington Square College of Arts and Science Physics Department Each of the physics units under Dr Zumino's direction will continue to have its own chairman. Prof. Sidney Borowitz has been appointed chairman of the University Heights Department to replace Dr Lyle Borst, who has assumed a professorship in the all-University Department of Chemistry Prof Morris H Shamos will continue as chairman of the Washington Square College of Arts and Science Physics Department.

Announcements

THE Executive Council of the Commonwealth Agricultural Bureaux has appointed Dr V Armstrong, representing New Zealand, as chairman in succession to Mr C K Reheem, representing Pakistan Mr W G Alexander, representing the United Kingdom, has succeeded Dr V Armstrong as vice-chairman

DR A W BALDWIN, formerly an associate research manager of the Dyestuffs Division, Imperial Chemical Industries, Ltd , has been co-opted as a governor of the J A. Radley Research Institute, 220–222 Elgar Road, Reading

DR ROBERT PHYFE MADDEN, chief of the Spectroscopy Section, Physics Research Laboratory, in the Engineer Research and Development Laboratories at Ft. Belvoir, Va , has been appointed to the Atomic Physics Division of the U S National Bureau of Standards His new group is to develop absolute radiation standards for the far ultra-violet spectral region

THE ninth annual Western Spectroscopy Conference will be held at Asilomar, Pacific Grove, California, during January 25–26, 1962 Further information can be obtained from Dr. Donald G Rea, chairman, Western Spectroscopy Association, Space Sciences Laboratory, Leuschner Observatory, University of California, Berkeley 4, California

ERRATUM In the communication by M R Droop entitled "Evidence for a Sexual Phase in the Life-Cycle of an Amœba" in *Nature* of October 21, p 281, par 2, lines 1 and 5, the names *Heteramœba* and *Heterochromonas* should be interchanged.

PHILOSOPHY OF SCIENCE

THE sixth annual conference of the British Society for the Philosophy of Science was held at St Hugh's College, Oxford, during September 22–24 The four formal meetings and discussions, once again organized by Dr Mary Hesse (Cambridge), were attended by eighty members and guests

The theme of the first meeting, with Dr B A Farrell (Oxford) in the chair, was explanation in psychology The first of the two papers was read by Dr M Treisman (Oxford), who challenged the widespread belief that, whereas the physical and biological sciences use only public data, the psychologist also uses private data of immediate experience Dr Treisman's method was to select topics from the history of psychology to show that the modern psychologist has refined the vague descriptive analyses of the past and now uses concepts as precisely definable and as objective as those of the physicist The first illustrative topic he discussed was the analysis of instinct He contrasted McDougall's classification analysis, vague and merely descriptive, with the present-day model of instinctive behaviour in animals based on the work of Lorenz and Tinbergen Here there was little to dispute though the argument edged close to the controversial issue of reductionism His second topic was the history of the measurement of sensation he described the experiments of Fechner and of Stevens and Davis which led to the apparently conflicting laws $S = K \log I$ and $S = K I^n$ respectively, where S is the 'measure of sensation' and I is the intensity of the stimulus Dr Treisman argued that these laws could be reconciled but that the second was preferable because it had more explanatory power

Dr. J O. Wisdom (London School of Economics) widened the scope of the kind of psychological explanation to be considered by discussing the hypotheses used by psychoanalysts to explain patients' associations Such an explanation, he argued, requires to be tested clinically by observing the patient's response to a presentation of an interpretation embodying the hypothesis But, following Seeborn Jones, he indicated that two problems then arise Does the presented interpretation cause a change ? Is the interpretation true ? He proffered some new criteria to distinguish between an interpretation that causes a change by suggestion and an interpretation that is true

A long and lively discussion ensued. The chairman began by challenging the assumption implicit in Dr Wisdom's paper that the reports of the psychoanalyst could be regarded as objective data, but, for an hour, discussion was focused on Dr Treisman's reconciliation of the two laws of sensation, a reconciliation which failed to satisfy the mathematicians present Eventually there was a return to psychoanalysis What are its basic statements ? To what extent could the reports of the analyst about his subject be regarded as objective data ? But again the discussion returned to the measurement of sensation How could 'half a feeling' be measured ?, the physicists demanded Various solutions to the many problems raised echoed along the corridors of St Hugh's until well after midnight

At the next session the president, Prof R B Braithwaite (Cambridge), took the chair for a discussion on learning machines Dr F H George (Bristol), treading delicately over this philosophically thorny field, outlined some of his recent work He gave a lively description of his method of programming a *Deuce* computer so that one-half of the machine taught the other half to play an optimal game of noughts and crosses—a by no means trivial game, as the chairman had later occasion to emphasize. Dr George went on to explain more generally how a computer can 'learn' to generalize from its own experience and to symbolize or 'name' the processes it goes through He suggested that such performances give the computer qualities analogous to the insight and flexibility of human thought-processes

Prof D M Mackay (North Staffordshire) gave a thoughtful elaboration of Eddington's account of 'the two tables' extended to persons (and their brains) and to machines He first considered the transition from the micro-description of a mechanism to its behavioural description in such terms as 'controlling' and 'goal-seeking' He argued that such behavioural terms presuppose an under-specification of the physical situation which becomes meaningless when applied to mechanisms specified in atomic detail He then extended his analysis to 'game-playing' mechanisms and explained how the crucial difference between playing against and manipulating a machine lies in the nature of one's commitment towards it Finally, he extended his analysis to human beings, regarded as persons and as cell-mechanisms, and discussed the degree to which it is possible to switch from one view to the other when one is confronted by another human being

In the discussion Dr George was asked to elucidate some technical points, but members' questions mainly sought clarification of Prof Mackay's concept of under-specification, its relation to entropy and its implications for personal commitment in dialogue

The third meeting was devoted to a discussion of genetic coding and information theory, with Mr F T C Harris (Middlesex Hospital Medical School) as chairman. Dr I Leslie (Belfast) gave the first of two papers Using a helpful series of slides, he reviewed with modest impartiality some recent developments in the biochemical analysis of the processes by which information is transferred from the genes to the cellular sites of protein synthesis He explained the background to the present conflict of ideas about the way in which ribonucleic acid (RNA) acts as an intermediary in this transference In the 'classical' view much of the RNA is in the form of stable templates which are coded by DNA for the synthesis of specific proteins but recent experiments on bacteria and bacterial viruses suggest that some of the RNA acts as a 'messenger' which itself carries genetic information from the genes to the protein-synthesizing mechanisms

The second main speaker, Dr S Brenner (Cambridge), remarking that "philosophers are not really concerned with the facts", bounded gaily from Dr Leslie's well-laid factual foundations into genetic space He delighted the audience with bold speculative leaps and a frank analysis of his own approach to the problem He based his study of genetic coding, he explained, on two main principles: (1) that "anything useless must be wrong", and (2) that "there is no security problem in Nature", that is, Nature is no enemy striving to conceal the truth from us. He then attacked the view that the stable RNA of the ribosomes acts as a template and argued that the ribosomes are structures which synthesize, at a given time, the protein dictated by the attendant messenger. Thus he rejected two- and three-process models of transfer of information in favour of a one-process model which he claimed was universal.

The discussion was at first confined to thrust and counterthrust between the two experts, deftly provoked by the chairman. But gradually the audience gained sufficient confidence to intervene. Questions about the evolution and the controlling mechanisms of this complex system (for example: How do you make a cat ?) led to lively discussions. It slowly became apparent that though genetic coding was concerned with transfer of information, information theory itself had little to offer in solving the problems of genetic coding apart from providing some descriptive terms. As Dr Brenner put it in his final remark, "It's now just a matter of linguistics", that is, of reading the code.

The fourth and final discussion was devoted to philosophical aspects of cosmology. Prof J. L. Mackie (Sydney) took the chair, and Mr R. Harré (Oxford) and Dr. W. Davidson (Battersea College of Technology) were the two main speakers. Mr Harré argued that the universe, defined as 'everything there is', was logically not a proper subject for scientific study. He carefully distinguished between the cosmo-sciences and the astro-sciences by analysing the logical form of typical propositions of these subjects; though cosmography, he thought, could be regarded as scientific, the other cosmo-sciences, cosmogeny and cosmophysics, were merely speculative. His main attack centred on the concept of infinity as used in or implied by the cosmo-sciences: he distinguished between the concept of an actual or referential infinity, which he rejected as logically improper, and the concept of a descriptive infinity which (analogously with the intuitionist concept of infinity in mathematics) has a finite starting point and a rule of progression which may continue without limit and thus be a growing but potentially infinite sequence.

Dr. Davidson argued that development of the cosmo-sciences was scientifically both valid and feasible, and that such questions as whether the universe is spatially infinite or not, or whether it had an origin in time or not, are not necessarily transcendental issues. He claimed that the unity of science as a whole may depend on the development of a satisfactory cosmology in which every science could find its place.

With the two protagonists thus apparently diametrically opposed, the chairman formulated their main differences and members of the Conference set about their task of resolving them. In the discussion, Mr Harré's dependence on Kantian antinomies, Russell's theory of types, and *a priori* arguments took some hard knocks. It seemed eventually that perhaps only the philosopher's concept of the universe was referentially infinite, while the cosmologist's universe, if not finite, was at worst only descriptively infinite and therefore not, logically, a wholly improper subject of study.

The conference ended with a business meeting at which, *inter alia*, members were able to express their thanks to St. Hugh's College as hosts and to Dr Mary Hesse as organizer for the parts they played in a pleasant and stimulating week-end.

B. C. BROOKES

IONIC MELTS

LIQUIDS still constitute one of the least well understood states of matter. Near the critical temperature various quasi-gaseous formulations of their behaviour are often quite successful, but near the melting point quasi-crystalline models offer more promise. Ionic melts have been much less studied than many other liquids. Their critical temperatures are usually not known, but are certain to be high. For this reason, current structural models for ionic melts show a bias towards quasi-crystalline concepts, which is strengthened by the strong statistical probability that each cation will have anions as its nearest neighbours, and vice versa, in order to lessen electrostatic repulsion energy.

However, really precise theories of the structure of ionic melts present difficulties which have not yet been surmounted. In addition to the theoretical difficulties, until recent years the amount and diversity of experimental work on ionic melts has been restricted by technical complications. Temperatures of study need to range from 100° to well above 1,000° C., and many of the melts attack practically every kind of containing wall. Despite these obstacles, interest in the science and technology of ionic melts has been growing rapidly. Sessions dealing with these systems have been included in a number of recent international scientific meetings, and two Gordon conferences in the United States have been wholly devoted to discussing their properties. At the discussion meeting of the Faraday Society, held in the University of Liverpool during September 5–7, seventy-five scientists from Britain and fifty from overseas had the opportunity of comparing progress in three main fields: structure, thermodynamic properties, and transport properties of ionic melts. The president, Sir Cyril Hinshelwood, was in the chair. Prof H. Bloom, of the University of Tasmania, gave the eleventh Spiers Memorial Lecture on "Structural Models for Molten Salts and their Mixtures".

A broad conclusion from the discussion was that interesting and important experimental work is being carried out in quite a few centres. Comprehensive theoretical treatments of the properties of ionic melts have by no means caught up with the range or the physical interest of the data now being obtained. Applied research on ionic melts in electrometallurgy or as heat-transfer fluids appears to be reasonably active in various countries. The number of substances capable of existence as stable liquids in the range 300–1,000° C. is comparatively small, which points to the profitability of exploring further fields of application of ionic melts. At present, basic research on ionic melts seems scanty in British universities, having regard to the importance of extending physicochemical research to higher temperatures, and to the properties of condensed states of matter generally.

The first group of six papers presented at the Liverpool meeting of the Faraday Society were concerned with structure and spectra. In dealing with problems of structure, methods of describing and measuring the nearest neighbour and next-nearest neighbour environment of any ions continue to be of prime importance. Although they are indispensable, X-ray techniques applied to ionic melts suffer from the weakness that as with other liquids they only yield information in the form of radial distribution functions, which give average environments. In a significant number of ionic melts, there is evidence for a closer approach between anion and cation, and an increase of separation between ions of like sign, compared with the crystals. This shrinkage of contact distance between ions of unlike sign has been explained in terms of the increased positional disorder in the melt, compared with the crystal. Disorder results in less complete compensation of electrostatic forces from the nearest neighbour shell of unlike ions, exerted on the ion at the centre of the shell. Greater local polarizations can be expected in the melt, even when the total volume it occupies is larger than when the same quantity of matter is in the crystalline state. Data were presented for radial distribution functions of molten carbonates and sulphates.

There was considerable discussion about the local arrangements of ions in close-packed melts. Spectroscopic techniques promise to give rather more specific information about local structure than is obtainable from X-ray determinations of radial distribution functions. Experimental studies presented for discussion included ultra-violet absorption spectra of transition metal ions in dilute solution melts. These yield information about ligand fields around the transition ion. A somewhat different type of ultra-violet absorption spectrum arises from so-called charge transfer effects, which involve excitation of an electron in the anion to an orbital the radius of which depends on that of the shell of cations immediately surrounding the anion. Such spectra can therefore yield valuable information about the configurational changes in this shell of nearest neighbours. One paper dealt with the modifications of charge transfer spectra that accompany various changes such as thermal expansion, the transition from one crystal form to another, and the transition from crystal to melt. Thermal expansions of crystalline nitrates observed by conventional means are found to match quite closely the expansions of the charge transfer orbitals as determined by the ultra-violet absorption spectra. This makes it all the more striking that volume changes on melting, as determined by conventional methods, are in the opposite direction to the changes in the charge transfer orbitals. Compared with the crystals, optical evidence for the melts thus points to a greater proportion of more tightly packed local assemblies of ions. Since the thermodynamic evidence shows an increase in volume, presumably this expansion results from increasing the proportion of vacancies and holes on melting alkali nitrates. Rubidium nitrate is one of the very few crystals which shrink on melting. Even here, the shrinkage of the charge transfer orbitals appears to be greater than the average contraction observed thermodynamically. For nitrates and other salts of polyatomic anions that have low melting points it is claimed that spectral evidence supports the general theory that the melting mechanism of these crystals includes the formation of 'association complexes', as well as the more usual increase of positional disorder and randomization of orientation of non-spherical ions. A second paper on charge transfer spectra described changes in mixed molten nitrates in terms of the effective electrostatic fields due to neighbours and next-nearest neighbours. Interesting information about the structure of liquids seems likely to emerge from extensions of such studies on mixtures of molten salts.

There was considerable but not very conclusive discussion about the usefulness or otherwise of attempting experimental and even conceptual distinction between 'association complexes' and 'complex ions'. It seems to be generally agreed that complex ions can be readily distinguished when they are formed in dilute solution in ionizing solvents, many techniques for their identification have been developed since Arrhenius. In condensed ionic assemblies, whether crystalline or molten, the distorting influence of nearest neighbours can make identification of a complex much less discriminating. 'Association complexes' can be the result of various ways of packing ions so as to minimize their potential energy. In discussing melting mechanisms, it is important to recognize that some of these packings are 'non-crystallizable', for these, addition of more and more ions according to the same rules of packing will not fill space economically. Only crystal nuclei can be extended by packing additional ions indefinitely in three directions in space, but so long as they are quite small, non-crystallizable association complexes may compete thermodynamically with potential crystal nuclei in the melt, which can be regarded as containing a mixture of the various kinds.

For discussing melting mechanisms, it may not be necessary to decide whether the reduction of potential energy on passing from random ionic packing to an association complex results primarily from electrostatic effects, or whether recognizable 'complex ions' are formed in which delocalization of valency electrons may extend over all the atoms in the complex.

But for the interpretation of other properties of ionic melts, the identification of strongly bonded complex ions may be more urgent. For example, cryoscopic measurements continue to yield suggestive structural information. One group of studies presented for discussion referred to the formation of complex ions such as $CdCl_3$ or $PbCl_3^-$ analogous to those encountered in aqueous ionic solutions.

A novel field is opened up by cryoscopic and other studies of the behaviour of solutions of metals in ionic melts. In some of these solutions the electron appears to leave the metal atom and may have a role analogous to that in solutions of alkali metals in liquid ammonia. Theoretical and experimental aspects of the electronic properties of such solutions received considerable discussion. Transport of electrons under the influence of electric fields applied to such systems has interesting contrasts and analogies with the movement of charge carriers in semiconductors. Conductances can be quite high even when the concentration of the metal atom is fairly dilute. Developments of these investigations promise to throw much additional light on conductance mechanisms generally that do not involve ion migration in condensed phases.

Papers presented at the meeting point to significant advances in the precision of measurement of basic thermodynamic parameters of ionic melts, such as activity coefficients Evaluations were reported of excess functions for the enthalpy and changes in volume on mixing ionic melts of the same or of different valency structure As a result, some of the current quantitative theories that have been developed for non-ionic liquid mixtures can begin to be adapted to ionic melts Considerable advances may be expected in the next few years in this branch of the theory of liquids

One-third of the papers presented dealt with transport properties of ionic melts, which have been studied for a number of years and which continue to receive much attention Use of radioisotopes has greatly enriched the possibilities of making measurements on diffusion, the correlation of which with other transport phenomena helps to unravel complicated structural problems In addition to their technological importance, transport properties can yield tests of theoretical models for ionic melts that can be much more sensitive than equilibrium properties to structural details assumed in the model Discussion of measurements of thermo-electric power of ionic melts suggested further interesting possibilities that seem likely to attract additional attention in the years ahead A R UBBELOHDE

WOOD EXTRACTIVES

A SYMPOSIUM on wood extractives was arranged by the Plant Phenolics Group and held at the Royal Holloway College, Englefield Green, Surrey, during September 20–22 The meeting proved valuable in bringing together workers in different fields, including several from overseas, having a common interest in the minor or extraneous components of woods After an introductory session the subject of wood extractives was dealt with under three headings, namely biosynthesis and taxonomy, the chemistry of extractives, and their influence on utilization of wood and wood products

Dr F E King (British Petroleum Co, Ltd) opened the symposium with an introductory address entitled "Perspectives in the Chemistry of Wood Extractives' Dr King surveyed the historical development of investigations on extractives, commencing with those of technical interest such as the tannins, dyestuffs and resins, and proceeding to the work of Erdtman on the heartwood components of coniferous trees and his own work on the corresponding components of hardwoods These investigations led to the conception of heartwood components as 'taxonomic tracers' which can be used to supplement the observations on morphological features employed by botanists in the taxonomic classification of trees Dr King emphasized the wide variety of C_6–C_3–C_6 compounds that are found in woods and discussed a few unusual structures that have been encountered

In the second paper, given by Prof F W Jane (Royal Holloway College), an account was given of the structure of the tree and the distribution of the various tissues in it This was helpful to the audience, consisting predominantly of chemists The extraneous substances are classified by botanists as gums, resins, tannins, oils, mucilages, colouring matters, mineral deposits, etc, and the location of these in the structure was described and illustrated There is clearly room for closer co-ordination between the observations of botanists, based largely on the microscopic staining reactions of these components, and their investigation by chemists

Prof E J Bourne (Royal Holloway College) described some new methods for the characterization and separation of extractives that have been studied in his laboratory These include the use of molybdates and tungstates which form complexes with polyhydroxy compounds and can assist in their separation by electrophoresis, the addition of phenylboronic acid to the solvents used in paper chromatographic separations of these compounds, the use of boron trichloride under very mild conditions to identify families of extractives by removing substituent groups, and the fractionation of mixtures by means of a new liquid/liquid countercurrent extraction technique, which was demonstrated

The session on biosynthesis and taxonomy opened with a paper by Prof W Sandermann (Bundesforschungsanstalt fur Forst- und Holzwirtschaft, Reinbek, West Germany) on the biogenesis of terpenes in plants Prof Sandermann discussed the part played by mevalonic acid and its conversion into geranyl and farnesyl pyrophosphates, from which the more complex terpenes may be derived He then described his work on the incorporation of labelled precursors in plants and showed how a distinction between alternative mechanisms can be made by these methods, for example, the biosynthesis of α-pinene and thujone was shown to take place by an electrophilic substitution rather than by an addition mechanism A series of terpenoid compounds has been found in teak and correlated structurally with the anthraquinones present in this wood

The systematic distribution of phenolic substances in plants was discussed by Dr E C Bate-Smith and Dr T Swain (Low Temperature Research Station, Cambridge) in a paper presented by Dr Swain The contribution that a study of the chemical components of plants can make to taxonomic classification was emphasized One of the results of an extensive survey of the occurrence of the common phenolics is the recognition that plants in some families or higher groupings are devoid of the capability of synthesizing certain types of compound In this connexion the occurrence of compounds containing vicinal trihydroxy groups and of ellagic acid in members of the Rosaceae was discussed

In continuation of this theme Prof C W L Bevan (University College, Ibadan, Nigeria) presented a paper entitled "Wood Extractives as Tracer Substances in Taxonomy', and referred particularly to the results of a survey carried out in his Department of components of woods of the family Meliaceae He also outlined the procedures followed in determining the structures of gedunin, isolated from *Entandrophragma angolense* and of khivorin from *Khaya ivorensis* and *K grandifoliola*

In the session on the chemistry of wood extractives four papers were presented, dealing respectively with the lignans, tropolones, stilbenes, and flavonoids, these being the principal types of phenolic compounds that are found in wood In discussing the lignans Prof R D Haworth (University of Sheffield) pointed out that the basic structure common to all lignans comprises two phenylpropane units linked in the β-positions The structure and stereochemistry of some of the naturally occurring lignans, of which about forty are known, was considered Where ring closure has occurred, with formation of phenyltetralin or furano-structures, the creation of a new asymmetric carbon atom renders the stereochemistry complex There is evidence that the lignan structure can be formed by oxidation of αβ-unsaturated phenylpropane compounds, such as ferulic acid.

A survey of the discovery and determination of structure of the tropolones found in various woods was given by Prof. J Gripenberg (Institute of Technology, Helsinki, Finland) The distribution of the tropolones, which appear to be confined to the family Cupressaceae, was discussed and their general properties and synthesis described. It is interesting to note that all the nine known tropolones occurring in wood contain an isopropyl (or isopropenyl) group and appear to be closely related to the terpenes A scheme by which they may be derived from carone has been proposed by Erdtman.

Dr D E Hathway (British Leather Manufacturers' Research Association) discussed the occurrence of stilbenes in wood and referred to the recorded incidence of hydroxystilbenes in conifers and dicotyledonous trees In a study of the extractives of the heartwood of *Eucalyptus wandoo*, 3 5·4′-trihydroxystilbene and its 3-glucoside were found to be present, and these compounds have been used as tracer substances in an investigation of the heartwoods of 60 species belonging to the *Longiores* sub-section of the genus *Eucalyptus*. This method confirms the existence of certain anomalies in the classification that have been suspected on other grounds

The fourth paper in this group, dealing with the flavonoids, was prepared by Dr. R A. Laidlaw and Dr J W W Morgan (Forest Products Research Laboratory) After a short account of the occurrence, function and nature of the flavonoids of wood, Dr Laidlaw went on to consider three classes of these compounds, namely the leucoanthocyanidins, dihydroflavonols and benzylcoumaranones, in greater detail. Methods for determination of the structure and stereochemistry of the leucoanthocyanidins and their characterization as flavan-3 4-diols were discussed. Among the dihydroflavonols, dihydromorin, phellamuretin and keyakinol were selected for special mention The naturally occurring aurones and hydroxybenzylcoumaranones were described and the behaviour of maesopsin (tetrahydroxybenzylcoumaran-3-one) on heating with alkali was discussed in detail.

The final section of the symposium consisted of two papers in which different aspects of the utilization of wood were considered. Dr R H Farmer (Forest Products Research Laboratory) discussed the influence of extractives present in different timbers on some of their technically important properties and applications A brief account was given of the manner in which the colours of woods (and the changes in colour occurring under certain conditions) and their natural durability are related to the chemical nature of the extractives present in them. Examples were then given of interaction between wood components and other materials with which the wood comes into contact These include the inhibition of the hardening of unsaturated polyester resins by certain timbers, shown to be due generally to quinones or hydroxystilbenes, and the retardation of setting of cement by water-soluble sugars and tannins

In the final paper Prof B Lindberg (Swedish Forest Products Research Laboratory) described the influence of extractives on the pulping of timber Extractives can influence the sulphite or the alkaline pulping processes in various ways Thus they may render the penetration of the cooking liquor into the wood more difficult, react with the cooking liquors and modify them, or condense with the lignin, preventing its dissolution Resinous and fatty components may give rise to 'pitch troubles', and can cause difficulty in bleaching because chlorination renders them more lipophilic R. H. Farmer

THE INTERNATIONAL SCIENTIFIC FILM ASSOCIATION

FIFTEENTH CONGRESS

The fifteenth annual congress of the International Scientific Film Association was held at Rabat, Morocco, during September 14–20, 1961 This was the first time that the Association had met outside Europe. The congress was under the patronage of H R H Prince Moulay Abdullah

Of the Association's twenty-five member countries, sixteen sent delegates and there were also observers from Guinea, India, Indonesia, the Lebanon and Mali. The largest delegation of twenty-two people was from Great Britain, followed by the U S S R with about twenty

All delegates, observers and friends enjoyed the gracious Moroccan hospitality From Rabat, where all were well housed in the new Cité Universitaire, excursions were made to Casablanca, Fes, Meknes and Marrakech As the sun shone without hesitation throughout each day on every day, expeditions were also organized to the delightful beach at Temarah

In his address of welcome, M Moulay Ahmed Alaoui, Minister of Information, Fine Arts and Tourism, expressed his country's pleasure at the honour being paid to Morocco as the scene of the congress This indicated also the importance of the African continent in the building up of the modern world For countries like Morocco, the cinema was not a diversion, but an important instrument in education and instruction

This congress has always two aspects one organizational, the other concerned with the problems of scientific film makers The former provides the machinery whereby the film makers can get together and see and discuss films in three main sections research, education and popular science For some time there has been a feeling that the organization has needed tightening, and this congress took certain steps to do so The new president is Mr Edgar Anstey (Great Britain), who replaces Mr Alexandre

Zgouridi (U S S R). The new honorary secretary is M Mohammed Afifi (Morocco), who replaces Mr John Maddison (Great Britain) It is Mr Maddison's guiding hand in the main which has been responsible for the steady development in the influence and prestige of the Association He has now been elected an honorary life member

The thirty films shown to the Research Section were described by its president, Dr. R Robineaux (France), as on a very high level Five films from France, two from Western Germany, and one each from Italy, the Netherlands and Czechoslovakia were specially selected by the Section for commendation At the discussions the special theme was new techniques The French described some new tools to aid research These included

(1) The light helper (this is a free translation of *l'asservisseur de lumière*), an instrument which allows the time of placing of the camera on the desired part of the curve of spectral sensitivity of the film to be fixed exactly and automatically, without acting on the diaphragm and without having to worry about rectifying the time of placing during the forward movement of panning of the camera It can be used in particular for taking shots automatically and slowly during daylight ; for example, during the complete phase of a solar eclipse when there are enormous differences in light. The instrument is a kind of 'cybernetic' tool

(2) The *brillancemètre*, which is incorporated in the camera to allow of instantaneous control of the mean brilliance of the image

(3) Motor regulated by transistor, a motor with continuous current fed by 8-, 12- or 24-V batteries, with transistorized regulation between 8 and 40 images a second, to which a small frequency emitter can be added to record sound on a separate magnetic tape at 24 or 25 images a second, even with differences in tension of several volts in the batteries

There was also a description of an apparatus to take under-water pictures with automatic illumination with synchronized flash It was developed primarily with biologists in mind, but has been of interest also to industrialists for the study of corrosion of submerged mechanical parts, and for the study of currents and their effects on public works and harbour installations.

Dr G Wolf, director of the Institut fur den Wissenshaftlichen Film, Gottingen, described how the film was being used for research in agriculture Evenly distributed rye was fed over an endless belt into the bar drum of a threshing machine In it the stresses to which the grain was subjected were observed and gauged With a circumference speed of the drum of 30 m /sec , the grain moves about two-thirds more slowly The flow of the threshed grain follows with irregular density due to congestion in the entrance An analysis was made of exactly what happens in the different parts of the threshing cage and productivity was increased Similar film analysis was done on the cutting capacity and cutting quality of the lawn-mower and hedge-cutting machine, which have developed empirically

Mr L Hallett and Mr John Maddison, both of Great Britain, read a paper on the "Use of Time Lapse in Research"

Thirty-eight films were exhibited for the Education Section Unfortunately, as the president, Prof J Jacoby (Poland), stated, there were no films from Great Britain or the U S S R these had been sent but had been mislaid Prof Jacoby was highly critical of the films seen He thought the quality was lower than that he had seen at any congress However, special mention was made of the films from Eastern Germany, which were commended for the high overall quality of their entry

Prof Jacoby said that screening conditions had made it impossible to appreciate the full technical quality of 16-mm films He wanted national branches of the International Scientific Film Association to take special care about the selection of films in future

Seventy-two films were shown to the Popular Science Section These were also of uneven quality and, as the president, Mr V Tosi (Italy), said, there was a great need for a clearer statement of the role and nature of the popular science film

Running at the same time as the congress, was a festival of popular science films which were shown nightly and were open to the public These films were included in the viewing by a special jury set up to recommend the outstanding popular science films of the congress. The following films were selected Great Britain, "Electron Microscopy", made by A E I , Eastern Germany, "Robert Koch", Bulgaria, "Life in a Drop of Water", Czechoslovakia, "Secrets of Heredity", Hungary, "Silver Threads", France, "Thaumetopoea", Rumania, "Coke", U S S R , "Colour TV", Poland, "The Gulf of Polar Bears" The British film, "Wild Highlands", made by the British Transport Commission, was mentioned for the high quality of its production and two Japanese films, "Lubricating Oil" and "Ancylostoma" were commended for their high technical quality. The Moroccan Scientific Film Association, as host country, awarded a diploma to the Japanese for their entries as best fulfilling the aims of the Association

There was a special session on television, at which the main speaker was Mr Aubrey Singer (Great Britain). of the B B C During the past year, a commission for the study of problems of the use of television has been working Its secretary, Mr. Jean Le Harivel (Great Britain), presented a detailed report based on information received from eight countries

Most countries have some kind of science programme which goes out generally in the evening and is intended for large audiences of adults Several countries produce science programmes for schools A few countries have used television for the education of adults This is true of Eastern Germany, with its television academy (mathematics and chemistry), of Czechoslovakia, of France, which has started technical education programmes and which is planning a series for workers on the social significance of technological developments, and of the United Kingdom, which is planning for 1962–63 a series of topical education programmes to supplement existing technical courses In the United States, "Continental Classroom" is a television programme for the teaching of teachers It goes out at 6 30 a m to a weekly audience of 1,700,000 through 150 stations

Mr Jan Varossieau (Netherlands) of the Stichting Film en Wetenschap Universitaire Film Institute, described the use of closed-circuit television for the teaching of science in the universities A van had been fitted out to tour the country It carried a Philips compact 'Vidicon' transistor camera This could be linked to monitors in the lecture hall

The congress agreed that closer co-operation between film and television producers would be of mutual benefit, and that there was a great need for centralized information services

Information was given of the detailed plan prepared by the Association for Unesco to facilitate the distribution of films to universities The purpose is to prepare a select list of films which could be used in first- or second-year university courses and of which it is possible to supply copies This is an experimental project and the number of subjects to be covered and the number of films to be selected is limited In co-operation with the International Council of Scientific Unions, a list of subjects has been prepared, reported Mr B Chibnall (Great Britain) the director of the project The list includes subjects in botany, zoology, geology, chemistry and physics

The International Film Library has been set up in Brussels, during the past year It is having some initial troubles, but the Belgian Government has given a generous grant and premises Among the directors are Mr Edgar Anstey and Mr John Maddison (both of Great Britain)

Among other decisions taken at the congress were to investigate the possibility of compiling and having published an international multilingual standard reference journal and vocabulary of scientific cinematography, and to standardize film catalogues An offer by the Moroccan Government to investigate the possibility of setting up a distribution centre in Africa for scientific films was accepted Two newly elected member countries of the Association are the United States and North Korea This brings the total of member countries to twenty-seven, with three corresponding member countries

The new officers of the Association are as follows *President*, Edgar Anstey (Great Britain), *Vice-presidents*, J Jacoby (Poland), S Okada (Japan), R Whaley (U S A), A Zgouridi (U S S R), *Hon Secretary*, Mohammed Afifi (Morocco), *Hon Treasurer*, Vladimir Vaclavek (Czechoslovakia)

The editor of *Scientific Film*, the official organ of the Association, is Mr Maurice Goldsmith, c/o The Scientific Film Association, 55a Welbeck Street, London, W 1. The 1962 congress is to be held in Warsaw, and the 1963 congress in Rome M GOLDSMITH

EDUCATION IN EMERGENT COUNTRIES

FORMERLY an outstanding educational administrator in New Zealand and now his country's Ambassador to France and permanent delegate to Unesco, Dr C E Beeby presented an interesting paper on September 5 to Section L (Education) of the British Association for the Advancement of Science meeting at Norwich The paper was based on Dr Beeby's experience with the growth of educational services in newly developing countries, and especially Western Samoa, but also drew from his wide acquaintance with the evolution of educational systems in Western countries

Dr Beeby showed that, while it has taken England a hundred years to work out a system of universal primary education fairly well adapted to the needs of the community and to the varied capacities of the children in schools, a number of emergent countries is now trying to do the same thing in a decade At first sight it might appear that, with Western aid and benefiting from Western experience, they could leap the century and establish straight away something approaching a good modern type of classroom without having to plod through the stage of dreary formalism that marked the first fifty years of the Western system of compulsory schooling

The position is not as simple as this, and the 'expert' advising an emergent country on its primary school system finds himself in the position of a butterfly teaching the chrysalis how to fly There is an intermediate stage which must be gone through before some of his advice is of much significance Dr Beeby became aware of this sixteen years ago when he had some responsibility for the control of two education systems that were, at that time, at least half a century apart He found himself, without any sense of inconsistency, encouraging in Western Samoa the introduction of the formal educational practices which he had for a long time been combating in New Zealand This led to the conception of stages of development in the life-history of an education system, stages through which all systems, at least of a certain type, must pass, and which, though they may be shortened, cannot be skipped

The inability of a primitive type of school system to profit immediately from all that has been learned about modern teaching methods is in no way due to any innate inferiority of either children or teachers in emergent countries It depends primarily on two professional factors—the level of general education of the teachers in the system, and the amount and kind of professional training they have received On this basis the development of an education system could be roughly divided into three stages (1) 'dame school' stage, where teachers are ill-educated and untrained, (2) stage of formalism, where they are ill-educated but trained , (3) stage of meaning, where they are relatively well educated and also trained At the dame school stage the syllabus consists of little but mechanical drill on the three R's and the memorizing of relatively meaningless symbols The unsophisticated and untrained teacher turns naturally to formal methods of teaching, and the real weakness of the teaching at the dame school stage is that it is confusedly and inefficiently formal It has all the defects of formalism and none of its virtues , it is formalistic in spirit without having form

At the other end of the scale the main characteristics of modern education, in spite of its failings, are the attempt to give the child a deeper and wider understanding of the meaning of the symbols with which he works Practice in a good modern school is free, informal, and apparently 'natural' , but it is based on a complex and sophisticated structure of theory, and, to succeed, it demands teachers who are both well-educated and well-trained This is a long step from the ill-educated and untrained teacher at the dame school stage and in between must inevitably come the stage of formalism where teachers are still ill-educated but are trained For sheer formlessness the only remedy is more form, however much one may dislike formalism as such in education

The freedom and informality of the modern classroom depend on the teacher being sure of himself and of his subject-matter, and a teacher who is teaching at the very limits of his knowledge can have no sense of security if he or his class wander beyond the bounds of a tight official syllabus or the paste-board covers of the one official text-book The only questions he can allow are those he asks himself, for they are the only ones of which he can be sure of knowing the answers So the classroom must become formal At the stage of formalism, symbols have more meaning for children than at a dame school stage, but it tends to be a narrow and restricted meaning Classroom facts and skills are thin disparate things that mesh poorly with one another and scarcely at all with the warm rich world of the child's personal life

It is tempting to try to find a short cut from the dame school stage or the lower levels of the stage of formalism to the best of modern teaching methods, but there seems little hope of this if the difficulty to be overcome is really the meagre education of the teachers who are themselves the products of the schools one is setting out to reform With help and advice, the stage of formalism can certainly be reduced in length, but the reform of a school system is a skilled and delicate business, where too much haste can create confusion, and where our own unanalysed experience may not always be the best of guides The difficulty is increased by the fact that not all the teachers in a system at any moment have the same capacity to take up new and complex methods

A new element of urgency has developed with the explosive demand for education in newly independent States that has been one of the striking characteristics of the past decade The demand for education, indeed, has become a new political force in the world, a force that no democratic Government can long resist, and countries are sometimes driven to launch ambitious schemes of universal education before they have teachers with enough education to make them effective Unless there is to be a great waste of effort and grievous disappointment with the results of popular education in emergent countries, there is urgent need for a scholarly study, from a new and practical angle, of the whole natural history of the educational system and of the factors that help or retard its progress from stage to stage

EXPECTATION OF LIFE IN THE UNITED STATES

A RECENT report issued by the Metropolitan Life Insurance Co , New York, directs attention to the fact that the average length of life (expectation of life at birth) among industrial policy-holders reached a new record of 70 5 years in 1960* This is slightly above the figure for 1959, and only half a year greater than that for 1955 But although recent increases have been limited, long-term gains have been very impressive Five years have been added to the average length of life of these policy-holders since 1946, ten years since 1937, and twenty-four years since 1909 In 1879–94, the earliest period for which data are available, the average lifetime was only 34 years—less than half that for 1960

The largest gains in expectation of life have occurred at the younger ages Thus, at the age of 5, the figure for 1960 was 5 9 years greater than that two decades ago At the age of 40, the increase since 1940 has been 4 3 years, while at 65 the average future lifetime of those insured has been extended 2 3 years to its present level of 14 2 years

The longevity of American wage-earners and their families has increased more rapidly than that of the general population of the United States In 1909, the expectation of life at birth of the industrial policy-holders was about six years less than that of the population as a whole By mid-century the two groups were on a par, at present industrial policy-holders have a slight advantage

The long-term achievements in the conservation of life are accentuated by the very low death-rates now experienced in early life Mortality of the coloured policy-holders, particularly beyond childhood, is considerably higher than that for whites Among females, the rates for the coloured are at least double those for the whites from age 23 to the end of the child-bearing period Even though the difference is not as large for males, the ratio of mortality among them for the age-range 24–39 is more than 3 2.

Similarly, the average length of life is appreciably greater for white than for coloured policy-holders, and in each race is greater for females than for males In 1960, the expectation of life at age 5 years was 70 7 for white females, compared with 66 2 years for coloured females, the corresponding figures for males were 63 8 and 61 9 years

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* Metropolitan Life Insurance Company *Statistical Bulletin*, Vol 42 (June 1961) *Voting Population at Record High Recent Variations in Pneumonia–Influenza Mortality Longevity of Industrial Policy-holders in* 1960 *Accident Death Rates Decreases in Most States* Pp 12 (New York Metropolitan Life Insurance Company, 1961)

THE AMERICAN ACADEMY OF ARTS AND SCIENCES

THE *Records of the American Academy of Arts and Sciences 1960–1961** includes, as usual, the list of officers and committees, lists of members (including those elected May 10, 1961) with alphabetical index, the statutes and charter of incorporation, and reports submitted at the annual meeting Special interest is given by the valedictory address of the retiring president, Kirtley F Mather, which, under the title "Assay and Perspective" reviews the activities of the Academy during the four years of his presidency before appraising the present position of the Academy and its responsibilities and opportunities during the next decade While it was doubtful whether the fullest possible use had been made of the Academy's new house, Mr Mather thought that the success in developing its publication *Daedalus* as a journal of ideas and opinion had been outstanding and the Academy had been most successful in its development of conferences on topics of wide importance and interest

As regards research, 119 grants totalling 91,903 dollars had been made in the past four and a half years, compared with 452 applications aggregating more than 500,000 dollars, and the Academy was seeking to increase the number and scope of its awards for recognizing excellence, as yet it had no such award in the social sciences corresponding with its Rumford Medals in the physical sciences, the Amory Prizes in the medical sciences and the Emerson–Thereau Medal in the broad field of literature. The Academy had made no public pronouncement during the period, but Mr Mather was not satisfied that there had been a suitable theme, and he inclined to think that the Academy was doing what it should more effectively by providing the information to illumine the problems on which policy makers must make their decisions.

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* American Academy of Arts and Sciences, Boston Records of the Academy (including new members elected 10 May, 1961), 1960–61 Pp 157 (Boston, Mass , American Academy of Arts and Sciences, 1961)

As regards the future, Mr Mather said that the Carnegie Corporation had made a grant of 50,000 dollars for each of the next two years for the continuation of the Academy's conference and study programme It would be necessary in the next four years to assess the value of the Academy's house and the function of the Academy in the State, but he thought that the Academy's outstanding opportunity and responsibility in the next decade lay in serving as a liaison between the community of practical affairs and the practitioners and scholars in the arts and sciences

BRITISH COLUMBIA RESEARCH COUNCIL

AMONG the varied research items described in the seventeenth annual report of the British Columbia Research Council is one into marine borer control (Pp 16 Vancouver British Columbia Research Council, 1961) During 1960–61 the stations recording marine-borer breeding were increased to more than thirty as more companies engaged the Division of Applied Biology for protecting logs and wooden installations against losses by borers By its larval inspection and log-scheduling service, the Division undertook the protection of more than 100 million feet of timber in sea-water storage for Canadian companies and a similar volume for a company in Puget Sound, where the intensity of marine-borer attack is the highest on the North Pacific Coast.

In instances where serious damage by borers would occur before the logs could be used, the rafts were treated chemically by a sprinkling system developed by the Council During 1960–61 more than 100 sections of high-grade stock were treated in this way prior to hauling from Alaska to Puget Sound, a total of 10 million board feet of wood were chemically treated

One of the outstanding achievements in this field in 1960 was the development of a sonic device for testing the internal condition of pilings This unit accurately reveals even minor damage by borers in piling. The unit has been used for testing the soundness of piling in wharves on Vancouver Island and in Vancouver Harbour Operating in conjunction with a diver, 130 piles a day can be examined

Also developed during the past year was a technique for enshrouding individual piles so that they can be treated chemically to kill embedded borers This method was developed to protect uncreosoted piles which are used in many logging operations on the coast and as fender piles on many wharfs. The shroud is all plastic to avoid corrosion in sea-water, and can readily be clipped on and off a pile without the use of hooks

A number of field studies on *Bankia setacea*, the local teredo-like borer, is being made These include the susceptibility of different species of wood to attack, the rate of loss by different species, the effect of water temperature on breeding, and studies on the borer itself

The Canadian Uranium Research Foundation has recently made a grant of 10,000 dollars a year for investigation into the use of uranium compounds as toxicant additives to wood impregnants for marine borers

RECRYSTALLIZATION OF LEAD AND ZINC SULPHIDES

By Dr. J. C. NIXON
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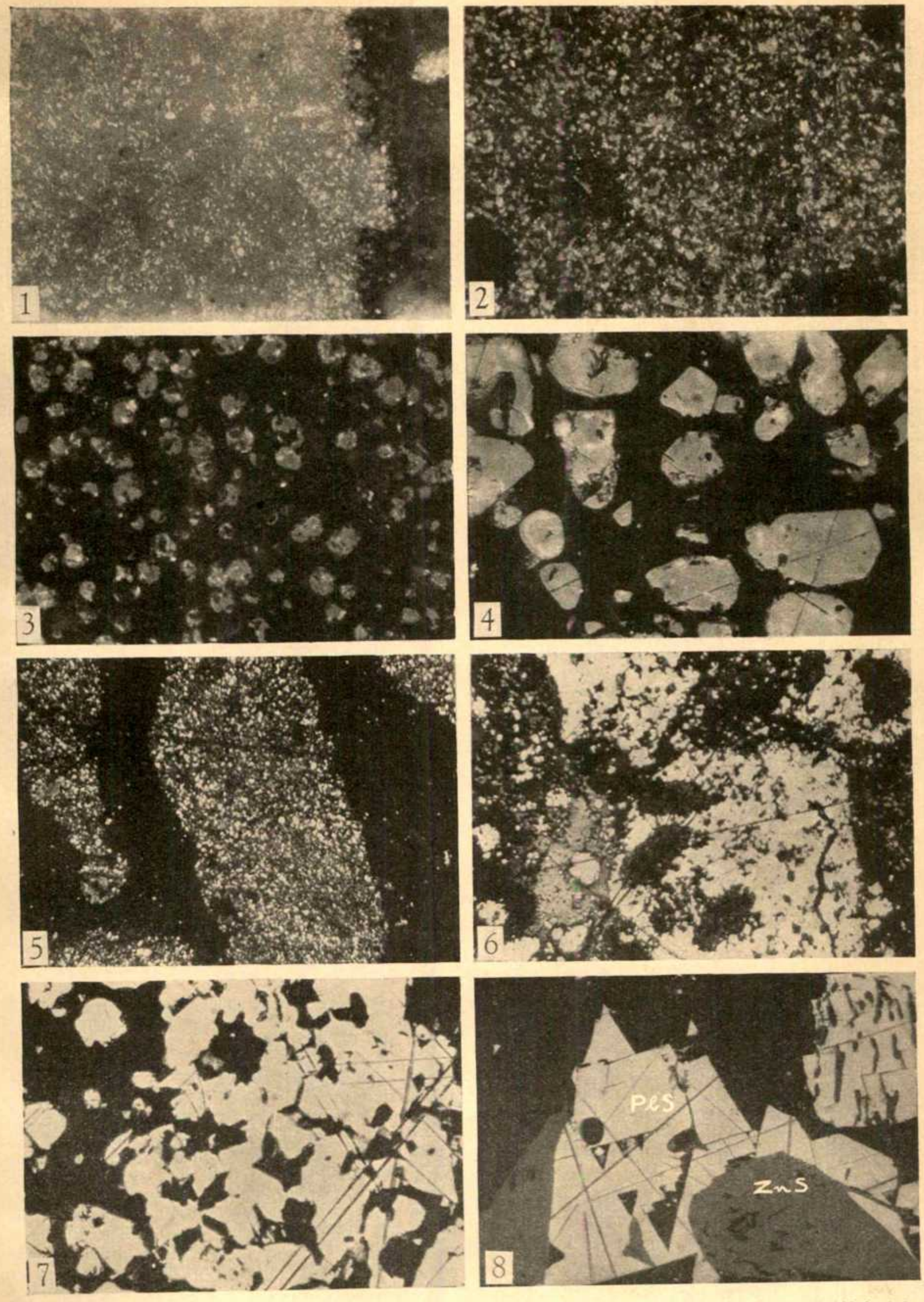
AND
J D HAYTON, D. C. LAWRIE and A E. TYNAN
Australian Mineral Development Laboratories, Parkside, South Australia

IDEAS advanced in recent years for the formation of conformable ore deposits of base metals (the Broken Hill lead-zinc deposit in New South Wales is one of these) imply that the observed ore textures were developed by crystallization or re-crystallization after initial deposition The earlier ideas depended in part on the assumption that the mineral textures were original and depositional Since a useful understanding of the history of these deposits could be of assistance in exploration, some simple experiments were conducted to find out whether the textural condition of lead and zinc sulphides was or was not affected by temperatures which might be attained in deep burial and in high-rank metamorphism

Chemically precipitated sulphides of lead and zinc were subjected to prolonged treatment in sealed vessels at elevated temperatures The sulphides were prepared by precipitation with hydrogen sulphide from solutions of lead and zinc salts The washed precipitates were dried at 90° C in an inert atmosphere, and stored in a vacuum desiccator Samples of each



Figs. : (1) Zinc sulphide heated at 200° C.; (2) heated at 400° C.; (3) heated at 600° C.; (4) heated at 800° C. (5) Lead sulphide heated at 200° C. ; (6) heated at 400° C. ; (7) heated at 600° C. (8) Zinc sulphide mixture (1 zinc : 1 lead) heated at 800° C. All at magnification 250

sulphide, alone, and with water were heated in a nitrogen atmosphere in stainless steel bombs for 3 weeks at temperatures of 200°, 400° and 600° C. A further sample of each sulphide with water and two mixtures of lead and zinc sulphides and water were heated (in bombs) at 800° C. for 3 days. The molecular ratios of zinc to lead were 1 and 2 in the respective mixtures. The temperature rose above 800° C. for part of the time—perhaps as high as 950°—and the bombs were so badly oxidized after three days that it was considered unsafe to continue heating. The initial sulphides and the materials discharged from the bombs were examined by X-ray diffraction and optical methods.

The X-ray patterns of the initial sulphides had broad peaks, indicating somewhat poor crystallinity. Patterns obtained for both sulphides after treatment at 200° C. for 3 weeks had sharper peaks, showing an improved degree of crystallinity. Materials from subsequent tests at higher temperatures gave diffraction patterns essentially the same as those obtained for the respective sulphides treated at 200° C., indicating that at this stage the materials were sufficiently well crystallized to show maximum sharpness in the diffractograph peaks. Products from heat treatment at temperatures higher than 200° C. were examined optically to determine the degree of crystallization. Optical examination revealed little significant difference between the initial sulphides and those treated at 200° C. In the latter it appeared that re-crystallization had begun in myriads of centres which were less than 1μ across in either sulphide. All these samples consisted of small granules, average size 2–3μ, which were aggregates of particles. Individual grains were detected in the materials heated at 400° C. and a few grains up to 80μ in width were seen in the zinc sulphide heated in the presence of water at this temperature. Some crystal faces were seen on the larger grains of this material. The zinc sulphides heated at 600° C. contained free grains showing no tendency to coalesce. The average particle size was approximately 12μ. The lead sulphides heated at 600° C. contained sintered aggregates of galena. The largest individual particles of galena in both samples were approximately 60μ across.

No really significant difference in resultant particle size was detected between wet and dry treatment. This would indicate that the higher pressures resulting from the presence of water vapour do not have any pronounced effect on the resultant particle size. The zinc sulphide heated at 800° C. for 3 days contained particles up to 90μ in width. The majority were between 30 and 50μ across, and well crystallized. The lead sulphide was reduced to metallic lead at 800° C. There are several possible reactions by which this could occur, but this aspect was not investigated. The lead sulphide in the mixtures was similarly reduced but there were some remnants of galena. In the mixture which contained the higher proportion of lead sulphide (1 zinc : 1 lead) many crystals of both sphalerite and galena were more than 0·5 mm. long and the galena contained numerous blebs of sphalerite typical of a eutectic textural intergrowth. The curved contacts between much of the two minerals were comparable with the mutual boundaries texture found in many natural galena–sphalerite ore bodies. Relatively more of the galena was reduced to metallic lead in the mixture which contained the lesser proportion of lead sulphide (2 zinc : 1 lead). However, the remnants of galena were well crystallized, but of smaller crystal size than in the other sample. The sphalerite was well crystallized and massive, with crystals of the same order of magnitude as in the other mixture.

It is clear that heating lead and zinc sulphides under the conditions described resulted in crystal growth, the effect being more enhanced at higher temperatures. The accompanying photomicrographs of polished sections of the briquetted materials illustrate this trend most effectively.

The work described was done by the Australian Mineral Development Laboratories on behalf of Consolidated Zinc Pty. Ltd.

BIOLUMINESCENCE IN THE SURFACE WATERS OF THE SEA

By RICHARD H. BACKUS, CHARLES S. YENTSCH and ASA WING

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RECENT work by Clarke *et al.*[1-4], who have lowered photomultiplier tubes to great depths at sea, have shown that flashing by bioluminescent organisms is present in varying degree at all depths in all localities of the ocean so far examined. Clarke's instrument detects bioluminescence resulting from the combined activities of a variety of organisms and only measures bioluminescence when the ambient light-level is low enough not to mask the flashes.

In order to make observations of bioluminescence independent of ambient light conditions we have devised an apparatus in which water from the sea surface is pumped at the rate of six gallons per minute through a darkened chamber fitted with a photomultiplier tube (*RCA* 6655–*A* with *S*11 spectral response). The tube is connected by appropriate circuiting to a recorder such that organisms luminescing while passing through the chamber are tallied and their numbers recorded at the end of either a 5-min. or a 1-hr. period. The effluent of the chamber may be strained with plankton nets for collecting its fauna and flora.

The plumbing system (Fig. 1) consists of the pump (Jabsco 2187), plastic intake and discharge hoses $\frac{1}{2}$ in. inside diameter, the viewing chamber (where the stream of water is about 2 in. wide and $\frac{1}{4}$ in. deep) and a flow-meter. The length of the intake hose was about 13 ft. in a harbour installation and about 50 ft. in a shipboard installation. In the harbour apparatus it took an organism somewhat more than 1 sec. to pass from the intake to chamber and in the shipborne apparatus about 5 sec. The intake of bioluminescent organisms into the plumbing system stimulates them to flash. Some flash at the point of intake and others all along the hose between intake and chamber. but, as our observations show, many flash in the chamber where the flow pattern is radically altered.

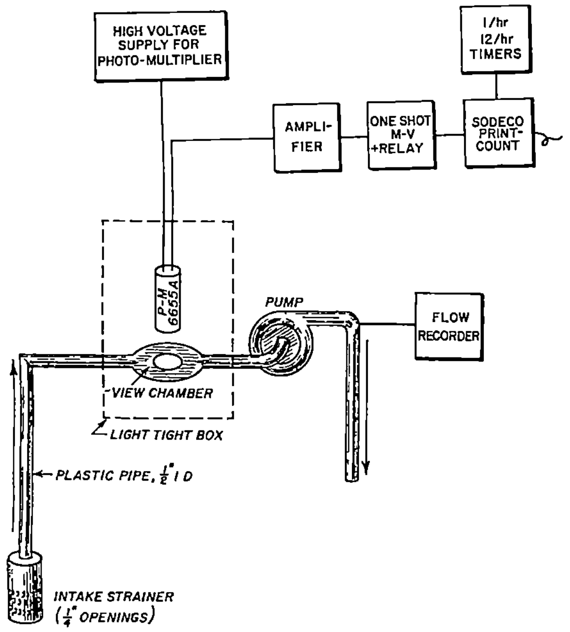

Fig 1

At the shore installation counts were recorded by either a Hewlett–Packard print-out counter (Counter 523 *BN* and Printer 560–*A*) or a Sodeco 1*Tpb*53 print-out counter At sea, counting was done with a simple solenoid-operated Sodeco counter which was read at appropriate times and re-zeroed

Counts of flashes for 1-hr periods for five days each in May and November 1960, in Great Harbor, Woods Hole, are shown in Fig 2 A pronounced diurnal rhythm in activity is obvious In May, maximum, or night-time, counts were about 700 times the minimum, or day-time counts, while in November these differed by a factor of about 100 Details of the rapid increase in count which occurs at sunset are shown in Fig 3 Here counts every 5 min are plotted relative to sunset for two days each in May and November 1960 and show that prior to sunset more bioluminescence occurred in May than November

A No. 20 plankton net was used to strain the effluent for 1½ hr at the time of minimum count and again for the same period at the time of maximum count on December 2, 1960 Counts of the principal groups found and the bioluminescence counts are given in Table 1 The numbers of organisms deemed potential sources of bioluminescence increased at

Table 1 BIOLUMINESCENCE COUNTS (PERIOD TOTALS) AND NUMBERS OF ORGANISMS (PER m ^{3} OF EFFLUENT) IN GREAT HARBOR, WOODS HOLE, DECEMBER 2, 1960

Time (E S T)	Bioluminescence	Diatoms*	Dinoflagellates†	Tintinnids*	Copepods‡
1330–1500	52	35,600	1,425	3,560	2,140
1730–1900	6,120	156,200	6,500	4,130	3,440

* Not luminescent
† All (?) species luminescent
‡ Some species luminescent

Table 2 STATION DESCRIPTIONS

Station	Position	Date (1960)	Time (E D T)	Depth* (fm)	Surf Temp (°F)
A	40° 39′ N , 72° 30′ W to 40° 35′ N , 72° 41′ W.	Sept 20	1230–2115	19–21	66 5
B	40° 29′ N , 72° 40′ W	Sept 21	0110–0130	24	62 8
C	40° 20′ N , 72° 39′ W	Sept 20–21	2310–0010	25	64 0
D	40° 06′ N , 72° 37′ W	Sept 21	0420–0445	29	—
E	39° 35′ N , 72° 32′ W to 39° 23′ N , 72° 45′ W	Sept 21	1115–2100	44–50	65 4
F	38° 55′ N , 72° 34′ W	Sept 22	0055–0130	920–970	73 5
G	38° 28′ N , 72° 27′ W	Sept 22	0425–0450	1,470–1,475	74 8
H	37° 35′ N , 72° 15′ W to 37° 26′ N , 72° 23′ W	Sept 22–23	1215–0730	1,680–1,760	71 9

* Bioluminescence observations and plankton samples were all taken from 1 m under the surface.

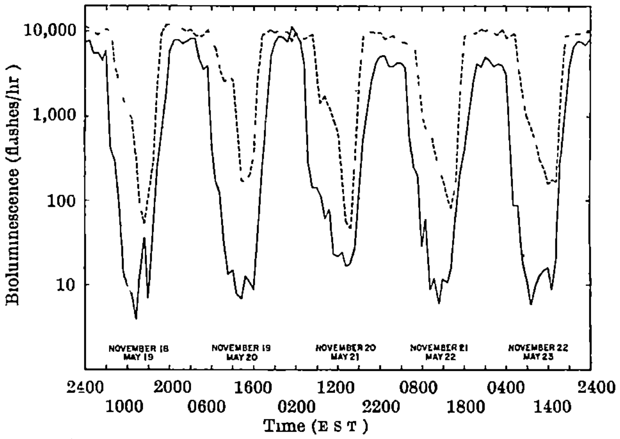

Fig 2

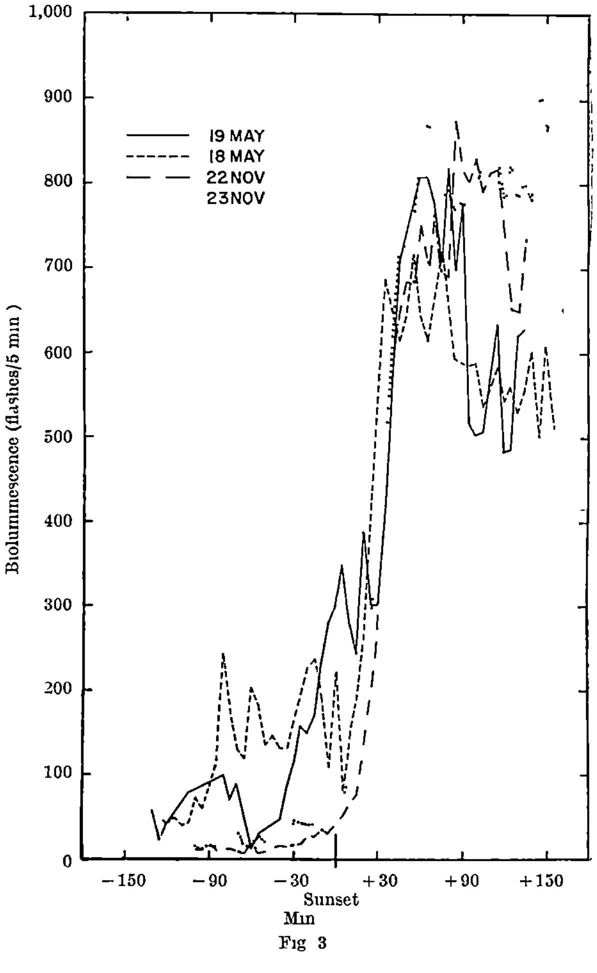

Fig 3

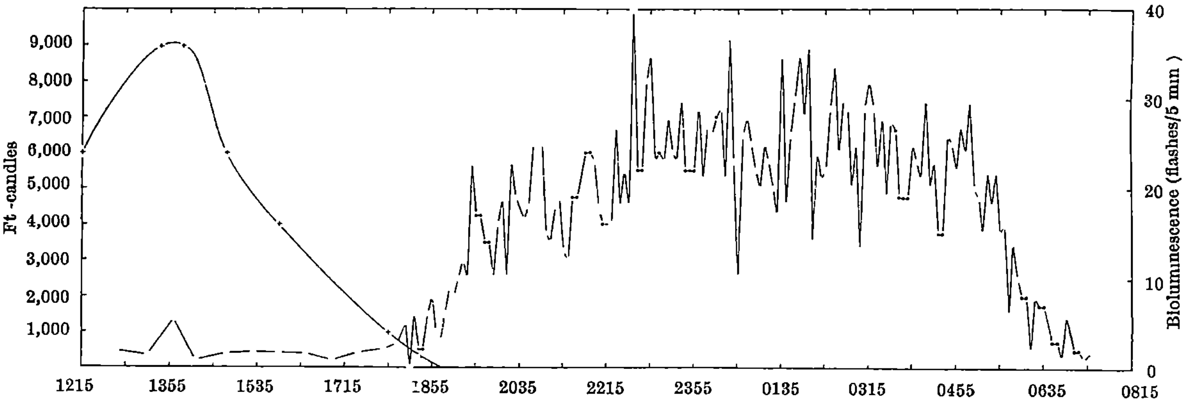

Fig 4

night by factors of about 1 2–5 While a part of the increase in numbers of organisms in the night sample may be due to vertical migration, the species composition was quite different from that during day, which suggests that the water mass changed between sampling times.

Similar observations of bioluminescence were made at eight stations (Table 2) in the continental shelf and slope waters south of New England in September 1960 The plankton community was collected simultaneously by passing the effluent of the pump through a No 20 plankton net Three stations (*A*, *E* and *H*) were occupied for 6–10 hr , including the time of sunset, in order to study the diurnal variation of bioluminescence The other five were used with these three for studying the geographical changes in night-time bioluminescence over the onshore–offshore section

At stations *A*, *E* and *H* a diurnal rhythm in bioluminescence was observed similar to that described for Great Harbor The observations for station *H* are shown in Fig 4 Here, as at stations *A* and *E*, sharp increases in counting-rate were observed when the daylight intensity fell below about 500 foot-candles. This appeared to be true regardless of the magnitude of radiation during the day Bioluminescence counts and counts of the principal organisms found in the effluent at station *H* during the afternoon and evening are shown in Table 3 Even considering the errors that may acrue due to plankton-counting techniques and to the loss of smaller organisms through the net it seems improbable that we would miss a vertical migration of plankton consistent with and of a magnitude sufficient to account for the diurnal changes in bioluminescence observed Results at both stations *A* and *E* were similar

Table 3 BIOLUMINESCENCE COUNTS (HOURLY TOTALS) AND NUMBERS OF ORGANISMS (PER M ³ OF EFFLUENT) AT STATION *H*

Time (E D T)	Bio-luminescence	Diatoms	Dino-flagellates	Green flagellates	Tin-tinnids	Copepods
1400–1500	15	1,600,000	3,063	700,000	396	6,030
1600–1700	16	1,150,000	1,975	300,000	1,088	1,877
1800–1900	46	567,000	2,322	437,000	838	4,440
1900–2000	143	1,475,000	3,510	271,000	811	3,780

Bioluminescence counts, counts of principal plankton organisms and amounts of net and total ('Millipore'-filtered) chlorophyll from the pump effluent during night hours at all stations are given in Table 4 Bioluminescence was at a maximum at *A*, the station nearest the coast, was low across the continental shelf (*B–D*) and rose to an intermediate value at the edge of the shelf (*E*) and remained moderately high at the stations in slope water (*F–H*) Numbers of dinoflagellates (mostly genus *Ceratium*) more or less followed this trend and it is probable that most of the bioluminescence we observed came from these organisms The copepods were more numerous in slope water than at the stations on the shelf, but the cladocerans were present at the shelf stations and absent in the slope water Tintinnids were absent at the shelf stations and present at the slope stations The principal non-luminescent organisms encountered were diatoms They were numerous on the shelf and, in general, diminished seaward except for the large population at station *H* The distribution of total chlorophyll and net chlorophyll was also higher on the shelf than on the slope

It has been our experience that the larger dinoflagellates, which are important producers of bioluminescence, are missed when counts are made on raw sea-water samples of 100–200 ml , thus a fine plankton net had to be used to concentrate these

Table 4 BIOLUMINESCENCE, PRINCIPAL PLANKTERS (NUMBER PER M ³) AND CHLOROPHYLL (μGM /L) AT STATIONS *A–H*

Station	Sampling period, E D T (bioluminescence)	Bioluminescence* counts/5 min	Sampling period, E D T (plankton and chlorophyll)	Dinoflagellates	Copepods	Diatoms	Tintinnids	Cladocera	Chlorophyll† Total	Chlorophyll† Net
A	1955–2115	33 2	2000–2100	514,000	1,710	32,600,000	—	2,020	0 36	0 0021
B	0110–0130	12 5	0108–0128	842,000	1,161	54,000,000	—	327	0 56	0 0063
C	2310–0010	1 9	2352–0012	2,000	1,183	53,000,000	—	312	0 37	0 0060
D	0420–0445	4 8	0420–0444	1,470	1,770	1,019,000	—	—	0 32	0 0020
E	1955–2100	16 0	2000–2100	4,270	5,400	8,010,000	344	98	0 21	0 0006
F	0055–0130	16 9	0113–0133	4,160	5,055	6,540	1,800	—	0 39	0 0018
G	0425–0450	12 7	0425–0445	1,839	4,500	3,150	1,300	—	0 21	0 0016
H	1955–2200	18 1	1900–2000	3,510	4,000	1,480,000	811	—	0 20	0 0013

* Flow-rate, 25 l /min

† Chlorophyll determinations by the methods of Richards with Thompson (ref 5) and Creitz and Richards (ref 6).

organisms. We are aware that a great fraction of the phytoplankton passed through the No. 20 net[11]. Comparison of the net to total chlorophyll (Table 4) shows that the net entrained less than 1 per cent of all the photosynthetic autotrophs.

Our observations are consistent with certain observations (summarized by Harvey[8]) of bioluminescence in sea-water samples containing dinoflagellates held under various conditions of light and dark, and with recent observations of circadian rhythms of bioluminescence in laboratory cultures of dinoflagellates[9,10].

Seliger, Fastie and McElroy[7] have recently published observations of bioluminescence in Chesapeake Bay using an instrument which integrates light intensity over short periods rather than counting flashes. They observed no diurnal rhythm and concluded that changes in intensity throughout the water column were due to the vertical migration of organisms. In contrast, we feel that the diurnal pattern of bioluminescence observed by us largely results from the inactivation of the bioluminescent system of the organisms responsible by daylight. The discrepancy between the Chesapeake Bay and Woods Hole results may be due in part to the difference in instruments but more than likely comes mainly from the geographical difference.

This work was supported by the National Science Foundation (grant *NSF*–G9579 and grant *NSF*–G8339) and the Atomic Energy Commission (contract *AT*(30–1)–1918).

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RESERPINE-INDUCED CIRCULATORY FAILURE

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DURING work on various drugs affecting adrenergic mechanisms, the first results of which have already been published[1], it was found that 24 hr. after the administration of 1 mgm./kgm. of reserpine a good number of cats, despite all care, died. Those animals which survived were very ill, lay immobile, unresponsive to sound, apparently lifeless. When handled and gently caressed, however, they opened their eyes and, with great difficulty although not paralysed, succeeded in making some movements, while purring and yet crying simultaneously. Despite all precautions, body temperature tended to fall, respiration was depressed, blood pressure was very low and the heart-rate markedly reduced. There was a loss of body-weight, possibly because the animal neither ate nor drank and, in addition, suffered marked diarrhœa. In other words, cats thus treated were very sick animals and presented a picture of great misery very similar in many ways to that of a human being during a stage of advanced shock (for example, late stages of acute circulatory failure), and definitely very much unlike the picture of an animal under the influence of an ordinary central depressant drug.

Reserpine is known to reduce or deplete the various tissues of their catecholamine content, and its pharmacological actions, especially those on circulation, have been, as a rule, attributed to this depletion[2]. Because of this, the cardiovascular system of animals pretreated with reserpine or isolated tissues from these animals have been extensively used for the study of the mechanism of action of various drugs. The cat has been a favourite experimental animal and, in order to 'reserpinize' it, doses of at least 1 mgm./kgm. of the drug have usually been used. The severe clinical condition, however, of the cats treated with reserpine was considered unlikely to be the result only of a depletion, however complete, of the animal's catecholamines. Experiments were therefore undertaken in which various parts of the cardiovascular system could be studied simultaneously *in vivo*.

Most cats were given a single dose of 1 mgm./kgm. of reserpine, either subcutaneously or intraperitoneally, on the day before the experiment. A few received the same amount of the drug spread over two or more days. Both the control and the reserpine-treated animals were anæsthetized with chloralose and prepared for the recording of blood pressure, peripheral blood flow (measured by recording the venous outflow from a hind limb) and force of cardiac contraction (recorded by means of a strain gauge arch attached directly to the muscle of either the right or the left ventricle).

The blood pressure of the cat pretreated with 1 mgm./kgm. of reserpine was low, rarely exceeding 70 and very often less than 50 mm. mercury and the heart-rate very slow. When the chest was opened, the heart was found to be enlarged, flabby and soft and the superior and inferior venæ cavæ and their tributaries engorged. The right side of the heart was, as a rule, at this stage more affected than the left. Moreover, the force of cardiac contraction was well below normal and the application of pharmacological tests clearly demonstrated that the heart was in failure. Furthermore, it was found that: (*a*) the sensitivity of the peripheral vessels to adrenaline, noradrenaline and isoprenaline, administered intravenously or close-arterially, was decreased; (*b*) any changes of blood pressure were, as a rule, secondary to changes in cardiac contraction; and (*c*) the peripheral blood flow passively followed the changes of blood pressure. In other words, 24 hr. after the administration of 1 mgm./kgm. of reserpine the cat exhibited a most spectacular circulatory failure, and furthermore any improvement of the circulation at this stage was found to be almost exclusively the result of an amelioration of the force of cardiac contraction.

Although circulatory failure was always present, its degree varied from animal to animal. For example, in older, lactating or pregnant animals the condition was at its worst. When the heart failure was less advanced, the force of cardiac contraction increased after the administration of adrenaline, noradrenaline or isoprenaline. However, as a rule, noradrenaline produced the largest inotropic effect, adrenaline was less effective, followed by isoprenaline. Moreover, in a number of experiments the force of cardiac contraction after isoprenaline was either unaffected or clearly depressed. Thus, in the animals treated with reserpine the ratio appeared quite different from that in the control cats, where isoprenaline is the most powerful of the three catecholamines. The reason for this rather puzzling behaviour of the catecholamines in the animal treated with reserpine became apparent when changes of heart-rate were recorded at the same time. It was found that the heart of the animal treated with reserpine, in contrast to the control, was unable to sustain an increased activity. Consequently, its response to isoprenaline varied according to its condition at the moment of the administration of the drug; if very poor, the heart faced the abrupt increase in heart-rate with a clear-cut depression in the force of its contraction; on the other hand, when failure was not very advanced, the heart contraction could still improve, but even then the response was always much smaller than that usually seen in the control heart. A similar variation, although less pronounced, was found with adrenaline. In the majority of experiments, adrenaline did produce a positive inotropic effect. Occasionally, however, when heart failure was very advanced, the effect of adrenaline on the heart was depressant. The only catecholamine the administration of which was always followed by a positive inotropic effect was noradrenaline.

In control cats a dose of 50–100 μgm. of ouabain produced very little or no change at all in either the force of cardiac contraction or the blood pressure. In contrast, in the animals treated with reserpine after ouabain, a gradual increase in the amplitude of the cardiac contraction occurred accompanied by a simultaneous rise in blood pressure. Furthermore, ouabain was found partially to restore the positive inotropic effect of various drugs, which in the animals treated with reserpine became ineffective or depressant to the heart. This was the case with both tyramine and isoprenaline.

Furthermore, histological examination of the heart muscle revealed in each case extremely severe and widespread degenerative changes (Fig. 1). The examination of various other organs is still in progress. However, the results already obtained indicate that the liver undergoes, at this stage, centrilobular (fatty) changes indicative of heart failure and raised venous pressure. Moreover, while the adrenal medulla appeared normal, the cortex in most animals showed a picture characteristic of severe stress with widespread degenerative changes affecting the cells of both zonæ fastigiata and fasciculata.

From these results it is quite obvious that, in the cat, reserpine in a dose of 1 mgm./kgm. produces a most spectacular poisoning of the heart muscle, leading rapidly to disastrous functional impairment. As the myocardium is of the very essence in the maintenance of the circulation, all other factors which contribute to the circulation of the blood, although important, are likely to be secondary to the function and integrity of the heart muscle. However, the reduced sensitivity of the peripheral vessels of these animals to the normal mediators must undoubtedly contribute to the circulatory debacle which develops in such an incredibly short period of time.

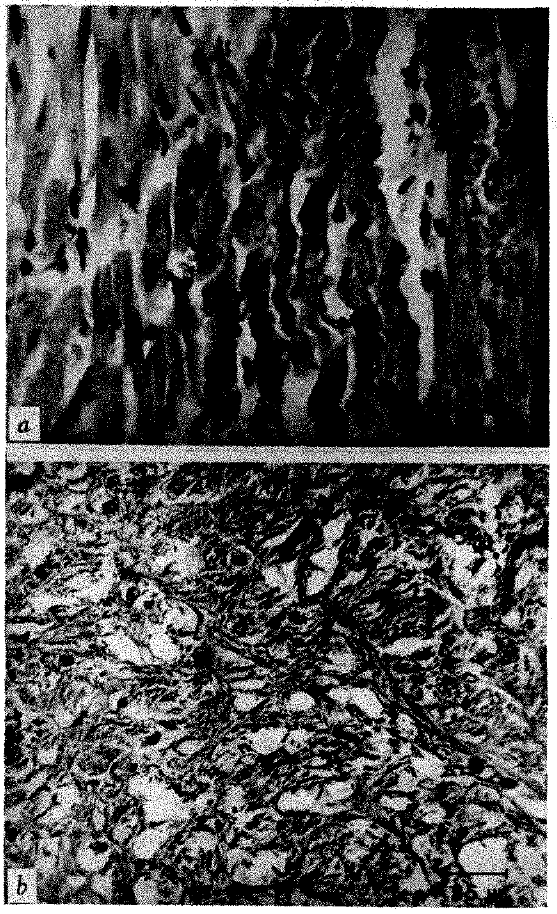

Fig. 1. Photomicrograph of myocardium (hæmatoxylin and eosin): (a) from a control cat; (b) from a cat treated with reserpine (0·25 mgm. per kgm. for 5 days)

The function and histological abnormalities found in the other organs of the animals treated with reserpine may be due to either direct toxic effects or to changes secondary to tissue anoxia, bound to exist in an acute circulatory failure.

The mechanism by which reserpine produces such powerful toxic effects remains to be elucidated. However, the functional and histological changes on one hand and the finding on the other hand that a metabolically 'active' tissue is more affected than a tissue relatively 'at rest' strongly suggest that the drug inhibits either some energy-yielding or some energy-consuming reaction. Kirpekar and Lewis[3] and Gillis[4] have already suggested a depressant effect of reserpine on the oxidative metabolism of various tissues.

Evidence that reserpine is a highly toxic drug has been previously reported. Unfortunately, such evidence has not received the attention it deserved. Saffran and Vogt[5] found that in rats a single intraperitoneal injection of reserpine (2·5 mgm./kgm.) produced a fall in the concentration of corticotrophin of the pituitary to 30 per cent of the resting value The authors reached the conclusion that this effect of reserpine is not due to interference with storage of adrenocorticotrophic hormone in the tissue, but was simply the result of the drug acting as a stressing agent In their experiments, the losses in adrenocorticotrophic hormone were of a degree not previously encountered after the injection of drugs, but of the magnitude reported after adrenalectomy, or after removal of one and enucleation of the other adrenal The only substance which in Saffran and Vogt's hands caused an even greater loss of adrenocorticotrophic hormone was a severely toxic drug—a nitrogen mustard, 24 hr after the injection of di(2-chloroethyl) methylamine hydrochloride ('Mustine', Boots) the rats which had survived exhibited œdema and weakness and the corticotrophin of the pituitary had fallen to 10 per cent

Goldin *et al* [6] have reported that reserpine exerts an anti-tumour effect They demonstrated that treatment with reserpine produced a significant increase in the survival-time of mice carrying advanced lymphoid leukæmia (*L* 1210) and caused regression of the local tumour which develops at the site of inoculation Robison *et al* [7] have made similar observations Furthermore, West *et al* [8] demonstrated that reserpine, in small doses, inhibited the growth of three different tumours in mice, and decreased uptake of oxygen in the intact animal They reached the conclusion, however, that the anti-tumour effect of reserpine is mostly non-specific and dependent on depression and reduction of food intake

Reserpine appears thus to be one of the most powerful 'poisons' known in pharmacology, and the 'reserpinized' animal, with both central and peripheral circulation in acute failure, a most abnormal experimental tool

I am grateful to my colleagues, Drs P P and M G Scott and Elizabeth Davis for their valuable advice and help with the histological studies This investigation was supported by a grant from the Palissade Foundation, Inc , of New York, and grant *B*-2474 (*C*1) from the National Heart Institute, National Institutes of Health, U S Public Health Service

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4-SULPHANILAMIDO-6-METHOXY AND 6-ETHOXY PYRIMIDINE (I.C.I. NOS. 32,525 AND 33,536): TWO NEW LONG-ACTING SULPHONAMIDES

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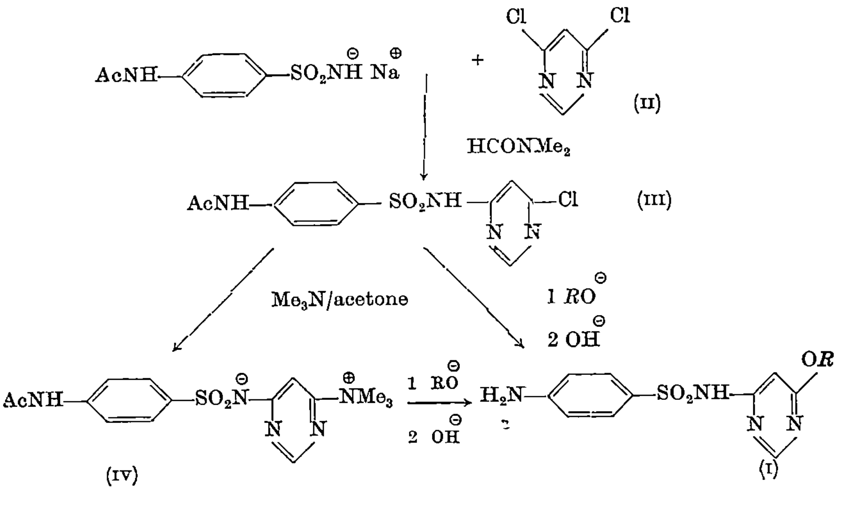
RENEWED activity in the sulphonamide field over the past few years has been aimed primarily at compounds with prolonged action The first compound with this characteristic, 2-sulphanilamido-4,6-dimethoxypyrimidine, was synthesized in these laboratories in 1947 (ref 1), and illustrated the importance of the methoxyl group in lowering the rate of excretion Sulphamethoxypyridazine[2] and sulphadimethoxine[3] followed from the laboratories of American Cyanamid and Hoffmann–La Roche and have attained wide clinical use

The one compound, closely related to these, which had not been prepared previously, was 4-sulphanilamido-6-methoxypyrimidine (I, *R* = Me) This and analogous compounds were readily available by the following route, starting with 4,6-dichloropyrimidine II (ref 4)



The zwitter-ionic trimethylammonium compound (IV) proved to be a useful intermediate in the preparation of higher homologues (compare ref. 5) since it is much more reactive to nucleophilic reagents than is the chloro-compound (III). 4,6-Dichloropyrimidine (II) reacts smoothly with ethanolic ammonia at room temperature to give 4-amino-6-chloropyrimidine. Replacement of the 6-chlorine with alkoxide followed by the standard sulphonylation procedure yielded I. The experimental details of these reactions have already been published[6]. The synthesis of the methoxy compound by similar routes was afterwards published by the American Cyanamid workers[7].

The 6-methoxy (32,525) and 6-ethoxy (33,536) sulphonamides are of outstanding efficacy against experimental infections in mice, as shown here, but this effect falls off as the size of the alkoxy group increases, presumably because of poor absorption from the gastro-intestinal tract. Substitution in the 5-position of the pyrimidine ring also lowered the antibacterial effect in most cases[8].

The antibacterial action *in vivo* and *in vitro* of 32,525 and 33,536 is compared here against three most widely used, long-acting sulphonamides. The effect of sulphaphenazole[9] against an experimental *Streptococcus pyogenes* infection was so poor that this compound was not included in further comparisons. The three organisms used in this work, both *in vivo* and *in vitro*, were *Salmonella dublin*, *Streptococcus pyogenes* (strain Kruger) and *Staphylococcus aureus* (strain Buttle 147).

Bacteriostatic action in vitro. Median inhibitory concentration of the sulphonamides mentioned were determined in Jewell and Pearmain's medium[10] (median inhibitory concentrations are expressed in µgm./ml. and are the mean of three concordant observations, determined graphically), they are shown in Table 1.

Table 1

Sulphonamide	Test organism *Strep. pyogenes*	 *Staph. aureus*	 *Sal. dublin*
32,525	13	3	5
33,536	19	3	15
Sulphadimethoxine	6	3·5	64
Sulphamethoxypyridazine	42	14	14
Sulphaphenazole	12	6	60

The results given in Table 1 show that 32,525 is as effective as the other compounds against the Gram-positive organisms and clearly illustrates its superiority against the Gram-negative.

Therapeutic action in vivo. Infected mice were treated orally with the sulphonamides, doses being given 1 hr. after the infection with subsequent doses at 24 and 48 hr. Deaths were recorded daily, mice alive on the eighth day (fifteenth in the *Staph. aureus* infection) being arbitrarily awarded a survival time of 8 (or 15) days. The figures given in Tables 2, 3 and 4 are 'effects', that is, mean survival time in days of the treated group minus mean survival time of the control group.

Table 2. *Streptococcus pyogenes*

Compound	Dose (mgm./20 gm.) 8	 4	 2	 1	 0·5
32,525	5·0	3·3	2·6	1·1	0·7
33,536	4·9	4·5	3·6	1·6	0·6
Sulphadimethoxine	3·7	1·2	0·4	0·2	0·0
Sulphamethoxypyridazine	3·8	2·3	1·8	0·6	0·2
Sulphaphenazole	1·9	0·4	0·2	0·0	0·0

Table 3. *Salmonella dublin*

Compound	Dose (mgm./20 gm.) 0·5	 0·25	 0·125	 0·06	 0·03	 0·015
32,525	5·5	5·0	4·8	3·5	2·3	2·0
33,536	5·1	4·9	3·9	2·6	2·1	0·9
Sulphadimethoxine	2·9	2·3	0·7	0·0	0·9	—
Sulphamethylpyridazine	3·0	2·6	1·4	1·6	0·9	—

Table 4. *Staphylococcus aureus*

Compound	Dose (mgm./20 gm.) 0·5	 0·25	 0·125	 0·06	 0·03
32,525	12·5	11·1	8·5	4·7	1·0
33,536	10·9	10·9	2·7	1·4	—
Sulphadimethoxine	7·8	3·6	—	—	—
Sulphamethylpyridazine	10·9	2·1	1·3	0·0	—

It can be demonstrated that the fraction of a sulphonamide which is bound to plasma proteins has no antibacterial activity[11]. Equilibrium dialysis experiments between buffer and human (or mouse) plasma show that the two new compounds are less extensively bound than the older ones. This fact, together with the high intrinsic activity and a persistence in the mouse about equal to that of sulphamethoxypyridazine, probably explains the superior effect of 32,525 and 33,536.

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GROUP RETENTION FACTORS IN THE GAS-LIQUID CHROMATOGRAPHY OF STEROIDS

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IT has been shown[1] that the retention time of a mono-unsaturated sterol methyl ether on the gas-liquid chromatogram, in which polyethylene glycol succinate is the liquid phase, may be expressed in the form

$$r_{(s+x)} = r_s \times k_x \qquad (1)$$

where $r_{(s+x)}$ is the retention time of the sterol having a double bond in position x, r_s is the retention time of the parent saturated structure and k_x is a constant factor, characteristic of the position of the double bond but independent of the molecular weight. The further relationship

$$r_{(s+x+y)} = r_s \times k_x \times k_y \qquad (2)$$

describes the retention time, $r_{(s+x+y)}$ of a dienol methyl ether with non-interacting double bonds in positions x and y, the second double bond being characterized by the factor k_y

These observations suggest that this type of relationship is a general one such that the introduction of a substituent group into a specific position in the steroid molecule influences the retention time by a factor which is a constant for the type of grouping and its particular site of attachment, but which is independent of the molecular weight of the total structure and of the influence of other functional groups, provided no interactions occur with them

If this is so, it is to be expected that equation (2) may be applied in its most general form to the steroids, so that the retention time of a poly-substituted steroid in which intramolecular group interactions are negligible can be expressed as

$$r_{(n+a+b+c+\ldots)} = r_n \times k_a \times k_b \times k_c \times \ldots \qquad (3)$$

where $r_{(n+a+b+c+\ldots)}$ is the retention time of the total structure, r_n is the retention time of the unsubstituted nucleus and $k_{a,b,c,}$ are group retention factors for a series of non-interacting substituents at positions a, b, c, etc It is the purpose of this communication to present the preliminary results of some work with compounds which have been selected with the specific object of testing equation (3)

The steroids were authentic samples which had been synthesized by standard methods Methyl ethers were prepared by reaction of the appropriate hydroxy-compounds with methyl iodide in the presence of potassium *t*-butoxide in diethyl ether Gas-liquid chromatography was carried out with 5 per cent polyethylene glycol succinate as the liquid phase under conditions exactly as previously described[1]

The retention times, relative to that of cholestane, of a series of steroid hydrocarbons, their 3β-methoxy-, 3β-methoxy-Δ^5-, and 5α-hydroxy-derivatives are presented in Table 1 In the last three columns of Table 1 are listed the retention factors for the 3β-methoxyl and 5α-hydroxyl groups and the Δ^5-bond, respectively These are the separation factors between the saturated parent hydrocarbons and their corresponding 3β-methoxy- and 5α-hydroxy-derivatives and between the saturated 3β-methoxy-compounds and their Δ^5-derivatives.

The retention factor for the 3β-methoxyl group in the saturated series is seen to be the same, within the limits of measurement, in all cases The factor associated with the introduction of the Δ^5 bond into the 3β-methoxy-compounds, which has already been shown to be constant for the C_{27}, C_{28} and C_{29} sterols[1], is seen from the present work to have the same value in the C_{19} and C_{21} series The retention factor of the 5α-hydroxyl group shows greater variation than can be accounted for by operational error but still remains constant over a range of 4 per cent as between the pregnane and stigmastane derivatives

Table 1 RELATIVE RETENTION TIMES OF SOME STEROID HYDROCARBONS AND DERIVATIVES

	Substituents			Retention factors		
Hydrocarbon	3β-methoxyl	5α-hydroxyl	3β-methoxy-Δ^5	3β-methoxyl	5α-hydroxyl	Δ^5
Androstane 0 12	0 42	—	0 461	3 55	—	1 09
Allopregnane 0 21	0 73	1 10	0 806	3 50	5 25	1 10
Cholestane 1 00	3 50	5 24	3 80	3 50	5 24	1 09
Ergostane 1 38	4 89	6 98	5 22	3 54	5 06	1 07
Stigmastane 1 72	6 11	8 70	6 60	3 55	5 06	1 08

Table 2 presents some results obtained with a series of bifunctional steroids, chosen to test the accuracy with which retention times may be predicted by means of equation (3) In the left-hand side of the table are listed a series of monofunctional compounds and their retention times relative to that of cholestane In the right-hand side a series of bifunctional derivatives are listed, together with their observed relative retention times and their calculated relative retention times derived from the data for the monofunctional steroids in the left-hand side of Table 2 and the appropriate values shown in Table 1 It is seen that in all except the last two cases (5α-hydroxy-Δ^2-cholestene and 5α-hydroxy-Δ^7-ergostene) there is close agreement (within 4 per cent) between the observed and calculated values

Table 2 OBSERVED AND CALCULATED RELATIVE RETENTION TIMES (r) OF SOME MONO- AND BI-FUNCTIONAL STEROIDS

Monofunctional steroids		Bifunctional steroids		
	r (obs)		r (obs)	r (calc)
17β-Methoxyandrostane	0 333	3β,17β-Dimethoxy-androstane	1 19	1 18
7α-Methoxycholestane	1 67	3β,7α-Dimethoxy-cholestane	5 65	5 83
7β-Methoxycholestane	2 25	3β,7β-Dimethoxy-cholestane	7 65	7 88
		3β,7α-Dimethoxy-allopregnane	1 19	1 22
		3β,7β-Dimethoxy-allopregnane	1 60	1 64
7α-Hydroxycholestane	6 83	3β-Methoxy-7α-hydroxy-allopregnane	5 20	5 00
Δ^2-Cholestene	1 20	5α-Hydroxy-Δ^2-cholestene	5 34	6 30
Δ^7-Ergostene	1 80	5α-Hydroxy-Δ^7-ergostene	7 26	9 06

The aberrant results obtained in the case of unsaturated 5α-hydroxy-compounds bear on the question of intramolecular group interactions The calculated values for the relative retention times of 5α-hydroxy-Δ^2-cholestene and 5α-hydroxy-Δ^7-ergostene exceed the observed values by 18 and 25 per cent respectively Measurements of Dreiding models indicate that the approximate distances of 3 1 and 2 9 Å between the 5α-oxygen atom and C_2 and C_3 respectively in the Δ^2-compounds are almost identical with the distances between the oxygen atom and C_8 and C_7 in the Δ^7-compound It is therefore reasonable that approximately the same degree of interaction should take place between the double bond and the homoallylic hydroxyl group in each case The precise nature of the interaction is not clear It may be in part a simple steric effect in which the hydroxyl group partially blocks the interaction between the double bond and the liquid phase but probably also involves a transannular π-bonded interaction between the double bond and the labile hydroxylic hydrogen. An evaluation of the relative importance of these two possible effects would require a study of analogues in which the 5α-hydroxyl group was replaced by an inert substituent of similar size

These results attest to the validity of equation (3) as a general expression of the relationship between structure and retention time in the steroid series. They are entirely consistent with the relationships between members of the same and closely related homologous series which have already been firmly established by the work of James and others[2]. It has been in the nature of the materials which have been most thoroughly investigated hitherto that emphasis has fallen more heavily on the logarithmic relationship between retention time and molecular weight within a homologous series than on these separation factors between different homologous series. In the present context the retention factors arising from the introduction of individual substituent groups into the molecule inevitably claim complete attention

As was pointed out previously[1], the precise influence of the liquid phase on the values of the group retention factors remains to be established. Lipsky and Landowne, in a valuable summary[3] of separations achieved with a wide range of steroids on a variety of the less polar polyester liquid phases, have reported separation factors which are approximately of the same relative order as those obtained in these Laboratories. Similar results have also been reported by Haahti *et al* [4]. It seems likely that the same general relationship expressed in equation (3) must hold with the less polar liquid phases but that the actual values of the group retention factors differ considerably according to the chemical nature of the liquid phase In view of the generally shorter retention times obtaining with the less polar polyester liquid phases used by the above authors it is evident that these are to be preferred for the separation of highly oxygenated compounds since the cumulative effects of highly polar substituents soon lead to excessive retention times For the analytical separation of simple sterols, in the form of their methyl ethers, however, there may well be some advantage in the use of the highly polar polyglycol succinate used in the present work

The extension and further refinement of this type of study seem likely to yield information which will supplement in a novel manner the existing body of knowledge concerning reactivity of substituent groups and their intramolecular interactions The practical value of the group retention factor method for the elucidation of hitherto unknown steroid structures is already established by its routine use in these Laboratories

A complete account of this and related work will be presented elsewhere. I wish to express my thanks of Prof Louis Fieser and Prof H B Henbest for generous gifts of steroid samples and to Prof Konrad Bloch for his interest

This investigation was carried out during tenure of an established investigatorship of the American Heart Association and was supported by grants from the American Heart Association and the National Institutes of Health

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SURVIVAL OF *RHIZOBIUM* ON CLOVER SEEDS

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THE high death-rate of *Rhizobium* inoculated on the surface of clover seeds is a serious problem where the survival of adequate numbers of rhizobia on the seed is essential for satisfactory nodulation, as in acid soils where the acidity further reduces the number of viable rhizobia[1] On lime-coated seed, which is used for establishing legumes on acid soils[2], the death-rate is also high[3] and in addition the seed is difficult to re-inoculate This article reports the survival of *Rhizobium* on seeds of subterranean clover for periods exceeding twelve months, in the presence or absence of a lime coat

A treatment designed to force rhizobia under the seed coat[4] was tested in experiments with the Mount Barker strain of subterranean clover (*Trifolium subterraneum* L) The method used was to immerse the seeds in a liquid culture of *Rhizobium*, and apply a vacuum of 700 mm of mercury for three successive periods The total period of immersion was 5 min

Rhizobium trifolii strain *NA*30 was cultured in a yeast mannitol medium[5] of *p*H 7 Immediately prior to inoculation, maltose or sucrose was added to the culture (nodulation was not affected by the form of sugar) to give a concentration of 10 per cent sugar Solutions containing 10 per cent sugar were used for all dilutions and for the uninoculated controls One set of replicates was prepared without a lime coat, and a corresponding set with a lime coat, using 50 mgm of methyl ethyl cellulose[6] plus 5 gm of precipitated calcium carbonate per 25 gm of clover seed The seeds were air-dried and stored in paper envelopes at room temperature, which seldom varied outside the range of 20°–27° C

Two lots of seed which had been prepared separately (Exps. 1 and 2, Table 1) were sown on an agar medium of *p*H 7 in test-tubes 64 weeks after treatment There were twelve tubes per treatment, with two seeds in each tube Table 1 shows clearly that the use of the immersion and vacuum treatment enabled rhizobia to survive on a proportion of the seeds for 64 weeks, and that lime-coating the seeds had no harmful effect on this survival

The results presented in Fig 1 show the beneficial effects of the immersion and vacuum treatment in

Table 1 NODULATION OF SUBTERRANEAN CLOVER SOWN ON AN AGAR MEDIUM 64 WEEKS AFTER TREATMENT

Inoculation treatment		Percentage of tubes with nodulated plants	
Method	No of rhizobia per ml of inoculum*	Without lime coat	With lime coat
	Experiment 1		
Uninoculated	0	0	0
Normal	$2\ 2 \times 10^{7}$	0	0
Immersion and vacuum	$2\ 2 \times 10^{6}$	8	17
Immersion and vacuum	$2\ 2 \times 10^{8}$	17	17
	Experiment 2		
Uninoculated	0	0	0
Normal	$2\ 4 \times 10^{8}$	0	0
Immersion and vacuum	$2\ 4 \times 10^{6}$	25	17
Immersion and vacuum	$2\ 4 \times 10^{9}$	42	42

* For the normal inoculation treatment, 1 ml of inoculum was added to 25 gm of seed (approximately 3,000 seeds) For the immersion and vacuum treatment 25 gm of seed absorbed about 3 ml of inoculum

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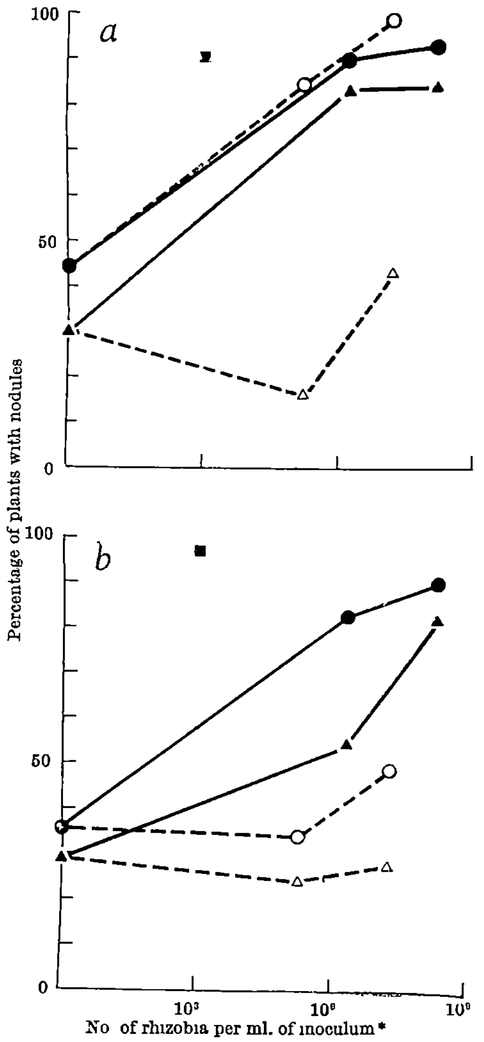

Fig. 1 Effects of treatments on the nodulation of subterranean clover sown on an acid soil in the field *a*, 1 day, *b*, 12 weeks after inoculation △, normal inoculation, ○, normal inoculation, with lime coat, ▲, immersion and vacuum inoculation, ●, immersion and vacuum inoculation, with lime coat, ■, freshly inoculated, drilled with lime * See corresponding footnote to Table 1

field sowings on a virgin soil of *p*H 5 4 The normal method of inoculation was ineffective on this soil when used without a lime coating, even when the seeds were sown one day after inoculation When the seeds were lime-coated the normal method of inoculation was effective for the first sowing, but was not effective after storage for twelve weeks When the seeds were immersed in *Rhizobium* inoculum and subjected to vacuum, the inoculation was effective at both sowings At the twelve-week sowing the highest percentage nodulation occurred where freshly inoculated seed was drilled with lime, but pre-inoculation by immersion and vacuum treatment combined with lime coating was nearly as good

At the first field sowing (Fig 1) there was a good response to inoculation by the immersion and vacuum treatment in the absence of a lime coat, suggesting that this method of inoculation had provided the *Rhizobium* with some protection against the harmful effects of the acid soil The response was also evident at the twelve-week sowing, although here a lime coat was needed for the best response to inoculation where the lower number of rhizobia was used

Further tests were made in an acid soil in a glass-house All the treatments shown in Fig 1 were included in a trial in pots sown 32 weeks after the treatment of the seed In this trial the only response to inoculation was obtained where the immersion and vacuum treatment was used in conjunction with a lime coat on the seed The lime-coated, vacuum-inoculated seed gave 75 per cent nodulated plants at each level of inoculation, compared with less than 20 per cent of nodulated plants for all other treatments

At a 52-week sowing in pots, where the immersion and vacuum treatment was tested only in the presence of a lime coat, the lower and higher levels of inoculation respectively gave 16 per cent and 51 per cent nodulated plants, compared with no nodules on the uninoculated controls

These results show that it is possible to inoculate clover seeds in a way that enables the rhizobia to survive for very long periods It is clearly important to define the factors concerned in the effect of the immersion and vacuum treatment used, and in the survival of the rhizobia

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CHOLINERGIC NEURONS IN VERTEBRATES

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CAVANAGH and Holland[1] have recently reported histochemical evidence showing that acetylcholinesterase (AChE) is more widely distributed in the nervous system of the chicken than in the nervous system of mammals They found that in the hen, in contrast to mammals, it was present in the cytoplasm of all central neurons examined and was in the dorsal root ganglion cells as well Because cholinergic cells characteristically contain AChE (ref 2), they suggest that all neurons in this species are cholinergic and that in this respect it differs fundamentally from mammals, in which non-cholinergic as well as cholinergic cells are found The additional possibility is adumbrated that an all-cholinergic nervous system is simpler than one to which non-cholinergic cells contribute and as such may be better suited to the requirements of a species less highly developed than mammals I would like to comment on these suggestions in the light of earlier published work[2] and of evidence which I have been collecting, as material becomes available, on acetylcholine (ACh) metabolism in representative vertebrate species This later work, which is referred to here, has been briefly summarized in an article now in the press[3]

First. it is relevant to consider how far biochemical data can be used as criteria in identifying cholinergic neurons. Known cholinergic cells of the periphery are found to contain both AChE and ChAc (choline acetylase, the enzyme that synthesizes ACh) By combined histochemical and biochemical analysis it can be shown that the AChE is present both in the perikaryal cytoplasm and in the axoplasm of cholinergic neurons and, in addition, is concentrated at their axon terminals, although at this site a proportion of enzyme is postsynaptic and apparently the product of the cholinoceptive cell which they innervate Where this characteristic disposition of AChE is observed it is highly probable that the cells in question are cholinergic However, the presence of enzyme in the cell body does not necessarily mean this since the cytoplasm of certain non-cholinergic neurons, including some sympathetic and spinal ganglion cells, also contain AChE in fairly high concentration Therefore, in order to find out if a cell is cholinergic, it is of greater importance to establish whether ChAc which has not been found in any proved non-cholinergic neurons is also present in it Unfortunately, there is no suitable micro- or histo-chemical technique by which to determine the cellular location of ChAc, so that in samples of the central nervous system it is usually not possible to identify the neurons from which it is derived and the information obtained from its measurement is quantitative only. In the central nervous system, expressed as the amount of ACh which can be synthesized per gm tissue per hr., its concentration may vary in some species of mammal from 0 to 5 μgm in tissues such as the optic nerve and parts of the cerebellar cortex to amounts equivalent to an activity of 3 mgm in the caudate nucleus; while in birds an even wider range of activity is found

These variations of ChAc have been interpreted to mean that the proportion of cholinergic to other neurons varies in different parts of the central nervous system, and that its concentration is larger or smaller depending on the numbers of such neurons To some extent it may also depend on which parts of them, the cell body, axon or axon terminals, are sampled since it appears that there is more enzyme at the axon terminals than elsewhere in the cell

In line with this interpretation all central structures containing ChAc have AChE associated with it, and in the case of some tissues containing a high concentration of ChAc it has been possible to show that the distribution of AChE in some of the constituent neurons conforms to what would be expected if they were cholinergic. In much of the brain and spinal cord it is not possible, however, to apply this test because of the difficulty of tracing neurons through to endings that are often remote from the cells of origin A new approach which helps to overcome this difficulty by determining the effect of chronic section on AChE in some central pathways has recently been described[4]; but at present, conclusions about the identity and course of cholinergic neurons in the central nervous system are largely a matter of inference

The ultimate test of whether a nerve is cholinergic is the demonstration that it releases ACh to transmit impulses to the cell or cells with which it synapses There is now evidence to show that ACh is released in the central nervous system, and in the case of the cerebral cortex it has been demonstrated that the rate at which this occurs is correlated with the level of cerebral activity as measured by the electroencephalograph and can be increased by electrical excitation applied locally, or at a distance to nerve pathways which have connexions with the cortex[5] In addition, some cholinoceptive neurons have recently been identified in certain cortical areas[6]. The biochemical evidence for the existence of central cholinergic neurons has therefore received substantial support from physiological experiment and there seems little reason to doubt that they form an important part of the central nervous system

There is, however, one other cause for uncertainty in interpreting the biochemical data This is the possibility, which has never been completely excluded, that some neuroglia may participate in ACh metabolism. As the presence of ChAc in some non-nervous tissues shows[7], ACh probably has some biological function other than that of neuro-transmitter and this could be true of the central nervous system as well as of other organs There is no question that all neuroglial cells can be concerned in ACh synthesis since there are many parts of the central nervous system which are nearly devoid of ChAc yet contain large amounts of glial tissue. Moreover, we find that certain fractions, obtained by low-speed centrifugation from homogenized brain tissue in which glial tissue is concentrated, have an extremely low specific activity of the enzyme So far as can be judged, therefore, this tissue does not participate to a significant extent in the production of ACh in the central nervous system Nevertheless, as will be appreciated, there are several kinds of neuroglia, and we cannot as yet be certain that the conclusion applies with equal force to all such tissue; and so, until more evidence is available on this point, some measure of uncertainty must remain in deciding whether the regional distribution of ChAc is related solely to the distribution of cholinergic neurons

Although there may be uncertainty in the positive identification of cholinergic neurons on biochemical data alone, there can be little or no doubt that if, as in the optic nerve and dorsal roots of most species, there is no measurable ACh or ChAc, such nerves cannot be cholinergic. It was on the basis of this kind of information that I concluded earlier that, at some central synapses, some transmitter other than ACh must be employed[2]; and, contrary to the suggestion made by Cavanagh and Holland, this conclusion applies to the hen as well as to mammals, because in this species the optic nerve has virtually no ChAc, synthesizing less than 0 1 per cent of the amount synthesized by pure cholinergic nerves. Moreover, in one experiment on a chicken in which I attempted the dissection of its spinal roots I found that the pooled dorsal roots synthesized only 4 per cent of the amount of ACh synthesized by the pooled ventral roots This small amount of activity could be accounted for by contamination of dorsal with ventral root tissue that had occurred in the course of the dissection, but even without this allowance the great difference in level of ChAc shows that a large proportion of the sensory fibres must be without enzyme and therefore non-cholinergic

Thus it seems reasonable to conclude that non-cholinergic as well as cholinergic neurons contribute to the nervous system of both birds and mammals.

Where there may be a difference between the two is in the relative numbers of cholinergic neurons, since the concentrations of both AChE and ChAc appear to be much higher in the chicken than in mammals In the cerebellum of the hen, for example, the ChAc activity is about eight times greater than the highest activity obtained from the cerebellum of any mammal Hence it is probable that relatively more cholinergic neurons contribute to the chicken cerebellum

It is of interest to consider this difference in relation to the comparative morphology of the cerebellum The virtual absence of ChAc from the outer layers of the cortex in many mammals indicates that few or none of its constituent neurons are cholinergic A similar conclusion can also be drawn from the AChE distribution, as Cavanagh and Holland stress[1] From our own evidence (Hebb, C O , and Mitchell, J F , unpublished work) based chiefly on ChAc measurements, it appears likely that the second-order sensory neuron from which the mossy fibres are derived are cholinergic and that these fibres supply most if not all of the enzyme in the white matter of the cerebellum. Thus, the concentration of ChAc would be expected to diminish as the cerebellum increases in size and complexity, largely owing to the addition of pathways which are not cholinergic connecting the cortex with other parts of the brain the relatively high concentration of ChAc in the hen cerebellum could then be attributed to its simpler structure and the relatively low numbers of non-cholinergic fibres in its white matter.

The alternative explanation, that the cortical neurons in the hen cerebellum are cholinergic, seems less likely It is suggested by the presence of AChE in some of the cortical neurons, the Purkinje cells in particular, but this argument loses force in view of the fact that in the pigeon cerebellum, which also has a high ChAc activity (about five times higher than in mammals), the cortical neurons appear to be devoid of AChE Another point to be considered is that the main cell groups of the cerebellar cortex seem to be homologous with one another in birds and mammals and in other vertebrates In view of this, it becomes less probable that they would alter in the specific chemical attributes that determine which transmitter substance they form, since from our knowledge of the peripheral and autonomic nervous systems the opposite conclusion may be drawn, namely, that anatomically homologous neurons are chemically homologous as well

Finally, I think it should be noted that the evidence provided by ChAc measurements does not support the idea that cholinergic neurons are in general of less importance in higher as compared with lower vertebrates Certainly the concentration of ChAc in the brain as a whole is greater in teleost fish and birds than in mammals, but in other vertebrates, the snake (*Natrix natrix*), the frog and the dogfish it is much lower. Within the mammalian group the enlargement of the cerebral cortex seems to have involved the addition of relatively more non-cholinergic than cholinergic neurons, but even in man it appears that cholinergic neurons form an important part of the cerebral cortex and in subcortical structures they have the same importance as in other members of the group

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BLOOD AND SERUM GROUPS IN REINDEER COMPARED WITH THOSE IN CATTLE

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DURING a search for possible markers for population genetic work on the reindeer species, blood samples were investigated from 64 reindeer at the State Reindeer Research Station, Ripats, Sweden. The samples were studied for the occurrence of cellular antigens, plasma protein and hæmoglobin types

Erythrocyte suspensions from 37 of the reindeer were tested against a battery of cattle blood-typing reagents, which detects 39 antigenic factors belonging to nine different genetic systems[1] Only one of the antisera (anti-*J*) gave specific hæmolytic reactions with any of the reindeer samples, while two reagents, *I′* and *Y′*, gave rise to some weak and probably unspecific cross-reactions The remaining samples were therefore only tested against anti-*J*

Of the 64 reindeer, 20 (31 per cent) were found to have cellular *J* Twenty-four calves, approximately 2 months old, were included in the investigation, and of these, 8 were found to have cellular *J* In the reindeer then, the *J* substance seems to be developed on the erythrocytes at least at two months of age

Anti-*J* occupies a peculiar position among the cattle blood-typing reagents It is obtained from normal sera and it reacts specifically with cells of types *A* and *AB* in man and with sheep *R* cells[2,3] The *J* substance, which behaves as a dominant genetic character, is primarily a soluble serum substance acquired by the erythrocytes after the birth of the animal[2,4] The concentration of the *J* substance in the serum and/or on the cells is, however, subject to considerable individual variation[4,5]

In order to study the presence of soluble *J* substance in reindeer serum, inhibition experiments were carried out according to standard techniques[4] Sera from nine individuals were examined Three animals had cellular *J*, while the remaining ones had no detectable *J* on their erythrocytes, neither had they anti-*J* in their sera One of our ordinary cattle *J* reagents was used as anti-serum, and the reindeer sera (in serial doubling dilutions) were used as inhibitors The inhibition tests were carried out in duplicates with *J*-positive reindeer and cattle erythrocytes as test cells The results (summarized in Table 1) show that the reindeer sera from animals having cellular *J* contain a soluble substance with the ability to inhibit the reaction between cattle

Table 1. ABILITY OF REINDEER SERA TO INHIBIT THE HÆMOLYTIC REACTION BETWEEN *J*-POSITIVE CELLS AND AN ANTI-*J* CATTLE SERUM DILUTED 1/16 (APPROX 4 UNITS OF HÆMOLYSIN)

Inhibitant	Highest dilution of the inhibitant causing inhibition	
	Cattle test cells	Reindeer test cell
Sera from 3 reindeer with cellular *J*	1/2–1/16	1/16–1/32
Sera from 6 reindeer lacking cellular *J*	No inhibition	No inhibition (3 cases) 1/4–1/8 (3 cases)

anti-*J* sera and cattle or reindeer cells of type *J* Three of the sera from individuals lacking *J* on their erythrocytes also inhibited the reaction between anti-*J* and reindeer *J* cells However, these three sera gave no definite inhibition when cattle cells were used as test erythrocytes The remaining three sera lacked inhibiting power entirely

Finally, a serum sample from each of the 64 reindeer was tested against erythrocytes from 30 reindeer and 10 cattle None of the 20 sera from animals with cellular *J* substance contained any antibodies However, 19 of the remaining sera contained antibodies against two or more of the red cell samples of type *J* One of these sera (No 5/55) was particularly strong In tests against all the reindeer samples and 90 cattle taken at random, the reactions of this serum exactly paralleled those of the cattle anti-*J* serum, which is in routine use in our laboratory The results from absorption experiments with the anti-*J* cattle serum and the reindeer serum No 5/55 are given in Table 2 As seen from the table, the *J*-positive cells from the reindeer species absorbed out the antibodies for the *J* cells in the cattle species and vice versa

Table 2 REACTIVITY OF ANTI-*J* SERA OF CATTLE AND REINDEER ORIGIN

Test cells	Cattle anti-*J* (No 451)			Reindeer anti-*J* (No 5/55)	
		Absorbed with			Absorbed with cattle *J* cells
	Un-absorbed	cattle *J* cells	reindeer *J* cells	Un-absorbed	
Reindeer					
J-pos.	+	–	–	+	–
J-neg	–	–	–	–	–
Cattle					
J-pos	+	–	–	+	–
J-neg	–	–	–	–	–

It can be concluded that the antigenic system in the reindeer species described here is remarkably similar to the *J* system of cattle and therefore the symbol *J* has been given to it Reindeer with cellular *J* also have soluble serum *J* However, as in cattle, soluble *J* substance seems to occur also in sera from some reindeer lacking cellular *J* Some reindeer sera contain normal antibodies with a specificity identical or very similar to cattle anti-*J* It is noteworthy that the serological similarity between the *J* systems in cattle and reindeer seems to be much greater than that between the cattle *J* and sheep *R* systems

No breeding data were studied, because the sires of the animals were unknown However, it seems justified to assume that the genetics of the *J* system in reindeer is similar to that in cattle[4,5]

The hæmoglobin and plasma proteins were investigated by starch-gel electrophoresis Using a continuous *tris*-buffer *p*H 8 9 (refs 6 and 7) only one kind of hæmoglobin was found among all the 64 reindeer It migrated a little faster than the cattle hæmoglobin *A*

The plasma proteins were analysed both with the continuous *tris*-buffer[6,7] and with a discontinuous buffer system[8] Six different types were observed regardless of the method used Fig 1 shows the six reindeer types compared with a bovine transferrin Tf^A/Tf^E type (ref 7). The variation seems to be in the β-globulins. By staining the starch gel with nitroso-R-salt reagent[9] the four varying zones, named *A-a*, *B-b*, *C-c* and *D-d*, were shown to bind iron and were therefore classified as transferrins Capital letters are used for the intensely stained zones and small letters for the less intensely stained zones. The six transferrin phenotypes can be designated ***aB***, ***bC***, ***cD*** ***aBC***, ***aBcD*** and ***bCD***

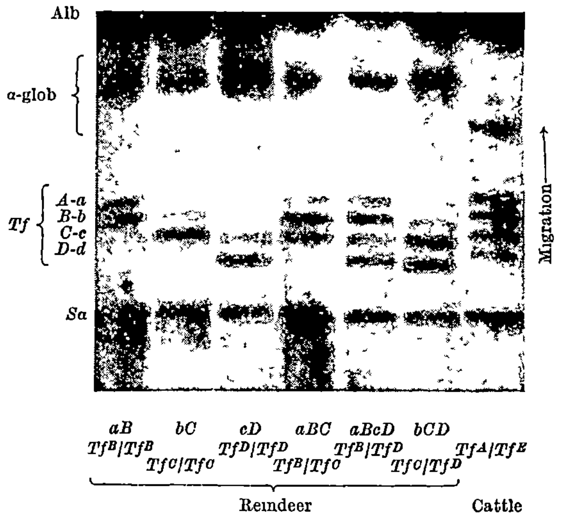

Fig 1 Photograph of the results of a starch-gel electrophoresis experiment with plasma from reindeer of the six transferrin types compared with the bovine Tf^A/Tf^E type Alb, albumin, α-glob, α-globulin, *Tf*, transferrin, *Sα*, slow α-globulin

A hypothesis is proposed that the transferrin types are determined by three alleles, called Tf^B, Tf^C and Tf^D Each allele gives rise to two transferrin fractions with a certain migration-rate in starch-gel electrophoresis The 64 reindeer are distributed in the following way with the phenotypes in parenthesis 6 Tf^B/Tf^B (*aB*), 18 Tf^C/Tf^C (*bC*), 4 Tf^D/Tf^D (*cD*), 15 Tf^B/Tf^C (*aBC*), 12 Tf^B/Tf^D (*aBcD*), and 9 Tf^C/Tf^D (*bCD*)

Among the reindeer tested there were 46 mother-offspring groups (several mothers had more than one offspring and sometimes daughters were mothers in other groups) The sires were unknown All offspring had a transferrin type compatible with that of their dam except in one case where a Tf^B/Tf^B daughter was assigned to a Tf^C/Tf^C mother This may be due to an error in the breeding records and therefore need not discount the three-allele hypothesis

A variation in the hæmoglobin-binding proteins was also observed but the results were difficult to interpret Some animals had a hæmoglobin-binding zone migrating slightly faster than the slow α-globulin while others had such a zone just behind the slow α-globulin However, in most animals no distinct hæmoglobin-binding zone appeared

We are much indebted to Dr M Nordkvist, of the State Veterinary Institute, Stockholm, who collected the samples of reindeer blood

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ADENOSINE DIPHOSPHATE IN RED CELLS AS A FACTOR IN THE ADHESIVENESS OF HUMAN BLOOD PLATELETS

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The initial reaction of hæmostasis[1] and arterial thrombosis[2–4] is adhesion and aggregation of blood platelets to the wound surface and to the site of intimal damage. The elucidation of the mechanism of this reaction is of great importance not only for diagnosis and management of hæmorrhagic disorders, but also for the problem of thrombosis. Several factors are involved: (1) a property of the platelets which enables them to become adhesive when exposed to the physiological adhesiveness-producing mechanism (thrombasthenia represents by definition the congenital lack of this property), (2) a tissue substrate for the platelets to adhere to, probably collagen and elastic fibres[5,6], (3) a plasma factor, which is lacking in von Willebrand's disease[7] and (4) calcium. A fifth factor, possibly triggering the adhesion–aggregation reaction, was reported by Hellem as being an acidic, dialysable and heat-stable factor which was found in red blood cells[8]. This factor produced strong agglutination of platelets in citrated or oxalated plasma and converted the non-adhesive circulating platelets to adhesive platelets showing immediate adherence to foreign surfaces.

This article describes the identification of this factor as adenosine diphosphate. The effect of a number of other nucleotides on the adhesiveness of human platelets has also been examined.

The semi-quantitative test for the adhesiveness of human blood platelets described by Hellem[8,9] was used throughout this work. 0.05 ml. of saline containing the material to be tested was added to 0.5 ml. of platelet-rich plasma, and the reaction considered positive if clumping of the platelets occurred.

An extract obtained by treating human red blood cells with boiling water[8] was passed through a 'Dowex 1 × 8' column in the formate form. The column was eluted stepwise with increasing concentration of formic acid and ammonium formate–formic acid mixtures. A typical elution diagram of ultra-violet absorbing material recorded at 260 mμ is seen in Fig. 1. The eluted fractions (I–VI) were concentrated to dryness in vacuum in the frozen state, and then dissolved in a volume of saline equal to that of the original extract. Only fractions V and VI were positive in the platelet test. Occasionally the elution diagram would contain a large inactive peak at the site of fraction IV.

Fractions V and VI both yielded adenine on hydrolysis with *N* hydrochloric acid for 1 hr. at 100° C. as shown by paper chromatography in isopropanol/concentrated hydrochloric acid/water (68 : 17.6 : 14.4).

In electrophoresis at *p*H 5.7 in 0.2 *M* ammonium acetate at 900 V. for 2 hr., fraction V gave two ultra-violet absorbing zones, one of which had the same mobility and ultra-violet absorption spectrum in 0.1 *N* hydrochloric acid and in 0.1 *N* sodium hydroxide as adenosine diphosphate. By chromatography of fraction VI in isobutyric acid/concentrated ammonia/water *p*H 3.7 (66.1 : 1.0 : 32.9), three spots which corresponded to adenosine monophosphate, adenosine diphosphate and adenosine triphosphate were found.

The presence of adenosine diphosphate in fractions V and VI was definitely proved by a specific enzymic test[10] for adenosine diphosphate using reduced diphosphopyridine nucleotide–phosphoenol pyruvate phosphokinase, and lactic acid dehydrogenase. (Reagents for testing adenosine diphosphate were purchased from C. F. Boehringer and Soehne G.m.b.H., Mannheim.) The total change in the ultra-violet absorption at 340 mμ was used in calculating the amount of adenosine diphosphate present. In fractions V and VI, assuming all ultra-violet absorption at 260 mμ in 0.1 *N* hydrochloric acid to be adenosine diphosphate, 86 per cent and 50 per cent respectively were present according to the enzymic test for adenosine diphosphate.

Thus adenosine diphosphate was present in both the active fractions V and VI and proved to be the substance responsible for effecting the adhesiveness of the platelets. It is active in the test solution in a concentration of 0.02 μgm./ml. A similar activity was encountered with commercial adenosine diphosphate.

The presence of adenosine diphosphate as the only active substance in fractions V and VI was suggested by the following experiment: the smallest amount of the two fractions which produced a positive reaction in the test system contained, when calculated from the amount of adenosine diphosphate present in the fraction, approximately 0.02 μgm./ml., in agreement with the amount expected.

A number of commercial nucleotides have been tested for activity. The results are shown in Table 1.

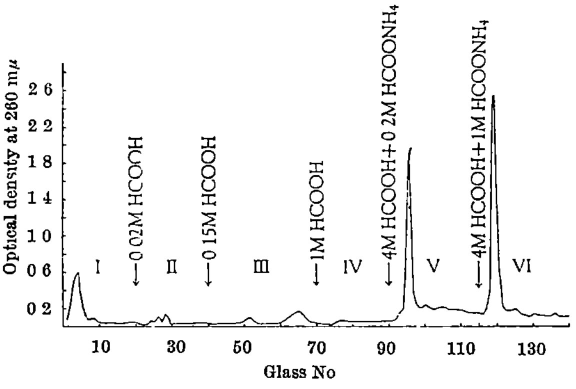

Fig. 1. Elution diagram from 'Dowex 1 × 8' (10 cm. × 1 cm.) in the formate form. 5-ml. fractions were collected. 27 mgm. of freeze-dried extract of red blood cells was dissolved in 5 ml. water and put on a column which was eluted stepwise as indicated in the diagram. The pooled fractions are indicated by I, II, III, IV, V and VI

Table 1 Effect of Unpurified Commercial Nucleotides on the Adhesiveness of Human Blood Platelets

The concentrations given for the active nucleotides represent the minimum concentration which will produce an effect

Nucleotide tested	Concentration of nucleotide in test solution (µgm /ml)	Clumping of blood platelets
Adenosine 5′-monophosphate sodium*	100	–
Adenosine 5′-diphosphate sodium†	0 02	+
Adenosine 5′-triphosphate disodium*	2	+
Deoxyadenosine 5′-monophosphate sodium‡	100	–
Deoxyadenosine 5′-diphosphate sodium*	7	+
Inosine 5′-phosphoric acid*	100	–
Inosine 5′-diphosphate sodium§	100	–
Inosine 5 -triphosphate sodium*	100	–
Guanosine 5′-monophosphate sodium*	100	–
Guanosine 5′-diphosphate sodium§	10	+
Guanosine 5′-triphosphate sodium*	100	–
Uridine 5′-monophosphate disodium $2H_2O$§	100	–
Uridine 5′-diphosphate trisodium $2H_2O$§	100	–
Uridine 5′-triphosphate sodium*	100	–
Cytidine 5′-monophosphate sodium $2H_2O$§	100	–
Cytidine 5′-diphosphate sodium $4H_2O$§	63	+
Deoxycytidine 5′-phosphoric acid‡	100	–
Deoxycytidine 5′-triphosphate sodium*	100	–
Thymidine 5′-phosphate calcium $4H_2O$‡	100	–
Diphosphopyridine nucleotide*	100	–
Triphosphopyridine nucleotide‡	100	–
Flavin adenine dinucleotide*	310	–

* Purchased from Sigma
† Purchased from Nutritional Biochemical Corporation
‡ Purchased from California Corporation for Biochemical Research
§ Purchased from Mann

It is seen that deoxyadenosine diphosphate, guanosine diphosphate and cytidine diphosphate exhibited activity To decide whether the activity was genuine or due to traces of adenosine diphosphate, the substances were purified by paper chromatography in the isobutyric acid–ammonia–water solvent system which will separate adenosine diphosphate from any of the three diphosphates After purification, deoxyadenosing diphosphate was the only active material It was active in a concentration of approximately 7 µgm /ml , whereas the other two were inactive at a concentration of 100 µgm /ml

Adenosine triphosphate was active in a concentration of 2 µgm /ml , but was found by the enzymic test to contain approximately 1 per cent of adenosine diphosphate, which would account for the activity The commercial adenosine triphosphate was purified by paper electrophoresis in 0 1 *M* phosphate buffer *p*H 5 7, a procedure which separates adenosine diphosphate and adenosine triphosphate The latter substance after purification was inactive at a concentration of 84 µgm /ml , whereas the material corresponding to the mobility of adenosine diphosphate exhibited activity It follows that the presence of adenosine triphosphate does not prevent the activity of adenosine diphosphate Furthermore, the platelet system provides an extremely sensitive test for trace amounts of adenosine diphosphate.

The results suggest that the effect on the adhesiveness of the platelets is very specific since out of twenty-two nucleotides tested, only adenosine diphosphate and deoxyadenosine diphosphate were active, and furthermore that the 6-amino group in the purine ring and the pyrophosphate group are required for activity.

As a naturally occurring intracellular substance with the highly potent and specific effect described, it is logical to assume that the release of adenosine diphosphate by cellular damage may play an important physiological part as a trigger mechanism for the initial stages of hæmostasis and thrombosis Investigations of the mechanism of its interaction with platelets are in progress

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DISSECTING ANEURISMS OF THE LARGE ARTERIES OF CHICK EMBRYOS DUE TO (SWEET PEA) LATHYRISM

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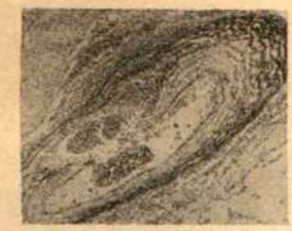
PONSETI and Baird in 1952[1] succeeded in producing dissecting aortic aneurisms in young rats fed on a diet containing 50 per cent *Lathyrus odoratus* Since that time, these vascular changes have been studied repeatedly by a number of workers β-aminopropionitrile has been found to have similar properties to the so-called *Lathyrus* factor By means of this compound, Lalich *et al* [2] were able to produce dissecting aneurisms in turkeys.

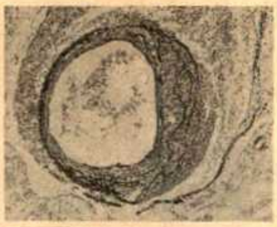
There is a high incidence of aortic aneurisms due to *Lathyrus* factor in developing rat fœtuses. We were able to demonstrate this fact clearly in some of our experiments[3] As a result of these experiments we attempted to produce vascular changes by means of fœtal chicken lathyrism.

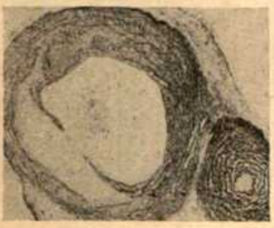
Lathyrus odoratus seeds were finely ground, and macerated in physiological saline solution (w/w, 1 2) at room temperature for 2 hr. The fluid obtained by pressing this thin, watery mash was rendered free from micro-organisms by using a bacteriological filter

Fertilized hen eggs (Leghorn white) were placed in special circular stands in a perpendicular position in an incubator, with the end of the egg containing the air space pointing upwards On the 7th day after placing the eggs in the incubator the top of each egg was opened so that a circular hole appeared having a diameter of 1–2 cm Each egg was covered by a glass vessel, allowing the access of air from beneath The embryos were examined twice a day

In our first experiment 50 eggs, treated as described, were divided into 5 equal groups Beginning on the 7th day, we applied different amounts of *Lathyrus odoratus* extract to each egg (using the hole at the top) belonging to groups 1–4 every day The eggs of group 5 were treated with saline solution only (0 4 ml. for one egg daily) The daily amounts of







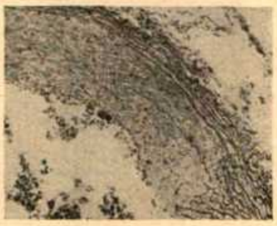


Fig. 1 (top, left). Ruptured dissecting aneurism of a branch of the pulmonary artery. (van Gieson's and Weigert's elastica stain, × *c.* 33)

Fig. 2 (top, right). Acute dissecting aneurism of ductus arteriosus. (Staining as in Fig. 1; × 50)

Fig. 3 (bottom, left). Acute, almost circular dissecting aneurism of the aorta. The ductus arteriosus is seen at the edge of the aorta. (Masson's trichrome and Weigert's elastica stain, × 25)

Fig. 4 (bottom, right). Scar in the aortic wall as a result of a healed dissecting aneurism. (Staining as in Fig. 3, × *c.* 83)

Lathyrus extract were as follows: Group 1, 0·05 ml./day; group 2, 0·1 ml./day; group 3, 0·2 ml./day; group 4, 0·4 ml./day. In the 3rd and 4th group, the fœtuses died very early, usually during the 10th or 11th day (that is, after 2–3 days of *Lathyrus* extract application). In group 2, 6 embryos died between the 11th and 13th day, the remainder of them dying between the 18th and 20th days. In the 1st experimental group, 7 embryos died between the 15th and 20th day, and the remaining 3 hatched out on the 21st day of incubation. In the control group, 2 chickens died on the 13th day, the remaining 8 hatched spontaneously on the 20th or 21st days.

Histological examination of the experimental animals that died during the course of the experiment very often disclosed extensive hæmorrhage, especially in the thoracic region. We were able to find acute massive dissecting aneurisms affecting one of the main branches of the pulmonary artery in 3 animals, belonging to the 1st and 2nd experimental groups (dying on the 19th and 20th days). In the control group, we failed to find any pathological changes.

In the second experiment 30 eggs, treated in the same way as above, were used. They were divided into 3 equal groups on the 12th day of their development. At this time, *Lathyrus* extract was injected in a single dose by a sterile needle into the albumen at the bottom of the eggs. The hole remaining after the puncture was closed by a paraffin plug. The amount of the *Lathyrus* extract injected was as follows: group 1, 0·5 ml.; group 2, 1 ml.: group 3, 2 ml.

In this experiment, the embryos died spontaneously even up to the end of their development, that is between the 18th and 21st day. Four chickens died in the first, 5 in the second, and 9 in the third experimental group. The remaining chickens hatched out on the 20th or 21st day. At birth, they required mechanical help, as they suffered from serious impairment of leg mobility. The surviving birds were killed on the following day by ether narcosis. The thoracic organs of all the chickens were serially sectioned *in toto* for microscopic examination.

In the animals of the first group minimal vascular lesions were found. In the second experimental group we were able to find extensive dissecting aneurisms in 4 chickens; both ducti arteriosi were affected in three chicks, the pulmonary artery once, and the aorta twice. In the third experimental group, dissecting aneurisms were disclosed in six chickens, the ductus arteriosi being affected most frequently.

Several workers have studied the effects of β-aminopropionitrile on connective tissue, using chicken embryos as the experimental animal[4-6]. Roy *et al.*[7] succeeded in producing aortic ruptures by means of 2-cyanopropylamine in young chickens after birth.

The method used in our second experiment seems especially suitable for the production of vascular lesions in chick embryos by *Lathyrus* factor.

We thank Dr. Z. Rychter and Dr. L. Lemez of the Institute of Normal Anatomy, Charles University, for technical advice.

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MODIFICATION OF THE EFFECTS OF AN AMPHETAMINE–BARBITURATE MIXTURE BY THE PAST EXPERIENCE OF RATS

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AMPHETAMINE – BARBITURATE mixtures have been used in psychiatry for many years, for example in the treatment of anxiety states, and sometimes in preference to either drug on its own. Why such mixtures might be especially effective is not well understood: barbiturates in the fairly small doses used in psychiatry are usually intended to act as mild depressants or 'sedatives', while amphetamine is a cerebral stimulant and its effects on most kinds of response tend to be the opposite of those of barbiturates. Clinically, the effects of the two kinds of drug when given in combination seem to be regarded as 'mutually corrective'[1], that is, as somehow reinforcing each other's beneficial effects while mitigating each other's undesirable side actions. But relatively little systematic work on the effects of such mixtures on the behaviour of animals or man seems to have been done in the laboratory.

The purpose of the experiments described here was to study the effects of such a mixture, and of the two constituents separately, on the exploration of a new environment by laboratory rats. There is an extensive experimental literature on 'exploratory behaviour'. Many factors can affect it. There is evidence for the following generalizations[2-4]: Novel environments evoke in animals exploratory activities like walking about, rearing and sniffing which can be attributed to curiosity. Novel environments may, however, also have the opposite effect on behaviour; they may induce 'freezing' and if, for example, a noxious stimulus is introduced into a novel situation exploration is inhibited and may stop completely. These effects can be attributed to fear or anxiety. Both curiosity and fear are apt to diminish as familiarity with an environment increases, though they may do this at different rates. The actual amount of exploration in a given situation can thus be regarded as the net result of competition between two opposing tendencies; for example, radical novelty has been shown to evoke less exploratory behaviour than mild novelty, presumably because with radical novelty fear predominates. Miller[5] has shown in various experiments that the effects of fear induced in rats by electric shocks can be mitigated by barbiturates.

On this kind of analysis one might expect that drugs which can alleviate fear or anxiety would increase exploration of a radically novel environment; and they might have less effect in a more familiar environment where fear has already been largely overcome. In order to investigate this, two groups of rats were used: 'inexperienced' rats which had never been in the apparatus before, and 'experienced' rats which had been familiarized with the apparatus and had developed a relatively stable and high rate of exploring.

The apparatus was a symmetrical Y-shaped runway, 13 in. high. Each arm was 15 in. long and 5 in. wide. A trial consisted of placing a rat in the centre of the Y and leaving it in the apparatus for 3 min.; the number of times it entered the arms was the measure of exploration. Rearing and defæcation were also noted. There were no rewards. The rats were 48 adult Lister hooded females which had been reared under standard conditions since weaning and had been little handled. Throughout they lived in their home cages with food and water *ad lib.* All experiments took place between 2.30 and 5.30 p.m.

The procedure was as follows: when the rats were 117–127 days old they were allocated at random to the two experimental groups: (1) 'Experienced' rats, which were given regular trials in the runway, twice weekly, for 16 weeks. (2) 'Inexperienced' rats, which received an equivalent amount of handling but were not placed in the runway. At the end of the 16 weeks each group was divided at random into four sub-groups of 6 rats; three of each of the four sub-groups received a drug: (*a*) amylobarbitone sodium 15 mgm./kgm., (*b*) amphetamine sulphate 0·75 mgm./kgm., (*c*) these two doses combined in a mixture; the fourth sub-group were saline controls. For rats these doses are relatively small, but the proportion of barbiturate is rather higher than in the mixtures used clinically. The drugs were dissolved in saline so that the volume injected, subcutaneously, was 2 c.c./kgm. 35 min. after the injection each rat was given a single 3-min. trial in the runway.

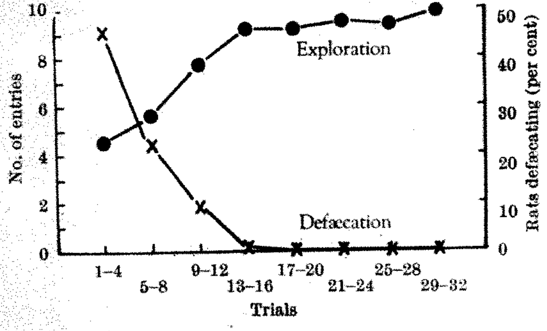

Fig. 1. Pre-drug trials of 'experienced' rats. The figure shows the mean number of entries into the arms of a Y-shaped runway made in 3 min. by 24 rats in 32 successive trials, and the percentage of rats which defæcated during these trials. The results have been grouped for four successive trials at a time, so that each point on the graph represents the mean results for four trials

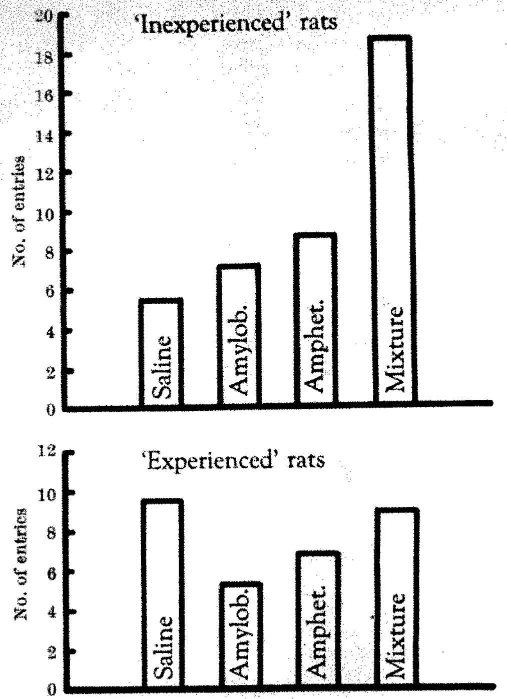

Fig. 2. Effects of drugs on exploration. The figure shows the mean number of entries into the arms of a Y-shaped runway made in 3 min. by four sub-groups of 'inexperienced' and of 'experienced' rats ($n = 6$). Three of each of the four sub-groups received a drug, respectively amylobarbitone sodium 15 mgm./kgm., amphetamine sulphate 0·75 mgm./kgm., and these two doses combined in a mixture; the fourth sub-group were saline controls

Pre-drug trials of 'experienced' rats. Fig. 1 summarizes the behaviour of the 'experienced' rats during the 32 pre-drug trials. At the first trial they explored relatively little (mean number of entries 5·79, *S.D.* 3·26), but in successive trials the amount of exploration gradually increased. From the 13th trial onwards the mean number of entries differed significantly from the first trial ($P < 0.02$) and soon remained fairly stable at about double the initial amount. Defæcation showed a corresponding decrease.

Effects of drugs. These are summarized in Fig. 2. The statistical significance of differences between sub-group means has been tested against the residual error, d.f. 40, discussed under (iii).

(i) 'Inexperienced' rats. The saline animals explored relatively little and spent much time sitting and 'freezing'. Their mean number of entries was 5·67, *S.D.* 2·25, which was similar to the mean number made by the experienced rats on their very first trial. Amylobarbitone and amphetamine barely increased exploration. The mixture, however, had a marked effect. the mean number of entries was 18·83, *S.D* 6·24, and this was more than three times the amount with saline ($t = 6\cdot11$, $P < 0\cdot001$). It was also noticed that the mixture induced unsteadiness of movement so that the rats had a rolling gait and sometimes toppled over, and some rats dragged themselves along on their bellies; in spite of this they continued to explore. Two of the amylobarbitone rats also showed some inco-ordination. It was further noticed that mixture rats were usually asleep in their home cages before being taken out for testing, and it was only in the new environment that they became active.

(ii) 'Experienced' rats. The experienced saline rats made a mean of 9·50 entries, *S.D.* 2·07, which was similar to their usual performance (cf., Fig. 1) and nearly twice the number made by the inexperienced saline rats. The drugs, however, had little effect on exploration. A few of the experienced rats showed motor inco-ordination with the mixture and with amylobarbitone, like rats in the corresponding inexperienced groups, but they explored no more than the experienced saline controls.

(iii) Interaction between 'experience' and 'drug' conditions. A two-way classification analysis of variance was carried out on the results for the four inexperienced and the four experienced sub-groups, and showed that the interaction between 'experience' and 'drug' conditions is highly significant ($F = 6\cdot64$, d.f. 3, 40, $P < 0\cdot001$). From Fig. 2 it is clear that the interaction arises in this way: among inexperienced rats the lowest mean was for saline controls while the highest mean was for the rats which had the mixture, the effect of 'experience' as compared with 'no experience' was to increase exploration under saline, but to inhibit the increase which the drug mixture induced in the inexperienced group.

These results suggest the following conclusions.

(1) Doses of amphetamine and amylobarbitone which separately barely affect exploratory behaviour can, when given in combination, have striking effects. These effects, moreover, seem to be in the same direction, that is the two drugs seem to act together to produce an increase in activity. Increased activity would not, of course, be surprising with amphetamine, but it is less usual with barbiturates. However, clinical reports have suggested that small doses of barbiturates, as of alcohol, can sometimes increase activity and excitement, and recently there have been some fresh and quantitative demonstrations of this kind of effect in laboratory animals[6-8].

(2) Whether an increase in exploratory activity occurs depends, however, not only on the drugs but also on the past experience of the animals: only rats which were unfamiliar with the new environment showed the increase. This is consistent with the assumption discussed at the outset (cf. ref. 9) that curiosity and fear may affect exploration in opposite ways and that the effect of the drug mixture should be to counteract fear. (i) Inexperienced saline rats, for whom the runway was a radically novel environment, explored less than experienced saline rats which had presumably already overcome their fear. That the experienced rats had indeed overcome their fear is also suggested by the gradual increase in exploration during the first 12 trials and by the corresponding decrease in defæcation. Defæcation has often been shown to be associated with fear and inversely related to activity (for example, ref. 10). Hence, by the time they were given drugs these rats were exploring at a rate consistent with a level of curiosity which had been stabilized by 32 previous trials, and so the drug mixture did not further increase it. (ii) But inexperienced rats given the mixture explored more than inexperienced saline rats, in other words, the drug mixture made them behave as though they were experienced and presumably unafraid. That these mixture animals explored even more than the experienced saline rats is not surprising since both curiosity and fear may be expected to be maximal on the very first occasion an animal meets a new environment, therefore, if fear is reduced, as by a drug, curiosity should more nearly show its maximal effect.

Even if further experiments do not confirm this interpretation in terms of curiosity and fear, the present results illustrate that (*a*) the effects of drugs may sometimes only become manifest if the experimental subjects have an appropriate past history, (*b*) 'inexperienced' animals can be more sensitive to drugs than highly trained animals. If the drug mixture had been tested only on experienced rats it would have been concluded that it had no effect on behaviour.

We wish to thank Profs. F. R. Winton, G. C. Drew and A. Summerfield and Dr. A. R. Jonckheer for discussing aspects of this work with us. The work was supported by research grant *MY*-3313 from the National Institute of Mental Health, U.S. Public Health Service.

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BIOCHEMICAL ASPECTS OF PHAGOCYTOSIS

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FOLLOWING a number of observations which indicated an increased rate of oxygen consumption by leucocytes during phagocytosis, Karnovsky and his colleagues[1-3] have shown that in the process of engulfment of particles by polymorphonuclear leucocytes, obtained from peritoneal exudates of guinea pigs, changes take place in the metabolism of the leucocytes. Increases in the rates of oxygen consumption and glucose utilization occur. The most significant change is the stimulation, during the phagocytic process, of the hexosemonophosphate shunt pathway of glucose oxidation. The ratio of glucose carbon 1 to glucose carbon 6 appearing in the respiratory carbon dioxide rises from a value of 8 in the resting (non-phagocytizing) leucocytes to a value of 22 during phagocytosis. Glycogen breakdown is increased[1,4] and there is some increase in lactate formation even though the rate of glycolysis in the leucocytes, both under aerobic and anaerobic conditions, is relatively high compared with that in many other mammalian cells. The energy for the phagocytic process seems to come from glycolysis[1]. Phagocytosis will take place under anaerobic conditions, or under aerobic conditions in the presence of a concentration of potassium cyanide that diminishes the respiration of leucocytes by 70 per cent (ref. 1). Acetate-1-^{14}C, uniformly labelled glucose-^{14}C, and phosphate-^{32}P are incorporated into the lipids of phagocytizing cells to a greater extent than into the lipids of resting cells[2,5], both under aerobic and anaerobic conditions. Incorporation of phosphate-^{32}P into phosphatidic acids is greatly increased as a result of phagocytosis[6].

The purpose of this communication is to report on certain other biochemical aspects of phagocytosis, particularly the stimulation of formate oxidation[7] and the possible bearing of this phenomenon on the mechanism by which oxygen consumption during phagocytosis is stimulated and its implications in the bactericidal processes accompanying phagocytosis.

The preparation of polymorphonuclear leucocytes was carried out by the method of Karnovsky *et al.*[1,8] with a few modifications. Guinea pigs weighing 400–500 gm. were injected intraperitoneally with 20 ml. 12 per cent sterile sodium caseinate solution. The largest numbers of leucocytes appear about 48 hr. after injection of the casein. In the following experiments, after 30 hr., the peritoneal exudates were collected in ice-cold calcium-free Krebs–Ringer medium containing 0·25 per cent sodium citrate, shaken and allowed to stand for 10 min. The cell suspension was slowly decanted from debris, centrifuged at 3,000 r.p.m. for 2 min., washed twice by resuspending in 8–12 volumes of ice-cold calcium-free Krebs–Ringer medium (*p*H 7·4). The packed cells were then diluted with the Ringer medium in the ratio 1 : 4. There was little or no contamination with red blood cells. For the preparation of a leucocyte homogenate, the following procedure was adopted. The leucocytes, after washing with calcium-free Krebs–Ringer medium, were diluted in the same medium in the ratio of 1 : 4. They were frozen, thawed and the thick suspension was homogenized for 3 min. in a Potter–Elvehjem glass homogenizer dipped in crushed ice, and the process of freezing, thawing and homogenization repeated. The homogenate was then centrifuged at 12,000 r.p.m. for 1 min. in the Sorvall centrifuge and the supernatant decanted off for use.

Much of the work described in this communication was carried out with leucocytes to which killed *E. coli* suspensions were added to induce phagocytosis. The *E. coli* cells were grown overnight on nutrient agar, collected, washed twice with 0·9 per cent sodium chloride solution, and then suspended in the same medium. They were killed by heating in a boiling water-bath for 30 min. and then centrifuged, washed with saline and suspended in calcium-free Krebs–Ringer phosphate medium.

The phagocytosis experiments were carried out in the conventional Warburg manometric apparatus at 37°. Krebs–Ringer medium (with 1·5 m*M* calcium chloride) was used as the suspending fluid. For respiration and glycolysis measurements, sodium phosphate buffer (0·02 *M*) and sodium bicarbonate (0·025 *M*) were used respectively. The initial *p*H was 7·4. The main compartment of the manometric vessel contained the leucocyte suspension (about 15 mgm. dry weight). 0·2 ml. (about 7 mgm. dry weight) heat-killed *E. coli* cells, radioactive substrates or other additions were usually tipped in from the side-arm. The centre well contained 0·2 ml. of 20 per cent potassium hydroxide with a roll of filter paper soaked in it; in experiments with potassium cyanide as inhibitor, the potassium hydroxide–cyanide mixture described by Umbreit *et al.*[9] was used. In aerobic experiments the gas phase was air and in anaerobic experiments purified nitrogen (or a mixture of 93 per cent nitrogen + 7 per cent carbon dioxide) was used. To free the nitrogen from traces of oxygen it was passed in succession through alkaline pyrogallol, Fieser's solution[10] and chromous chloride, and finally washed with lead acetate and barium hydroxide solutions. Such a rigorous purification was found necessary since the system oxidizing formate was found to be sensitive to the traces of oxygen still contained in commercial 'purified' nitrogen. After equilibration of the manometric vessels for 10 min. the contents of the side-arm were tipped into the main compartment of the vessel, which was incubated for 1 hr. at 37°.

Carbon-14 dioxide produced by the oxidation of ^{14}C-labelled substrates was absorbed in potassium hydroxide in the centre well of the Warburg vessel, trapped as bicarbonate and then liberated by addition of 0·3 ml. of 30 per cent trichloracetic acid which also served to terminate the reaction. The carbon dioxide was precipitated as barium carbonate in the presence of carrier, plated on aluminium planchets, and the radioactivity assayed using a thin-window gas-flow counter. The results were corrected for background and to infinite thinness of the barium carbonate layer. In experiments with ^{14}C-labelled formate, it was found that a small amount of radioactivity was always fixed by potassium hydroxide even in the absence of leucocytes; a correction had, therefore, to be applied in a routine manner for this.

The catalase we have used was a preparation from horse liver, kindly supplied by Dr. E. Margoliash of this Institute, and had an activity of 1,200 units/ml. (ref. 11).

Metabolism of Glucose-U-^{14}C by Guinea Pig Polymorphonuclear Leucocytes

Different particles were tested for their effects on the respiration of guinea pig polymorphonuclear leucocytes in the presence of glucose and on the rate of formation of carbon-14 dioxide when using uniformly labelled glucose. Typical results are shown in Table 1. Stimulated oxygen uptake has already been shown to take place with insoluble starch particles and polystyrene latex spherules about 1·17μ in diameter[1] and with a number of different bacteria; for example, *Mycobacterium tuberculosis*[12], *Bacillus subtilis*[12] and *Sarcina lutea*[13]. Results in Table 1 show that the highest stimulation of formation of carbon-14 dioxide among the particles tested was obtained with heat-killed *E. coli* cells. High rates of stimulation were also obtained with polystyrene latex spherules (obtained from the Dow Chemical

Company) and heat-killed *M tuberculosis* (B.C.G.) cells grown in a Dubos albumin medium for 7 days. It should be noted that carbon and sulphur particles stimulate leucocyte metabolism, but that freshly prepared barium sulphate and manganese dioxide particles have little or no effect under the given experimental conditions. Possibly the extra oxygen consumption found when sulphur particles are added to the leucocytes is due to some oxidation of the sulphur itself. Extracts of *E. coli* were also without effect. Addition of serum (final concentration 20 per cent) or peritoneal fluid (final concentration 10 per cent) to the suspending medium made no difference to the phagocytic stimulation due to killed *E. coli* cells.

Table 1. EFFECTS OF THE PRESENCE OF DIFFERENT PARTICLES ON THE OXYGEN UPTAKE AND CARBON-14 DIOXIDE FORMATION FROM GLUCOSE-U-^{14}C BY GUINEA PIG POLYMORPHONUCLEAR LEUCOCYTES

Each manometric vessel contained 4 m*M* glucose-U-^{14}C (10^5 c./min./vessel) and leucocytes (15 mgm. dry weight) *p*H 7.5, 3 ml., 1 hr.

Particles	Amount used (mgm.)	Oxygen uptake (µl./vessel)	Carbon-14 dioxide c./min./vessel
None	—	29	450
Carbon	20	58	1,670
Polystyrene (spherules)	15	124	3,430
Sulphur (powder)	60	565	1,220
$BaSO_4$ (pptd.)	95	24	660
MnO_2 (powder, fresh)	50	13	340
B.C.G. (heat-killed)	15	105	2,850
E. coli (heat-killed)	13	155	4,620

The largest accelerations of metabolism occur with fresh leucocytes. If these cells are incubated in a nitrogen atmosphere, in the absence of glucose, before the killed *E. coli* and labelled glucose are added, marked falls in the subsequent stimulated rates of oxygen consumption and carbon-14 dioxide production occur. The latter process is affected more than the former. The metabolic rates in the resting cells are apparently unaffected by this preliminary incubation procedure. The optimum *p*H for phagocytic stimulation was found to be between *p*H 7.5 and 8.5. It is noteworthy that the velocity of oxygen consumption by leucocytes, during phagocytosis of killed *E. coli*, remains nearly constant for about 40 min. at 37°, after which the velocity falls until, after 80 min., it has the same rate as that of the resting cells. Thus the stimulating process is of relatively short duration.

There is little doubt that the cationic concentrations in the media in which the leucocytes are suspended are of importance in the phagocytic stimulation of metabolism. Some typical results, given in Table 2, show that magnesium ions must play an important part in the stimulation, and that optimal effects are obtained only when magnesium, calcium and potassium ions are present with sodium chloride.

It has been pointed out[1] that phagocytic stimulation of metabolism by starch particles is proportional to the number of particles until a maximal value is reached. This is the case with killed *E. coli*. With 17 mgm. dry weight of leucocytes, the rates of oxygen consumption and carbon-14 dioxide are increased proportionately to the amount of killed *E. coli* added until approximately 6 mgm. dry weight bacteria have been added. Little further change occurs when larger quantities of bacteria are added. Similarly the increase in oxygen consumption and carbon-14 dioxide formation during phagocytosis is proportional to the numbers of leucocytes present, a maximum being reached with approximately 15 mgm. dry weight leucocytes (for the addition of 9 mgm. dry weight bacteria). With larger quantities of leucocytes (using this amount of bacteria to induce phagocytosis) there is no increase in the phagocytic stimulation of respiration and there is a fall in the phagocytic stimulation of carbon-14 dioxide formation from labelled glucose. This is possibly due to an increased demand on the glucose for glycolysis by the increased number of leucocytes. The results show that, within certain limits, phagocytic stimulation of metabolism is proportional both to the number of dead bacteria engulfed by the leucocytes and to the number of leucocytes used. It is therefore possible that the process of phagocytic stimulation may be employed for an assay method for phagocytosis.

Table 2. EFFECTS OF CATIONS ON METABOLISM OF LEUCOCYTES IN ABSENCE AND IN PRESENCE OF HEAT-KILLED *E. coli*

Glucose-U-^{14}C 4 m*M*, 120,000 c./min./vessel, leucocytes, 10 mgm. dry wt., *p*H 7.4, 1 hr.

Leucocytes washed and incubated in	Oxygen uptake (µl./vessel)		Carbon-14 dioxide (c./min. vessel)	
	Control	Phagocytosis	Control	Phagocytosis
Krebs–Ringer medium	13	101	386	5,290
Krebs–Ringer medium No magnesium ions	12	56	329	3,640
Krebs–Ringer medium No calcium ions	8	74	264	5,070
Krebs–Ringer medium No magnesium or calcium ions	12	63	244	2,560
Sodium chloride (0.9 per cent)	9	28	286	1,800

Aerobic and Anaerobic Energy of Leucocytes

Polymorphonuclear leucocytes exhibit both Pasteur and Crabtree effects[1]. The presence of glucose depresses the respiration of leucocytes (Crabtree effect), but has no diminishing effect on the stimulated respiration during phagocytosis. Thus, in a typical result, the Q_{O_2} of resting leucocytes in the absence of glucose was 3.6, and in the presence of glucose 2.1, whereas with phagocytizing leucocytes the Q_{O_2} in the absence of glucose was 15.4 and in the presence of glucose 16.0. The Crabtree effect is evidently eliminated (or masked) by the phagocytic change of metabolism.

Examination of the relationship between aerobic and anaerobic glycolysis and the Crabtree effect in resting leucocytes shows that the rate of formation of adenosine triphosphate aerobically in the absence of added glucose is identical with that in the presence of added glucose, assuming a P/O ratio of 3, in leucocytes. This is seen in a representative result given in Table 3. This relationship is already known to hold with tumours[14]. Moreover, the adenosine triphosphate made available by anaerobic glycolysis is of the same order of magnitude as that produced

Table 3. RELATION BETWEEN AEROBIC GLYCOLYSIS $Q_{CO_2(O_2)}$ ANAEROBIC GLYCOLYSIS $Q_{CO_2(N_2)}$ AND INHIBITION OF RESPIRATION (CRABTREE EFFECT) OF RESTING POLYMORPHONUCLEAR LEUCOCYTES

	No glucose added	Glucose (4 m*M*)
Q_{O_2}	4.1	2.7
$Q_{ATP} = 6Q_{O_2}$	24.6	16.2
$Q_{CO_2(O_2)}$	4.2	12.0
$Q_{ATP(O_2)} = 6Q_{O_2} + Q_{CO_2(O_2)}$	28.8	28.2
$Q_{CO_2(N_2)}$	5.5	20.5
$Q_{ATP(N_2)} = Q_{CO_2(N_2)}$	5.5	20.5

Table 4 EFFECTS OF PHAGOCYTOSIS ON OXIDATION OF DIFFERENT CARBON-14-LABELLED SUBSTRATES
Substrates 100,000 c /min /vessel, leucocytes 10 mgm dry wt , 1 hr 37°
Phagocytosis of killed *E coli*

Labelled substrate used	Glucose (4 m*M*)	Oxygen uptake (µl /mgm dry wt leucocyte)		Carbon-14 dioxide (c /min /mgm dry wt leucocyte)	
		Control	Phagocytosis	Control	Phagocytosis
Glucose-U-^{14}C	+	2 5	11 2	40	470
Glucose-1-^{14}C	+	2 5	11 4	103	1,360
Glucose-6-^{14}C	+	2 6	11 4	8	22
DL-Lactate-1-^{14}C, 2 m*M*	−	4 4	11 3	252	253
DL-Lactate-1-^{14}C, 2 m*M*	+	2 1	13 1	154	180
Pyruvate-1-^{14}C, 2 m*M*	−	4 0	9 0	97	101
Fructose-U-^{14}C, 4 m*M*	−	2 9	8 5	22	64
Galactose-1-^{14}C, 4 m*M*	−	3 0	8 1	6	22
Glycine-2-^{14}C, 2 m*M*	−	3 6	11 2	1	5
Glycine-2-^{14}C, 2 m*M*	+	2 3	12 9	2	7
L-Leucine-1-^{14}C, 2 m*M*	−	2 8	12 2	60	28
L-Leucine-1-^{14}C, 2 m*M*	+	1 9	15 5	45	35
L-Glutamine-U-^{14}C, 4 m*M*	−	3 4	10 9	228	232
L-Glutamine-U-^{14}C, 4 m*M*	+	2 2	11 5	206	217
DL-Glutamate-1-^{14}C, 4 m*M*	−	3 9	12 2	88	85
DL-Glutamate-1-^{14}C, 4 m*M*	+	2 8	15 3	75	60
Ethanol-1-^{14}C, 1 m*M*	−	2 4	9 7	198	230
Ethanol-1-^{14}C, 1 m*M*	+	1 8	10 2	159	202
Formate-^{14}C, 2 m*M*	−	3 1	12 9	63	280
Formate-^{14}C, 2 m*M*	+	1 5	13 5	69	315

aerobically (in the presence or absence of glucose) If adenosine triphosphate, made available aerobically or anaerobically, is an essential factor for the operation of the phagocytic process, it is evident that the process should take place as well anaerobically as aerobically, other conditions being the same There is considerable evidence[1] to support this conclusion

Evidently, since respiration is suppressed by cyanide or antimycin A (ref 1) without interference with phagocytosis, the adenosine triphosphate produced by respiration may be localized so that it is not available for the phagocytic process

Substrates affected by Phagocytosis

Representative results recorded in Table 4 show the effects of phagocytosis on the oxygen consumption and carbon-14 dioxide formation from a number of labelled substrates in the presence of guinea pig polymorphonuclear leucocytes and killed *E coli* In confirmation of earlier findings[1], it will be noted that the rate of formation of carbon-14 dioxide from glucose-1-^{14}C greatly exceeds that from glucose-6-^{14}C, justifying the conclusion that the hexosemonophosphate shunt is greatly stimulated by the phagocytic process Only quantitatively minor effects are seen with other substrates, with the exception of labelled formate, the rate of oxidation of which is greatly increased during phagocytosis

Aerobic and Anaerobic Oxidations by Resting and Phagocytizing Polymorphonuclear Leucocytes

During aerobic phagocytosis, the rate of oxidation of formate, as estimated by the increase of rate of formation of carbon-14 dioxide from formate-^{14}C is increased 3–6 fold Typical results recording the quantities of substrate (glucose or formate) oxidized by 1 mgm dry weight leucocytes per hr are shown in Table 5 Although the oxidation of formate is markedly stimulated, there is little or no increase (within the experimental error) in the rate of oxygen consumption during its oxidation The stimulation of oxidation of formate by phagocytosis is considerably less than that of oxidation of glucose

Under strictly anaerobic conditions, the rates of oxidations of glucose and formate are very greatly reduced and the phagocytic process, anaerobically, brings about only relatively small increases of these rates (Table 5) However, the addition of 0 3 per cent potassium ferricyanide, well known as an oxidant of reduced phosphopyridinenucleotide[15], to the leucocytes under strictly anaerobic conditions brings about an increased rate of formation of carbon-14 dioxide from glucose-U-^{14}C with the resting cells and a large stimulation of this rate with the phagocytizing cells The ratio of formation of carbon-14 dioxide from glucose-U-^{14}C by phagocytizing cells to that of resting cells is 9 2 in the presence of oxygen and 5 4 in presence of ferricyanide anaerobically The rate of carbon-14 dioxide formation, however, aerobically from glucose-U-^{14}C is at least twice that obtained anaerobically in presence of ferricyanide, with both resting cells and phagocytizing cells (Table 5) It is evident that, whereas ferricyanide can replace oxygen as an oxidant in the phagocytic processes, it is, under the given experimental conditions, much less effective What is also evident from the results given in Table 5 is that there is no stimulation, within experimental error, of formate oxidation by ferricyanide under anaerobic conditions

Experiments carried out with sodium pyruvate show that the addition of this substance to the leucocytes under strictly anaerobic conditions increases

Table 5 OXIDATION OF GLUCOSE AND OF SODIUM FORMATE BY GUINEA PIG POLYMORPHONUCLEAR LEUCOCYTES RESTING AND PHAGOCYTIZING, UNDER AEROBIC AND ANAEROBIC CONDITIONS

Gaseous phase	Substrates	Micromoles of substrate oxidized per mgm dry weight leucocytes per hour × 10^4	
		Resting	Phagocytizing
Air	Glucose-U-^{14}C	38	351
	Formate-^{14}C	24	96
Nitrogen	Glucose-U-^{14}C	5	12
	Glucose-U-^{14}C + potassium ferricyanide (0 3 per cent)	15	81
	Glucose-U-^{14}C + sodium pyruvate (5 m*M*)	32	37
	+ sodium pyruvate (10 m*M*)	27	35
	Formate-^{14}C	1	2
	Formate-^{14}C + potassium ferricyanide (0 3 per cent)	0 5	1 2
	Formate-^{14}C + sodium pyruvate (5 m*M*)	0 5	1 3

Each manometric vessel contained 15 mgm dry weight leucocytes, 0 6 ml Ringer medium (5 times normal strength), 0 5 ml 0 1 *M* phosphate buffer solution *p*H 7 4, 15 micromoles glucose Final vol = 3 ml Incubation = 1 hr at 37° Glucose-U-^{14}C, 10^6 c /min / vessel, 2 m*M* sodium formate, 1 5 × 10^5 c /min /vessel Phagocytosis induced by addition of heat-killed *E coli* suspension (7 mgm dry weight) the rate of formation of carbon-14 dioxide from glucose-U-^{14}C obtained with the resting cells almost to the aerobic level (Table 5). This fact lends support to the conclusion that pyruvate may be the normal oxidant for hexosemonophosphate oxidation via the shunt system[3,17]. On the other hand, the addition of pyruvate is less effective than ferricyanide in increasing the rate of formation of carbon-14 dioxide anaerobically with phagocytizing cells. Moreover, the amount of glucose-U-^{14}C converted to carbon-14 dioxide during phagocytosis aerobically is at least nine times that found on the addition of pyruvate anaerobically. It is therefore evident that whereas pyruvate can replace oxygen for glucose oxidation in resting leucocytes, it can only do so partially in phagocytizing cells. Mention should be made of the fact[21] that pyruvate can accelerate the aerobic rate of formation of carbon-14 dioxide from labelled glucose (or lactate) with ascites tumour cells, the effect being ascribed to exogenous pyruvate acting as an additional electron acceptor.

Effects of Metabolic Inhibitors and Stimulators on Glucose and Formate Metabolism during Phagocytosis

A large variety of substances has been tested for their effects on the stimulation of the rates of oxygen consumption and carbon-14 dioxide formation from glucose-U-^{14}C in presence of guinea pig polymorphonuclear leucocytes.

Antibiotics such as penicillin, streptomycin, chloromycetin, polymixin, bacitracin, gramicidin, sarcomycin were without effect at concentrations that were effective in suppressing bacterial growth.

Sodium amytal (5 m*M*), sodium malonate (10 m*M*) and sodium fluoracetate (10 m*M*) were without effect on the phagocytic stimulation of metabolism. On the other hand, those substances capable of suppressing glycolysis, such as phenylarsenoxide thioglycollate (30 μgm./ml.), glyceraldehyde (8 m*M*), phloretin (0.2 m*M*), as well as iodoacetate[1] and fluoride[1] bring about large inhibitions of stimulated phagocytic respiration and of carbon-14 dioxide formation from glucose-U-^{14}C. Thus the phenylarsenoxide thioglycollate brought about 87 per cent inhibition of carbon-14 dioxide formation from glucose-U-^{14}C, glyceraldehyde 72 per cent and phloretin 47 per cent. Phloretin also exercised a large inhibitory effect on the respiration of the resting cells. Again, sodium tartronate, known as a glycolytic inhibitor through its competitive suppression of lactic dehydrogenase[18], exercises a considerable inhibition of both glycolysis and phagocytic stimulation of leucocytes. At a concentration of 50 m*M* it inhibited glycolysis by 40 per cent and carbon-14 dioxide formation from glucose-U-^{14}C by 34 per cent in resting cells, and 68 per cent in phagocytizing cells. Sodium oxalate, another inhibitor of lactic dehydrogenase[19], behaved similarly to tartronate.

These results support the conclusion that, although the energy required for phagocytosis is obtained by glycolysis, the phagocytic stimulation of metabolism consists largely of an acceleration of the hexomonophosphate shunt pathway of oxidation and to a minor extent of an acceleration of lactic acid formation[1]. As the former process is mediated by triphosphopyridine nucleotide, it was desirable to test the effects, on the process, of substances known to stimulate oxidation of triphosphopyridine nucleotide, for example, menadione and pyruvate[16,17]. It was found that a variety of quinones, including menadione, increase markedly the rates of oxygen consumption and of carbon-14 dioxide formation from glucose-U-^{14}C of resting leucocytes with but little effect on the phagocytic stimulated rates of oxygen consumption and of carbon-14 dioxide formation. Representative results given in Table 6 show that naphthoquinone (0.3 m*M*) and menadione (0.3 m*M*) increase the rates of oxygen consumption and carbon-14 dioxide formation from glucose-U-^{14}C with resting cells several-fold, menadione being the more effective substance, while the phagocytic stimulation is only increased about 20 per cent. Vitamin K_1 and α-tocopherol quinone have little effect on the rates of oxygen consumption but increase the rates of carbon-14 dioxide formation from glucose-U-^{14}C with resting cells more than 100 per cent, and with phagocytizing cells 43 and 24 per cent respectively at the concentrations tested. It is therefore evident that quinones can stimulate the formation of carbon-14 dioxide from resting cells, sometimes to the phagocytic level, but they have much less effect on the formation of carbon-14 dioxide from phagocytizing cells. The accelerating effect of menadione on formation of carbon-14 dioxide is seen with glucose-1-^{14}C and not with glucose-6-^{14}C (Table 6), as would be

Table 6. PERCENTAGE EFFECTS OF QUINONES, ETC., ON METABOLISM OF POLYMORPHONUCLEAR LEUCOCYTES

Percentage changes on metabolic rates (1 hr. incubation at 37°) only are given, phagocytosis being induced by addition of heat-killed *E. coli*

Substrate	Additions	Change in Q_{O_2}		Change in carbon-14 dioxide	
		Resting cells	Phagocytizing	Resting cells	Phagocytizing
4 m*M* Glucose-U-^{14}C	Naphthoquinone, 0.3 m*M*	+ 276	+ 22	+ 142	+ 24
	Menadione bisulphite, 0.3 m*M*	+ 520	+ 21	+ 565	+ 22
	Vitamin K_1, 0.5 per cent	+ 2	+ 18	+ 222	+ 43
	α-Tocopherol quinone, 0.5 per cent	− 35	− 4	+ 133	+ 24
	Phenazine methosulphate, 0.3 m*M*	+1240	+ 154	+1910	+ 236
	Methylene blue, 0.3 m*M*	+ 364	+ 15	+ 420	0
4 m*M* Glucose-6-^{14}C	Menadione bisulphite, 0.15 m*M*	+ 264	+ 30	− 3	− 18
4 m*M* Glucose-1-^{14}C	Menadione bisulphite, 0.15 m*M*	+ 330	+ 38	+1300	+ 44
,,	Phenazine methosulphate, 0.3 m*M*	+ 930	+ 46	+2500	+ 104
,,	Methylene blue, 0.3 m*M*	+ 250	+ 12	+1210	+ 12
,,	Sodium pyruvate, 8 m*M*	0	0	+ 74	0
,,	Sodium iodoacetate, 0.1 m*M*	+ 20	− 35	− 49	− 57
,,	Sodium iodoacetate, 0.1 m*M* +Sodium pyruvate, 8 m*M*	+ 14	− 24	+ 47	− 42
,,	Sodium iodoacetate, 0.2 m*M*	+ 5	− 48	− 64	− 63
,,	Sodium iodoacetate, 0.2 m*M* +Sodium pyruvate, 8 m*M*	0	− 33	+ 13	− 57

See Table 4 for control values

expected if the acceleration due to menadione were confined to the hexosemonophosphate shunt

Phenazine and methylene blue are also accelerators of the rates of oxygen consumption and carbon-14 dioxide formation in the presence of labelled glucose, their effects on the metabolism of resting cells being greater than on that of phagocytizing cells (Table 6) Phenazine is much more active than methylene blue in this respect It is noteworthy that phenazine permits the direct oxidation of reduced diphosphopyridine nucleotide[20]

Sodium pyruvate has little or no effect on the rates of respiration of resting and phagocytizing cells, it increases (at 8 mM) the formation of carbon-14 dioxide from glucose-1-^{14}C by 74 per cent with resting cells, but has no effect on that obtained with phagocytizing cells Its effects are very clearly seen on leucocytes treated with iodoacetate, for it nearly abolishes the inhibition of the rate of formation of carbon-14 dioxide by iodoacetate in resting cells (Table 6) On the other hand, it has a relatively small effect in diminishing the inhibition by iodoacetate of formation of carbon-14 dioxide with phagocytizing cells.

While these results indicate that pyruvate will increase the rate of formation of carbon-14 dioxide from labelled glucose with resting cells by at least 74 per cent, both with normal cells and with cells treated with iodoacetate, and that it may, therefore, act as an oxidizer of reduced triphosphopyridine nucleotide, it is clearly very ineffective as compared with a quinone such as menadione or a carrier such as phenazine Moreover, the fact that it cannot increase the rate of oxygen consumption, or of carbon-14 dioxide formation, of resting cells to the phagocytic levels indicates that phagocytic stimulation cannot be wholly due to an increased rate of liberation of pyruvate in the leucocyte during the process of phagocytosis It should be noted that menadione (Table 7) increases the rate of formation of carbon-14 dioxide from formate-^{14}C with resting leucocytes almost to the phagocytic level

Table 7 EFFECTS OF INHIBITORS AND STIMULATORS ON AEROBIC GLUCOSE AND FORMATE OXIDATION DURING PHAGOCYTOSIS

Conditions as in Table 5

Additions	10^4 × μmoles substrate oxidized/hr / mgm leucocytes			
	Glucose-U-^{14}C		Formate-^{14}C	
	Resting	Phagocytizing	Resting	Phagocytizing
None	41	484	18	56
Flavin mononucleotide (2 mM)	52	472	37	68
Menadione (0 2 mM)	240	627	46	72
Sodium azide (1mM)	37	442	1	2
Potassium cyanide (1 mM)	36	451	6	10
Sodium iodoacetate (0 1 mM)	26	259	10	25

Evidence for Hydrogen Peroxide Formation during Phagocytosis

Experiments, typical results of which are shown in Table 7, show that the formate-oxidizing mechanism is greatly inhibited by concentrations of sodium azide and potassium cyanide that have but little effect on the oxidation of glucose, either with resting or phagocytizing cells Iodoacetate has an inhibitory effect on formate oxidation approximately equal to that on glucose oxidation The facts indicate that formate oxidation in the leucocyte is dependent on that of glucose, that it requires molecular oxygen as an oxidant and that it is blocked by inhibitors that affect enzymes containing hæm As it is already known that leucocytes are rich in peroxidase[22], and as the optimum pH for phagocytic stimulation of formate oxidation is 6 0, which is the optimum for peroxidase, it is reasonable to conclude that oxidation of formate is accomplished by hydrogen peroxide formed during the oxidation of glucose by the hexosemonophosphate shunt It is already known[23] that in the presence of peroxidase or catalase, hydrogen peroxide oxidizes formate and that azide and cyanide will inhibit the activity of verdoperoxidase

Evidence that hydrogen peroxide is actually formed during phagocytosis comes from experiments the results of which are shown in Table 8 In the first place it is evident that the addition of catalase more than doubles the rate of oxidation of formate in the presence of resting or phagocytizing leucocytes, whereas the addition of heated catalase has no effect Such an accelerating effect must be attributed to the action of catalase on hydrogen peroxide leaking from the leucocyte, as we have found no evidence that the catalase content of the leucocyte is increased on exposure to catalase Secondly, the addition of sodium nitrite (2 mM) which will act as competitor to formate[23] brings about an inhibition of oxidation of formate by resting or phagocytizing leucocytes. Moreover, the system oxidizing formate is to be found in the leucocyte homogenate, which is capable of extensive oxidation of formate in the presence of a system producing hydrogen peroxide such as is afforded by a mixture of glucose oxidase (notatin) and glucose (Table 8)

Table 8 FACTORS AFFECTING AEROBIC OXIDATION OF FORMATE BY LEUCOCYTES AND LEUCOCYTE HOMOGENATES

Formate-^{14}C (2 mM) present throughout Glucose = 5 mM Notatin = 0 01 per cent

	10^4 × μmoles formate oxidized/hr /mgm leucocytes
Resting leucocytes	
Leucocytes only (pH = 6 0)	36
Notatin + glucose	0
Leucocytes + notatin + glucose	715
Leucocytes + notatin + glucose + sodium azide (1 mM)	1
Leucocytes only (pH = 7 4)	18
+ sodium azide (1 mM)	0 8
+ potassium cyanide (1 mM)	6
+ catalase (1/750)	40
+ heated catalase (1/750)	18
+ sodium nitrite (2 mM)	3
Phagocytizing leucocytes	
Leucocytes only (pH = 7 4)	86
+ catalase (1/750)	198
+ heated catalase (1/750)	87
+ sodium azide (1 mM)	2
+ sodium nitrite (2 mM)	12
+ potassium cyanide (1 mM)	9
Leucocyte homogenate	
Homogenate only (pH = 6 0)	65
+ glucose + notatin	529
+ glucose + notatin + catalase (1/15,000)	708
Glucose + notatin + catalase (1/15,000)	105
Homogenate + glucose + notatin + sodium azide (1 mM)	5
Homogenate + sodium azide (1 mM)	3
Homogenate + sodium nitrite (2 mM)	6

The evidence indicates that the systems oxidizing glucose (by the hexose monophosphate shunt pathway) and formate are present in the non-particulate cytoplasmic fraction of the leucocytes, a fact in agreement with observations concerning the localization of glucose-6-phosphate and 6-phosphogluconic acid dehydrogenases in many other tissues[25]

Oxidation of Sodium Sulphite by Resting and Phagocytizing Leucocytes

A substance that greatly increases the rate of oxygen uptake of leucocytes undergoing phagocytosis is sodium sulphite In a typical experiment this substance (at 30 mM) increased the oxygen consumption per hour of 12 mgm (dry weight) resting leucocytes from 30 µl to 60 µl, and of phagocytizing leucocytes from 120 µl to 700 µl. At the same time it diminished carbon-14 dioxide formation from glucose-U-^{14}C, with phagocytizing cells, by 90 per cent. Oxidation of formate-^{14}C to carbon-14 dioxide was also suppressed by sodium sulphite, an inhibition of 47 per cent occurring with 10 m*M* sodium sulphite. The reasons for these effects are now being investigated.

Participation of Flavin in the Formation of Hydrogen Peroxide in Leucocyte Homogenates

Results, which will be described in detail elsewhere (Iyer, G. Y. N., and Quastel, J. H., to be published), show that homogenates of guinea pig polymorphonuclear leucocytes are capable of oxidizing in the presence of air the reduced forms of di- and triphosphopyridine nucleotides to hydrogen peroxide, as indicated by oxidation of formate-^{14}C to carbon-14 dioxide. Reduced triphosphopyridine nucleotide is more effective than the reduced diphosphopyridine nucleotide in this respect. It seems likely that this mechanism underlies the formation of hydrogen peroxide during phagocytosis. The addition of flavin mononucleotide (0.01 m*M*) accelerates the rates of oxygen consumption of the leucocyte homogenate in presence of reduced di- or tri-phosphopyridine nucleotide. Moreover (Table 7), the addition of flavin mononucleotide increases the rate of formation of carbon-14 dioxide from formate-^{14}C in the presence of the resting leucocytes. These facts support the conclusion that a system catalysed by flavin is involved in the oxidation of the reduced di- and triphosphopyridine nucleotides by leucocytes to form hydrogen peroxide.

The liberation of a reduced di- or tri-phosphopyridine nucleotide oxidase, capable of producing with these dinucleotides, hydrogen peroxide, during the process of phagocytosis, or that of a quinone linking reduced di- or tri-phosphopyridine nucleotide with molecular oxygen, would account for the observed phenomena. Possibly the release of enzymes from the granules of polymorphonuclear leucocytes, shown by Cohn and Hirsch[26] to occur during phagocytosis, is involved in these phenomena[3,6].

Formation of Hydrogen Peroxide in Leucocytes during Phagocytosis

Calculations indicate that if the increased oxygen consumed by the guinea pig leucocytes on phagocytosis is wholly converted to hydrogen peroxide, the amount of the latter formed will amount to 0.39 micromole per mgm. dry weight leucocytes at *p*H 7.4. This corresponds to a concentration of approximately 100 m*M* in the leucocyte, assuming no leakage. If, however, the increase in oxidation of formate by the leucocytes on phagocytosis may be considered as the measure of hydrogen peroxide formation, this will amount to 1·3 m*M* at *p*H 7.4. At a lower *p*H, for example, 6.0, the corresponding values are 26 m*M* hydrogen peroxide (calculated from the increased amount of oxygen uptake) and 6.2 m*M* (calculated from the increased oxidation of formate. It is evident that the amount of hydrogen peroxide formed will vary according to the amount of phagocytosis and the intracellular *p*H and that it may vary from 1 m*M* to 100 m*M*. The latter concentration of hydrogen peroxide is known to be highly toxic to *E. coli*[27], but whether such an amount is actually formed is still uncertain. Nevertheless, in the consideration of the various factors that may operate in bringing about bactericidal action during phagocytosis, the possibility that hydrogen peroxide is formed during this process must be taken into account. It is unlikely that hydrogen peroxide is wholly responsible for the non-specific bactericidal effects of phagocytosis[28,29], but it will surely be one of the responsible agents.

Summary

Phagocytosis by guinea pig polymorphonuclear leucocytes is accompanied by an increased rate of oxidation of formate. Formate oxidation is linked with the oxidation of glucose and is sensitive to cyanide and to azide. Whereas glucose oxidation, anaerobically, may be stimulated by the presence of ferricyanide or pyruvate, formate oxidation under these conditions does not occur. The evidence indicates that whereas added pyruvate can replace oxygen as an oxidant with non-phagocytizing leucocytes, it is relatively ineffective with phagocytizing cells. The results also indicate that oxidation of reduced phosphopyridine nucleotides in leucocytes is accompanied by formation of hydrogen peroxide which is responsible for oxidation of formate. It is possible that the stimulation of glucose oxidation that occurs during phagocytosis is due to liberation of oxidase systems reacting with molecular oxygen to form hydrogen peroxide. The implications of the latter process on the non-specific bactericidal effects occurring in phagocytosis are discussed.

The award of Colombo Plan Fellowships to two of us (G. Y. N. I. and M. F. I.), under the technical assistance programme of the Canadian Department of Trade and Commerce is gratefully acknowledged.

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LETTERS TO THE EDITORS

ASTROPHYSICS

Atmospheric Scale Height as a Modifying Influence on Linear Trend of F-Region Electron Density with Sunspot Number

IN the prediction of maximum usable frequencies a linear increase in *foF*2 with sunspot number is generally assumed for practical purposes[1] However, an analysis of maximum usable frequencies, estimated for a sunspot cycle interval from Christchurch ionospheric vertical sounding data obtained during 1947–58, indicated that the trend of twelve-month running average values of *foF*2 monthly medians with twelve-month running average sunspot number showed significant departures from linearity especially in the daylight hours and above running average sunspot number 170 at the 1957–58 sunspot maximum Chun-Ming Huang[2] has observed a similar marked departure from linear trend at the 1957–58 maximum in an analysis of vertical sounding stations in the Western Pacific area, the time of onset relative to sunspot number varying with time of day and station latitude

A critical examination of Christchurch trends in noon values of maximum *F*2 ion density ($N_{\max}$) and true height ($h_{\max}$) was made to discover possible modifying influences contributing to the observed *foF*2 departures The plot of twelve-month running average values of $N_{\max}$ vs running average sunspot number (Fig 1) showed that departure from a linear trend is clearly defined for median, calm and disturbed conditions above running average sunspot number 170

The noon maximum ion density at a fixed pressure-level can be assumed to be an approximation to the equilibrium value·

$$N = \frac{q}{\beta}$$

where q is the rate of ion production and β is the rate of loss by an attachment-like process

On this assumption, if height changes, and changes in β throughout a cycle are neglected, an increase in q with sunspot number due to an increasing incident ionizing radiation could explain the main linear increase in $N_{\max}$ of the order of 4 1 for calm conditions. Height changes, however, indicative of scale height variations appear to be significant in modifying the electron density increase due to increasing q

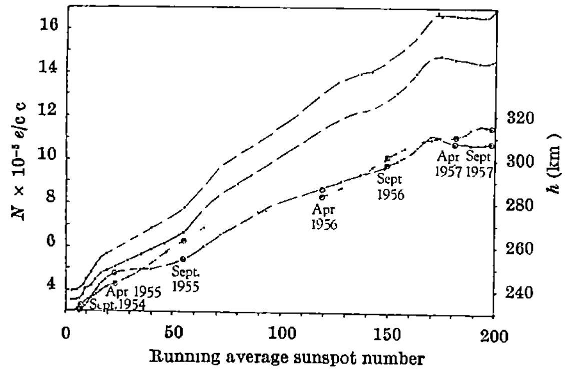

Fig 1 Twelve-month running averages of $N_{\max}$ vs running average sunspot number 1954–58 showing non-linearity above running average sunspot number 170 and annual residual variation. Full lines, ion densities, dotted line, median heights Upper, middle and lower curves are for calm, medium and disturbed conditions respectively

True height values of $h_{\max}$ for representative calm days 1954–58 were available from *N′h* profiles computed by Cummack using the method of King[3] Where the coverage was not complete for this period a relatively quick computation of $h_{\max}$ using the median trace method[4] combined with a slider technique was used The latter values were in reasonable agreement with the more rigorous calm-day computations and satisfactory for the purpose required

Twelve-month running average noon values of $h_{\max}$ for the 1954–58 interval were plotted on the ion density graph and the height trend coincided with the disturbed ion density trend (Fig 1). This latter exhibited similar departure from linearity to the median and calm ion density trends and was therefore taken as the ion density standard of reference for purposes of comparison

Above running average sunspot number 170 the height trend as shown in Fig 1 was maintained for a time after the linear increase in ion density had degenerated

Assuming that (1), height increases are indicative of increased scale height (H), and (2), that $qH \propto I$, where I is the incident ionizing radiation, it is postulated that critical increases in scale height near the 1957–58 sunspot maximum have been large enough to modify the increase in q resulting in a relative decrease in $N_{\max}$ at this stage of the cycle. As the ion density departures were similar under both calm and disturbed conditions, the effect of increased incidence of storms near sunspot maximum is not considered to be a major contributing factor to this phenomenon.

Fig 1 also shows that a residual annual variation remains during the years 1954–56 after the main seasonal variation has been eliminated by the smoothing process Relative ion density maxima and height minima centre on April while corresponding ion density minima and height maxima centre on September It can be considered that twelve-month running averages for April and September include the same equinoctial and winter months April–October. In addition, the April average includes the effect of the preceding summer months and the September average the effect of the following summer months As a result of the smoothing process, therefore, any marked departures from linearity occurring in the trend due to the effect of modifying influences in succeeding summer seasons could be at a maximum at the April and September points on the graph The observed residual annual departures then can imply that summers nearer sunspot maximum are characterized by a relative decrease in $N_{\max}$ associated with increase in scale height This deduction was supported in an examination of the declining phase of the cycle where height and ion density departures for April and September were found to be reversed, again implying that summers nearer sunspot maximum show a greater relative decrease in $N_{\max}$ due to relatively increased scale heights than those nearer sunspot minimum.

As the increase in summer heights from sunspot minimum to maximum (240–350 km.) is much greater than the increase at other seasons (230–280 km. in winter) a modifying influence due to increasing scale heights would be expected to be greater for summer as suggested However, a more refined discussion, including the effect of changes in β, is needed to account for the detailed seasonal behaviour pattern of $N_{\max}$ observed at Christchurch throughout the sunspot cycle investigated

A full account of the analysis of estimated values of maximum usable frequencies throughout a sunspot cycle and associated electron density trends at Christchurch will be published in the *New Zealand Journal of Geology and Geophysics* The survey includes detailed observations of seasonal trends in $N_{\max}$ throughout the cycle and examines sunspot cycle changes in diurnal trends of ionospheric storm departures

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PHYSICS

Triple Resonance Phenomenon in Plasmas

INTERACTIONS between electromagnetic waves and weakly ionized plasmas in the presence of a steady magnetic field have been widely investigated[1] The Appleton–Hartree formula for the equivalent complex dielectric constant of the plasma is partially reproduced in graphical form in Figs. 1 and 2 These graphs are restricted to the case that the propagation vector and E-polarization of the electromagnetic wave are both perpendicular to the direction of a uniform steady magnetic field With these restrictions, the relative dielectric constant can be written

$$\varepsilon' = \frac{\varepsilon}{\varepsilon_0} = \varepsilon_r - \frac{j}{\omega\varepsilon_0} = \frac{\varepsilon_{11}{}^2 + \varepsilon_{12}{}^2}{\varepsilon_{11}} \tag{1}$$

in which

$$\varepsilon_{11} = 1 + \frac{\omega_p{}^2}{j\omega}\,\frac{\nu + j\omega}{(\nu + j\omega)^2 + \omega_b{}^2} \tag{2}$$

$$\varepsilon_{12} = -\frac{\omega_p{}^2}{j\omega}\,\frac{\omega_b}{(\nu + j\omega)^2 + \omega_b{}^2} \tag{3}$$

where ω is the radian frequency of the wave, ν is the collision frequency. $\omega_b = eB/m$ is the cyclotron frequency, $\omega_p = \sqrt{\frac{ne^2}{m\varepsilon_0}}$ is the plasma frequency, e and m are the charge and mass of an electron B is the strength of the steady magnetic field, n is the volume density of free electrons in the plasma, and ε_0 is the permittivity of free space A dependence on time of $e^{j\omega t}$ has been assumed

When $\nu = 0$, the insertion of (2) and (3) in (1) leads simply to the result that the relative dielectric constant alternates between regions of positive and negative values as the electron density is increased Thus the electromagnetic wave is propagated if the free electron density is less than the value corresponding to $\omega_p{}^2 = \omega(\omega - \omega_b)$ It is cut off in the range $\omega(\omega - \omega_b) < \omega_p{}^2 < (\omega + \omega_b)(\omega - \omega_b)$ and is then propagated again in the range $(\omega + \omega_b)(\omega - \omega_b) < \omega_p{}^2 < \omega(\omega - \omega_b)$ For all electron densities greater than the value corresponding to $\omega_p{}^2 = \omega(\omega + \omega_b)$ the electromagnetic wave is not propagated It is apparent that increasing ω_b serves to separate these boundaries farther from each other In the laboratory, it is possible to achieve a sufficiently large ω_b/ω to make this triple resonance effect detectable

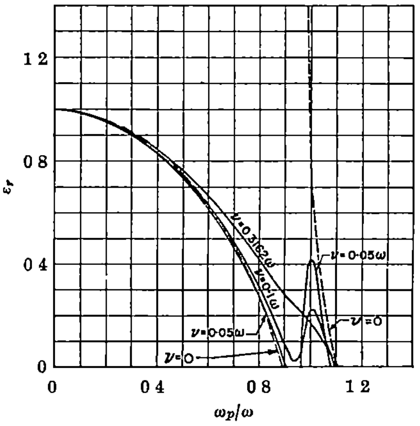

Fig 1 Relative dielectric constant versus normalized plasma frequency ($\omega_b = 0\ 2\ \omega$)

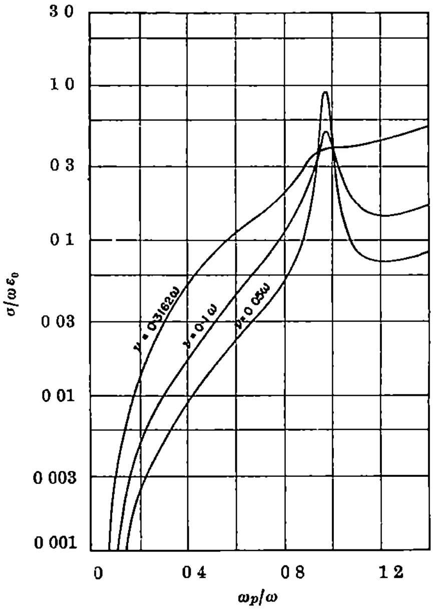

Fig 2 Normalized conductivity versus normalized plasma frequency ($\omega_b = 0\ 2\ \omega$)

The inclusion of collision losses serves to broaden these resonances, as in any problem in which Q is lowered However, it is interesting to note that a sufficiently high value of ν will erase the triple resonance effect entirely, as shown in the ε_r curves of Fig. 1 This proved to be a formidable stumbling block in the experimental work which was undertaken to demonstrate this phenomenon

An apparatus similar to one used earlier by Anderson[2] was employed. A 19-mm. outside diameter 'Pyrex' tube with a 1.5 mm. wall was placed inside a square brass wave-guide. The glass tube was 42 in. long with the last 3 in. on each end tapered to a point. The inside dimensions of the wave-guide were 0.805 in. by 0.805 in. with 0.035-in. walls. 5.5-in. wave-guide transitions coupled each end of the square guide to standard X-band wave-guide for connexion to the remainder of the microwave system. A klystron source was operated at 8,600 mc.p.s. and unmodulated.

The tube was attached through a hole in the wave-guide wall, with a 10-mm. nipple, to a spherical bulb of 3 in. diameter. The latter contained the ignitor electrode, auxiliary anode, and mercury pool cathode from a *GL*-5779 'Ignitron'. A second hole in the wave-guide wall, at the other end of the plasma column, accommodated a 5-mm. nipple which housed the main anode, a 0.1-in. diameter tungsten rod. The rod did not project into the plasma column, its end was even with the inside wall of the main tube. Continuous pumping was used to maintain the vacuum between 10^{-6} and 10^{-7} mm. of mercury.

A transverse d.c. magnetic field was impressed on the plasma by inserting the square wave-guide between the 1-in. wide by 57-in. long poles of an electromagnet. The magnet core was constructed of 1020 cold-rolled steel. The 17-gauge heavy 'Formvar' winding was continuous around the 57-in. dimension of the magnet, giving a uniform magnetic field along the entire length of the plasma column. A 0–150 V. d.c. generator supplied a maximum of 4 amp. to the winding to give a maximum field of 1,200 gauss.

The ignitor electrode was pulsed at 60 c.p.s. from a 600 V. supply and the main anode was energized from a composite source—5 kV. to provide a d.c. current plus a variable 60 c.p.s. supply to superimpose a sinusoidal variation on the d.c. current. In this manner, the plasma properties were made to vary at a 60-c.p.s. rate with controllable excursions. The results of this experiment were recorded by photographing the traces of a dual beam oscilloscope for various values of d.c. magnetic field. A representative set of traces is reproduced in Fig. 3. In each display the upper trace is the a.c. component of tube current and the lower trace is the detected microwave signal which has traversed the plasma column. All the oscillograms shown in Fig. 3 were taken with 420 m.amp. d.c. and constant amplitude a.c. through the discharge tube. The negative peak of the upper trace corresponds to minimum electron density in the plasma, a value small enough to cause essentially full transmission of the microwave signal in the absence of a d.c. magnetic field.

The lower traces have many features in common. The 8,600-mc.p.s. wave is cut off until such time as the current through the plasma tube (and thus the electron density) lowers to the point where propagation begins; this is a resonant point. The propagation then rises to a peak, giving a second resonant point. The propagation again decreases, the minimum point giving a third resonance. The propagation then rises to a maximum value of transmission. As the tube current increases in value this sequence is repeated in reverse order.

It is evident that as the d.c. magnetic field is increased, and both the d.c. and a.c. current-levels are kept constant, the transmission-level decreases. This is attributable to the increases in electron density and collision frequency which accompanied the increases in the d.c. magnetic field. It is interesting that Beckman[3] has predicted just such an effect.

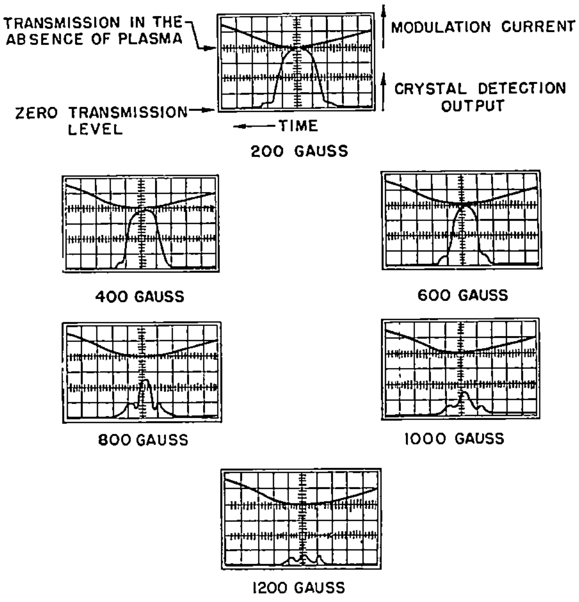

Fig. 3. Oscillograms showing the variation of modulation current and transmitted power during one de-ionization and ionization process for six different magnetic field values. (Reading from right to left, de-ionization occurs in time before ionization)

For this reason no attempt was made to infer that there were specific values of plasma frequency at the three resonant points. No claim can be made that the three resonances separated on the ω_p scale as ω_b was increased. However, the triple resonance phenomenon showed up very clearly and this was the principal goal of the experiment.

Considerable difficulty was experienced in obtaining the results shown in Fig. 3. It was found that the apparatus attained a considerable temperature due to heat generated in the plasma and in the winding of the d.c. magnet. This caused the collision frequency to increase to the point that the triple resonance effect was obscured as predicted by the data in Figs. 1 and 2. Indeed, when the apparatus was first turned on, the oscilloscope trace would clearly show a triple resonance, and as one watched, these features would subside until finally no resonance phenomena were displayed.

This difficulty would not necessarily be present in measurements on a plasma which was not supporting a large current. Often the collision frequency is small in problems concerning the plasma sheath around a missile, or the wake behind it, or in problems concerning the ionosphere. Such plasmas do not contain large currents and their exact laboratory stimulation must be accompanied by techniques different from the one used here. In such applications it can be expected that joint use of a d.c. magnetic field and electromagnetic waves can invoke a triple resonance effect which will prove useful in diagnostics.

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Excitation of the 2*s* State of Atomic Hydrogen by Electron Impact

In a recent publication Kingston, Moiseiwitsch and Skinner[1] have applied the second Born approximation, retaining only terms up to the third order in the interaction energy, to the calculation of the total cross-section Q ($1s$–$2s$) for the $1s$–$2s$ excitation of hydrogen atoms by electrons Their results are displayed in Fig 1 Curve 1 is the cross-section obtained by using the first Born approximation while curves 4 and 6 are respectively the cross-sections given by the second Born approximation with allowance for (*a*) distortion due to the coupling to the $1s$ and $2s$ states, (*b*) polarization due to the coupling to the $2p$ states as well as distortion due to the $1s$ and $2s$ states

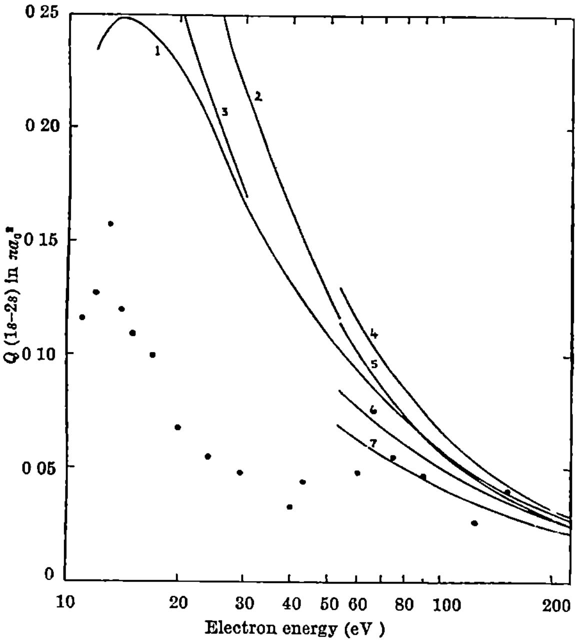

Fig 1

Wu[2] has reported first Born approximation calculations of Q ($1s$–$2s$) in which allowance for exchange has been made From the tables given by Wu the contribution resulting from exchange can be readily calculated and incorporated into the second Born approximation calculations of Kingston, Moiseiwitsch and Skinner The resulting cross-sections are displayed in Fig 1 Curve 5 includes the effects of distortion and exchange while curve 7 includes in addition the effect of polarization Allowance for exchange reduces the excitation cross-section Smith, Miller and Mumford[3] have carried out exact solutions of the coupled differential equations for the $1s$–$2s$ electron impact excitation of atomic hydrogen derived by making full allowance for the distortion due to the $1s$ and $2s$ states but neglecting exchange The cross-section obtained by them is given by curve 2 and is in satisfactory accord with the second Born approximation curve 4 calculated by Kingston, Moiseiwitsch and Skinner

Smith[4] has solved the coupled integro-differential equations for the s, p, d partial waves making full allowance for exchange as well as distortion The resulting total cross-section Q ($1s$–$2s$) is given by curve 3, the higher-order partial cross-sections having been estimated with the first Born approximation Curve 3 lies somewhat below curve 2, and thus allowance for exchange reduces the cross-section in agreement with the previously noted effect of exchange on the second Born approximation cross-sections

The experimental points of Stebbings, Fite, Hummer and Brackmann[5] for Q ($1s$–$2s$) are also displayed in the figure. Lichten[6] has shown that the original data of Stebbings *et al* are in error and should be multiplied by a factor 3/2 This correction was therefore introduced (cf Hummer and Seaton[7])

There is satisfactory agreement, at least at energies greater than 60 eV, between the modified experimental results of Stebbings *et al* and the cross-section curve 7 in which allowance for exchange as well as distortion and polarization has been included Noting that curve 4 lies slightly above curve 2, it is to be anticipated that curve 7 is slightly in excess of the actual $1s$–$2s$ excitation cross-section, which would then bring calculation into still closer agreement with the absolute experimental data of Stebbings *et al.*

The experimental cross-section obtained by Lichten and Schultz[8] does not extend above 45 eV, and in any event is not absolute Hence no comparison between theory and their experimental results is feasible However, their relative cross-section is in good agreement with the shape of the cross-section obtained by Stebbings *et al* as was pointed out by the latter authors

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Measurement of Stopping-Power Ratio for Cavity Ionization Chambers

The energy absorbed in a medium per unit mass, E_m, exposed to X- or γ-radiation can be related to the ionization produced per unit mass of gas, J_m, situated in a cavity in the medium by the Bragg–Gray equation

$$E_m = \frac{1}{F_m} W J_m \qquad (1)$$

where W is the average energy associated with the production of one ion pair in the gas, F_m is the mass stopping-power ratio of the gas to the solid for electrons F_m has been evaluated theoretically by assuming electrons lose energy continuously[1,2] and also by allowing for discrete losses of energy[3,4] However, experimental investigation has shown that these theoretical treatments are only useful for a limited range of size of cavity[5], as indeed the initial assumptions in their derivations imply. In many situations the greatest uncertainty in measuring exposure or absorbed dose arises from uncertainty in this factor. Thus a direct measurement of F_m is desirable

Several workers have measured the relative stopping-power ratio, but Whyte[6] devised a method

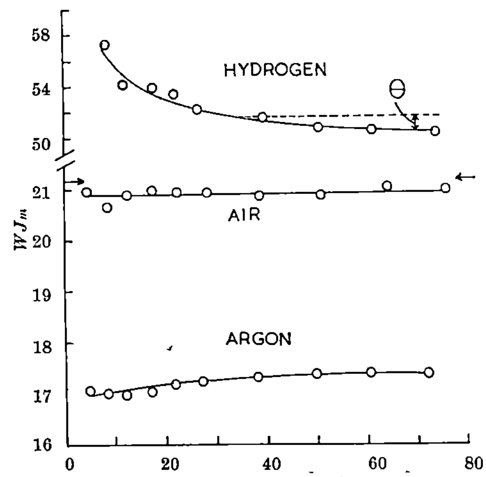

Fig. 1. Variation of the ionization/gm multiplied by the average energy associated with the production of one ion pair with the gas pressure in a graphite chamber irradiated by cobalt-60 γ-rays. θ is the change in the ionization/gm between 70 and 35 cm of mercury pressure

of measuring the stopping-power ratio absolutely by varying the air pressure in a series of ionization chambers with walls of different atomic number.

An absolute measurement of the stopping-power ratio can also be made by varying the pressure of different gases introduced into a single ionization chamber in the following way. Fig. 1 shows the variation in the ionization/gm of gas with the gas pressure for hydrogen, air and argon in a graphite ionization chamber irradiated by 1.25-MeV. photons (cobalt-60 γ-rays). The experimental technique has been described elsewhere[7]. Hydrogen with an atomic number less than graphite yields a graph with a negative gradient, while air and argon with an atomic number higher than graphite yield a graph with a positive gradient. The magnitude of the variation in the ionization/gm with the gas pressure increases with the magnitude of the difference between the atomic number of the gas and the wall and hence increases with the factor, F_m.

To measure this variation of the ionization/gm with the gas pressure a parameter, θ, has been chosen. θ is the change in the ionization/gm between 70 and 35 cm of mercury pressure. The value of θ for each gas is plotted against the value of WJ_m at 70 cm of mercury pressure for each gas in Fig. 2. Now if it were possible to fill the cavity with a gas exactly equivalent to the graphite wall, F_m would be unity at all gas pressures. Thus the ionization/gm would be independent of the gas pressure and in Fig. 1 a straight line parallel to the x-axis would result. In this case θ would be zero. It follows that the value of WJ_m in Fig. 2 where $\theta = 0$ is the value that would occur when the gas in the chamber is equivalent to graphite, namely, when $F_m = 1$.

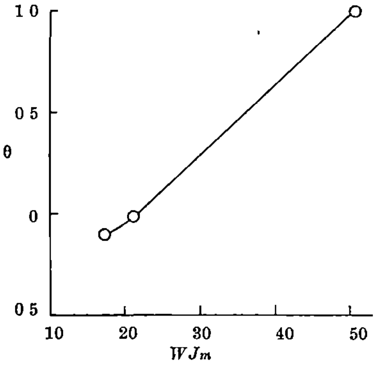

Fig. 2. Variation of the change in the ionization/gm with the ionization/gm multiplied by the average energy associated with the production of one ion pair

For the three gases used in this work W is known reasonably accurately and the values adopted are 36.3 eV for hydrogen, 33.8 eV for air and 26.1 eV for argon. From equation (1) it may be seen that F_m is proportional to WJ_m. In Fig. 1 the ionization/unit mass of gas has been multiplied by the appropriate value of W for each gas. Thus the ordinates in Fig. 1 are proportional to the stopping-power ratio, F_m. Now the value of WJ_m when $F_m = 1$ can be determined as described here and is represented by the arrows in Fig. 1. The absolute value of the stopping-power ratio, F_m, for any of the gases in graphite at a particular pressure is given by the ratio of the ordinate of the appropriate curve at that pressure to the ordinate of the arrows, for which $F_m = 1$.

The values of the stopping-power ratio, F_m, obtained in a preliminary investigation of the method were 2.39 ± 0.05 for hydrogen, 0.99 ± 0.02 for air and 0.82 ± 0.02 for argon for a 0.5-cm^3 cavity in graphite at atmospheric pressure irradiated by 1.25 MeV photons (cobalt-60 γ-rays). A more refined technique would considerably reduce the uncertainty quoted for these figures. In very accurate work it would probably be necessary to consider the effect of the transfer of slow electrons between the electrodes[8].

Although this method of measuring the stopping-power ratio requires data concerning W, it does offer the following advantages over Whyte's method: (1) gases are more easily interchanged than the chamber wall material; (2) there can be no errors arising from differences in volumes of the chambers as in Whyte's method; (3) to perform the interpolation in Fig. 2 it is obviously an advantage to obtain values of θ well above and well below $\theta = 0$. Thus, for example, to determine the stopping-power ratio for a graphite chamber containing air (a combination used frequently) materials are required with an atomic number, Z, well separated from the range $Z = 6$–8, as well as close to this range. By interchanging the wall materials Whyte's method is limited to conducting solids and therefore can only go down to beryllium ($Z = 4$). However, provided the appropriate relative values of W are available, this method can use hydrogen ($Z = 1$) and helium ($Z = 2$) both well separated from $Z = 6$–8. Above $Z = 6$–8 there are several suitable materials for both methods; (4) there can be no error arising from differences in the photon attenuation in the walls or from differences in the absorption coefficient of the wall materials.

I thank Prof. J. E. Roberts for his supervision and Dr. J. R. Mallard for his encouragement.

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BIOPHYSICS

Estimation of Mass Thickness of Biological Samples from their Electron Micrographs

DURING the past several years a number of investigations[1-13] have been made to compute the mass thickness of biological samples, from the contrast in their electron microscope images The basis of all these measurements is the well-established relation

$$I_t/I_0 = \exp(-S.\ \rho t) \qquad (1)$$

where I_t/I_0 is the image contrast of an elemental object area of mass thickness ρt and S is the scattering cross-section per gm of the object By using carbonaceous samples, for example, spheres of polystyrene latex, of known diameter and density and measuring their image contrast, several authors[3,12] have estimated the value of S under various operating conditions of the electron microscope From these results, curve 1 in Fig 1 has been plotted, showing the variation of S with the angular aperture α, for 50-kV energy electron beam A few values of S for (i) carbon film calculated from the experimental data of Lippert[14] and (ii) 'Formvar' film (from data of Bahr[5]) have also been plotted in Fig 1

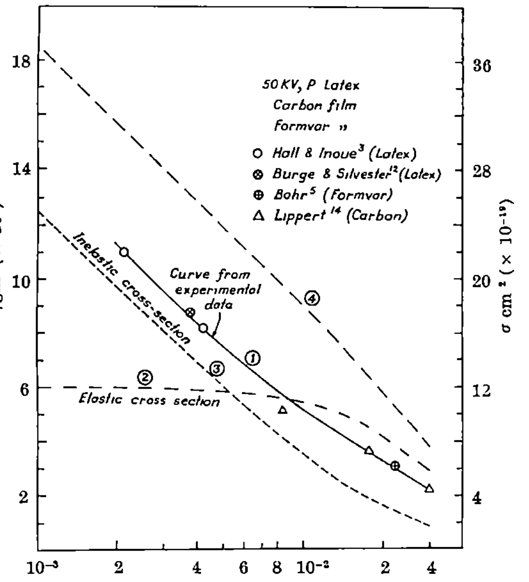

Fig. 1 Variation of scattering cross-section of carbonaceous samples for 50-kV electron beam

Considering the samples to be composed mainly of carbon, the scattering cross-section per atom is $\sigma = AS/N_A$, that is, 2×10^{-23} S cm ², and the same curve (1) represents σ versus α, with suitable change of scale in the ordinate

For comparison, the values of the elastic and inelastic cross-sections of the carbon atom, calculated from the electron scattering formula of Lenz[15] as modified by Sadhukhan[16], are shown as curves (2) and (3), while curve (4) represents the sum of the two cross-sections

The following conclusions can be drawn from these curves The scattering cross-section varies with angular aperture even in the range of 10^{-3}–10^{-2} radian, the nature of the variation indicates that the inelastic component of scattering seems to play an important part in the small angular region The experimental values of the scattering cross-section are not in quite satisfactory agreement with Lenz's equations Hence, any attempt to compute the mass thickness by making use of the theoretical electron scattering formula may give only the order of magnitude, but there is a possibility of a wide discrepancy as regards the right value

I wish to thank Prof N N Das Gupta for his kind interest in this work and Mr P Sadhukhan for helpful discussion Thanks are also due to the Ministry of Scientific Research and Cultural Affairs, Government of India, for financial assistance

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RADIOCHEMISTRY

Isolation of Radioactive Bismuth from Metallic Lead and its Compounds

IT is self-evident that the isotopic radionuclide lead-210 will remain an inseparable part of the bulk lead metal which is produced during the extraction of this metal from its various mineral ores

This lead isotope, having a half-life of 22 years, is a direct daughter product of the radium decay chain, and is a natural precursor of bismuth-210, the latter radionuclide decaying by β-decay to the α-emitting polonium-210

$$\begin{array}{ccccc} {}^{210}\text{Pb (Ra}D) & \xrightarrow{\beta} & {}^{210}\text{Bi (Ra}E) & \xrightarrow{\beta} & {}^{210}\text{Po (Ra}F) \\ \text{22 years} & & \text{5 days} & & \text{138 days} \end{array}$$

It has been established experimentally that factual information regarding the concentration of various radioactive isotopes in normal samples of metallic lead may be obtained by the radiochemical separation and determination of the bismuth isotope This is achieved in a quantitative manner by the acidic dissolution of 10–20 gm of the lead material, adding approximately 10 milligrams of bismuth as carrier in the form of the nitrate, and precipitating the bismuth with an *n*-propyl gallate solution in 0 01 normal nitric acid solution at boiling point The yellow crystalline precipitate formed consists essentially of the salt, $C_{10}H_9O_5$ Bi H_2O (molecular weight =436 188), and contains 0 4790 gm bismuth per gm of precipitate dried at 110° C

In experimental practice, the precipitate may be adequately dried by washing with ethanol with suction prior to weighing During the present experiments the filtration was carried out on a

Whatman 541 filter paper of 22 mm diameter, this being mounted on a 1-inch aluminium planchet and counted under a conventional end-window Geiger tube

Alpha particles from any contaminating polonium-210 would be satisfactorily excluded by the Geiger window

Extrapolation of the count-rate to zero time (time of precipitation) shows that the emission decays with the characteristic half-life of 5 days, thus positively identifying the bismuth-210 isotope Typical experimental data are illustrated graphically in Fig 1

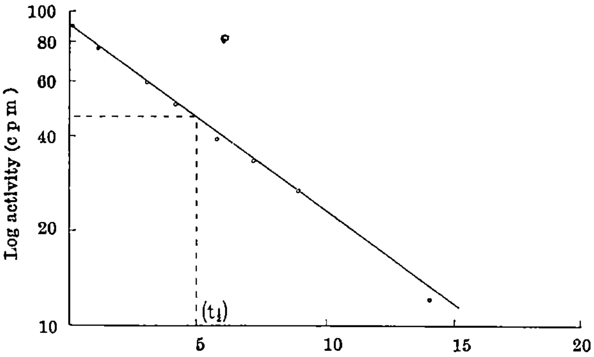

Fig 1 Decay curve of bismuth-210

The count-rates are related on a quantitative basis with those given by a standard solution of radium D, E, F, from which bismuth has been precipitated in the same way The chemical yield of the precipitated bismuth is invariably 100 per cent

Table 1 gives some of the results of these investigations on various samples of lead and its oxides It may be noted that sample No 280 was carefully counted using an anti-coincidence type Geiger assembly, whereas all the other samples were done by normal Geiger tube counting

Table 1 RADIOACTIVITY OF SAMPLES OF LEAD

Laboratory No.	Lead sample	Bismuth-210 β-activity $\mu\mu$c /gm	Total α-activity $\mu\mu$c /gm
279	Remelt ingot	3 32 ± 0 15*	4 7 ± 0 5
280	Scrap lead piping	0 17 ± 0 02	<0 5
281	Remelt lead	1 46 ± 0 2	1 6 ± 0 6
282	Scrap window flashing	1 44 ± 0 08	2 54 ± 1 0
283	Remelted scrap	2 4 ± 0 07	5 1 ± 0 5
284	Litharge	2 08 ± 0 06	1 56 ± 0 5
286	Lead (BSS 602)	15 3 ± 0 3	16 5 ± 0 5
288	Litharge	4 2 ± 0 8	2 9 ± 0 7
291	Red lead	4 50 ± 0 15	3 8 ± 0 8
292	Litharge	13 0 ± 0 5	12 0 ± 1 0
294	Litharge	2 7 ± 0 2	2 6 ± 0 6
295	Virgin lead	2 3 ± 0 1	4 3 ± 1 0

* 90 per cent confidence limits, errors on counting only

The order of radioactivity shown is less than the figure of 20 µµc /gm , which is the proposed limit for natural lead given in the Radioactive Substances Act, 1960 (Sec. 18, Schedule III)

W E Grummitt *et al* [1], by an alternative method, quote the figures of 0 17 µµc total β-activity per gm for sixteenth-century lead, and 1 7 µµc /gm for modern lead These values are in complete accord with those obtained by the bismuth method in the present investigation

In the final column of Table 1, experimental values are given for the total alpha activity of the individual lead samples used These activities were determined by counting infinitely thick sources in a scintillation counter and comparing the results with those obtained from an artificially prepared lead standard containing a known amount of radium D in equilibrium with its daughter products This was prepared by precipitating inactive lead sulphide in the presence of a known amount of radium D, E, F, and the $Z^{2/3}$ relation of J L Glasson[2] was used for correcting the results for the lead compounds of different average atomic number to the lead sulphide standard

The numerical agreement between the measured alpha and beta activities in the final and penultimate columns of the table strongly suggests that the equilibrium product, polonium-210, is the main α contaminant in commercial lead samples, and that radium and other emitters can only be present in small concentrations

The method described in this paper for the separation of bismuth radionuclides from abundant lead has many obvious applications which are now being explored in this Laboratory

In a recent paper presented by A Turkovitch *et al* [3] it was shown that radioactivity induced in the lead sheath of the recovered satellite *Discoverer XVII* by solar-flare on November 12, 1960, was largely due to the production of the 15-day bismuth-205 which emits X-rays in the 70-keV region

Separation was carried out by the electro-deposition of bismuth carrier on a platinum electrode immersed in the lead electrolyte obtained by acid dissolution of the satellite sample (cf A D R Harrison *et al* [4])

It may be confidently predicted that the method now described here could be usefully employed in satellite investigations of this character, and would afford a radiochemical alternative to the electrochemical method of separation

It may be mentioned that occasionally samples of lead are naturally contaminated with inactive bismuth, and where this occurs, the appropriate correction must be applied for the radiochemical yield

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CHEMISTRY

Radiation Effects on Lithium-doped Nickel Oxide Catalysts in Carbon Monoxide Oxidation

EFFECTS of nuclear radiation on heterogeneous catalysts has been the subject of various investigations in the past few years[1,2] These investigations have shown that significant changes due to irradiation are produced in the catalytic behaviour of some oxides and metals The phenomenon is due to the fact that catalytic activity is strongly dependent on the structure of solids and this structure may be modified on irradiation Nuclear radiations may create in semi-conductors new energetic levels, which change the position of the Fermi-level in the solid This leads to changes in various structural sensitive properties, for example, the activation energy of the reaction catalysed by the surface of the solid[3]

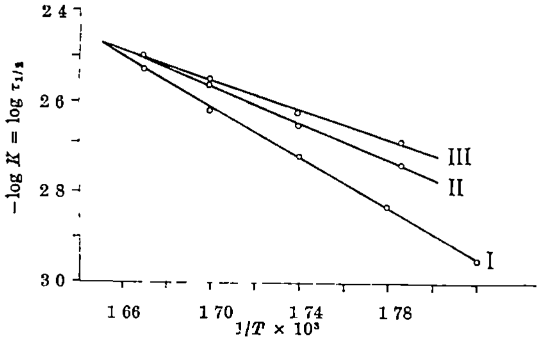

Fig 1 Arrhenius plot for catalytic oxidation of CO on NiO + Li_2O catalyst before (I) and after (II) 80-hr irradiation in the nuclear reactor and after one further irradiation (III) of 80 hr Catalyst NiO + 2 5 mol per cent Li_2O

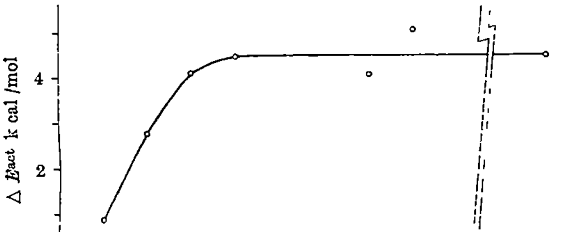

Fig 2 Activation energy differences ΔE_{act} (before and after irradiation) versus irradiation time

However, the major part of the cited papers deals with the variation of the reaction-rate after irradiation at a given reaction-temperature, and only in three papers are there presented results about variation of the activation energy[4–6]. In our opinion, from the changes in the activation energy following irradiation one can get more information about how irradiation affects catalytic behaviour of solids and, on the other hand, how to correlate the change of catalytic and electrical properties via activation energies

In the present communication, we present the results of radiation effects on catalytic behaviour of solid solutions of nickel oxide (NiO) and lithium oxide (Li_2O) in carbon monoxide oxidation The choice of NiO was determined by the fact that its electrical and catalytic properties are better known, so that it is easier to give an interpretation of the experimental results Doping with lithium enhances the efficiency of nuclear radiations through the reaction $Li(n,\alpha)^3H$ Thus, in the neutron flux the lithium atoms become an important source of internal irradiation On the other hand, the results of the catalytic oxidation of carbon monoxide (CO) yield more reproducible results on NiO + Li_2O than on pure NiO (ref 7)

The effects of thermal neutron irradiation of NiO + Li_2O catalysts for the decomposition of nitrous oxide (N_2O) have been examined by Saito *et al* [6], but this work does not indicate a clear variation of the activation energy

Our experimental conditions were the following irradiations were made in the thermal column of the *VVR–S* 2,000 kW water-cooled and water-moderated enriched uranium reactor of the Institute of Atomic Physics, Bucharest The fluxes were thermal neutrons 2×10^{11} *n* /cm ^{2} sec , and fast neutrons 7×10^9 *n* /cm ^{2} sec

The catalyst samples were obtained from the pyrolysis of $Ni(NO_3)_2$ The NiO powder was mixed with Li_2O (from Li_2CO_3), in proportion of 2 5 mol per cent, and sintered at 850° C for 3 hr The oxidation of carbon monoxide was followed in a closed system[7] as indicated by Dry and Stone[8] The results of a typical experiment are represented in Fig 1 Fig 2 shows the variation of the activation energy differences (before and after irradiation) versus irradiation time From these figures, it is clear that irradiation significantly affects the activation energy of the catalytic oxidation of carbon monoxide and that the effects increase with increasing irradiation time The experimental values from Fig 2 seem to imply the existence of a saturation effect revealed by a plateau beginning with irradiation times of the order of 40 hr

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Hydrogen Overvoltage at Cathodes of 77 per cent Palladium and 23 per cent Rhodium

CATHODIZING an equilibrium Pd/H/H_2 electrode (25° C 1 atm hydrogen zero volt potential with respect to a Pt/H/H_2 reference in the same solution) further increases its hydrogen content (H/Pd, atomic ratio) and its corresponding hydride vapour pressure *p*. These increases are reflected as a component of overvoltage (volts) given[1–3], the symbols η_2 or η_d (termed[1,3] the diffusion or hydride overvoltage) and related to *p* by $\eta_2 = 0\ 029 \log p$ The large capacity of palladium for hydrogen has enabled η_2 to be obtained by extrapolation, back to zero time, of the increase of electrode potential, *E*, which follows interruption of cathodization, using established relationships between *E*, *p* and H/Pd (refs 1–3) From such measurements it has been established[1,3–5] for surfaces active for rapid equilibration with hydrogen molecules dissolved in solution that at current densities *i* less than 0 040 amp cm $^{-2}$ the retarding kinetic step maintaining H/Pd in excess of its equilibrium value (1 atm hydrogen 25° C) is the slow diffusion from the cathode of hydrogen molecules formed during electrolysis Under these conditions, η_2 is related to *i* by

$$\eta_2 = 0\ 029 \log \left(\frac{i + i_0}{i_0}\right) \qquad (1)$$

where under conditions of vigorous stirring $i_0 \sim 3$ m amp cm $^{-2}$ The observation[1–3] that η_2 attains an almost constant value for *i* greater than 0 040 amp cm $^{-2}$, when hydrogen is evolved as bubbles, has been associated[1] with either critical supersaturation of the diffusion layer with dissolved molecules or saturation of chemisorption sites Experiments reported here appear to eliminate the latter possibility.

The general effect of increasing the amount of rhodium in the Pd–Rh–H system (ref 6 and unpub-

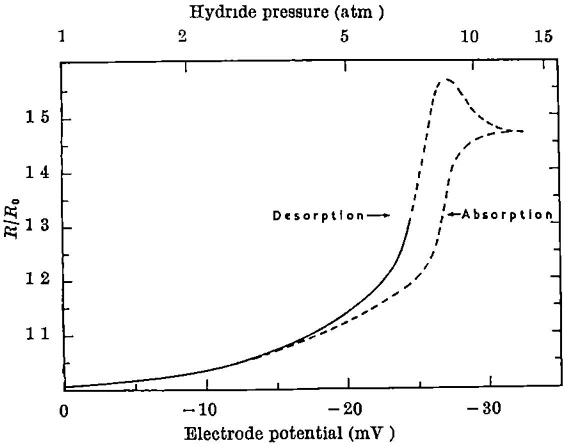

Fig. 1

lished results) resembles that of platinum in the Pd–Pt–H system[8] in increasing the hydride vapour pressure over regions where α- and β-phase hydrides co-exist (and the solids can show marked changes in hydrogen content) and is paralleled (unpublished results) by changes in electrical resistance (recorded as R/R_0 where R_0 is the resistance of the hydrogen-free alloy). Relevant relationships (25° C.) for the 23 per cent rhodium (by weight) system are shown in Fig. 1, where the desorption data were estimated with specimens of slightly less than homogeneously diffusion-controlled activity (see later). Cathodes were in the form of wire (0·0122 cm. diameter, about 10 cm. long) and the apparatus has been described[7]. R/R_0 was recorded, in conjunction with electrode potential both during and subsequent to cathodization, using an a.c. bridge[8] and measurements carried out in 0·002 N and 0·01 N hydrochloric acid to avoid complications due to solution-conduction of the bridge current[7,8]. Pre-activation was either (i) by pre-flaming at close to white heat, or (ii) by plating palladium black from aqueous palladium chloride, or (iii) by plating 'bright' palladium from aqueous tetramminepalladous nitrate under conditions recommended by Philpott[9]. All electrode potentials were zero in hydrogen-saturated solutions prior to cathodization.

As anticipated, values of η_2 (from steady-state values of R/R_0) attained by palladized specimens were in accord with equation 1 for $i \leqslant 0·020$ amp. cm.$^{-2}$ ($\eta_2 \sim 26$ mV.). Furthermore, in support of Knorr's contention[5] that the overvoltage at all platinum group cathodes (homogeneously activated) should be generally governed by the rate of diffusion of hydrogen molecules from their surfaces, pre-flamed specimens could be easily prepared which obeyed equation 1 in this range of i, not only attaining virtually identical steady-state values of R/R_0 to palladized specimens for the same i but also exhibiting virtually identical rates of decrease of $|E|$ and R/R_0 on interruption of cathodization. When $i \sim 0·050$ amp. cm.$^{-2}$, dense clouds of tiny bubbles began to evolve from all cathodes with surfaces sufficiently active for η_2 to be given by equation 1 at lower current densities with palladized specimens, η_2 could thereafter be increased little further, since R/R_0 never exceeded 1·2 even on increasing i to 0·260 amp. cm.$^{-2}$ (equation 1 giving $\eta_2 \sim 56$ mV.), in one specimen R/R_0 was constant at 1·13 from 0·050 to 0·260 amp. cm.$^{-2}$. These results were not the consequence of insufficient chemisorption sites, however, because $R/R_0 \sim 1·4$ was rapidly attained both by less surface-active pre-flamed specimens (even for $i < 0·020$ amp. cm.$^{-2}$) and also by cathodes which had been quite thickly coated with bright palladium but which exhibited, after interruption of electrolysis, only slightly slower rates of decrease of $|E|$ and R/R_0 (over comparable ranges of values) than cathodes for which R/R_0 was not found to exceed 1·2.

It is difficult to measure differences, resulting from hysteresis over the region of co-existence of α- and β-phase hydride (unpublished results) between the absorption/desorption relationships (Fig. 1), so that the precise value of η_2 during cathodization must be assessed with caution. So far, however, results suggest that the limiting hydride vapour pressure, and thus the pressure of dissolved hydrogen molecules in the diffusion layer, can be as low as 10 atm., which is slightly less than that indicated from work with pure palladium[1,3]. In further experiments with this and other palladium alloys, it is hoped to define these limiting pressures even more precisely.

We are indebted to the International Nickel Co. (Mond), Ltd., for providing the palladium/rhodium alloy.

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Kinetics of Dissociation of Ethyl Halides in Self-quenching Geiger–Müller Counters

By using the experimental procedure described in ref. 1 mass-spectrometric work on electron-impact dissociation of ethyl halides C_2H_5X (X = Cl, Br, I) in a working Geiger–Muller counter has been carried out.

Simple analysis of the respective decay diagrams shown in Fig. 1 leads to the conclusion that, as in the case of saturated hydrocarbons, a regular, essentially first-order, slow decomposition process follows an initial very fast consumption of parent molecules which practically comes to an end after the

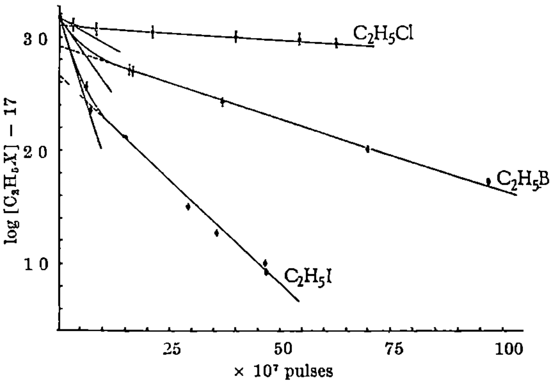

Fig. 1. Decay curves of ethyl halides used as quenching vapours

first 10^7–10^8 pulses have been registered by the counter It is interesting to note that from our mass-spectrometric data evidence was obtained that in the case of ethyl iodide the large initial loss of quenching parent molecules amounts to about 98 per cent For both ethyl chloride and bromide the respective values lie between 85 and 50 per cent

Assuming that the observed fast consumption process is due primarily to strong adsorption phenomena occurring on freshly degassed electrode and glass surfaces, it seems reasonable to expect that only very few undecomposed parent molecules will reach the wall of the counter The average life-time before decomposition for an excited polyatomic molecule is less than 10^{-13} sec (ref 2) The time required by the same molecule to reach the critical distance from the wall (where the probability of neutralization equals unity) being about 10^{-12} sec , it may be assumed that only about one in 10^9 molecules of ethyl halide can reach the cathode surface before decomposing Furthermore, it is apparent that heavy organic molecules such as ethyl halides have a large probability of pre-dissociating at all energies

In order to illustrate the primary decomposition of excited ethyl halide molecules or ions in a working Geiger counter use may be made of the following scheme

$$\begin{array}{ccccc} (M^0, M^+)^* & \underset{k'_i}{\overset{k_i}{\rightleftharpoons}} & (R^0{}_i, R^+{}_i) & + & (R^+{}_j, R^0{}_j) \\ & & \downarrow k_{R_i} & & \downarrow k_{R_j} \end{array}$$

where M^0 and M^+ are parent molecules or their ions, R_i and R_j—free radicals, the usual primary decomposition products, k_i and k'_i—the decomposition and recombination-rate constants, k_{Ri} and k_{Rj} rate-constants of subsequent free-radical secondary reactions

If the concentration of the molecular species M^0 and M^+ is denoted by $[M]$ and those of $[R^0{}_i + R^+{}_i]$ and $[R^0{}_j + R^+{}_j]$ by $[R_i]$ and $[R_j]$ respectively, then

$$\frac{\mathrm{d}[M]}{\mathrm{d}n} = k_i[M] - k'_i[R_i][R_j]$$

Now, at the beginning of the rapid-loss portion of the decay curve it may be assumed that $[M] \gg [R_i][R_j]$ which leads to a first-order equation for the rate of disappearance of parent molecules from a fresh quenching gas

$$\frac{\mathrm{d}[M]}{[M]} = k_i \mathrm{d}n$$

where n is the total number of counts registered by the scaling unit The straight lines, tangent to the decay curves at their origins, shown in Fig 1, represent the kinetics of the initial decomposition of the respective quenching agents It need scarcely be said, however, that with continued use of the counter, the composition of its quenching gas is steadily changing The main features involved are the recombination of free radicals to form a wide assortment of heavier molecules and the adsorption-desorption equilibrium Hence the over-all decay rate-constant is gradually approaching the k_p^s value of the first-order slow-decomposition process

Table 1 QUENCHING CHARACTERISTICS OF ETHYL HALIDE VAPOURS

Quenching molecules	C_2H_5Cl	C_2H_5Br	C_2H_5I
Filling pressure (at 298°) in mm mercury	60	60	60
Counter volume (ml)	71 2	71 2	71 2
Cathode	graphite	graphite	copper
Average No of electrons/ pulse, $\times 10^{-8}$	2 75 ± 0 27	7 2 ± 0 72	17 2 ± 1 7
Initial rapid decomposition rate-constant $k_p^r \times 10^9$/ pulse	5 06	13 2	29 2
Initial rapid decomposition rate-constant $k_e^r \times 10^{17}$/ electron	1 84 ± 0 18	1 83 ± 0 18	1 70 ± 0 17
Initial specific electron yield $S_e \times 10^{-3}$ molecules/ electron	2 7 ± 0 3	2 7 × 0 3	2 55 ± 0 26
Slow decomposition rate-constant $k_p^s \times 10^9$/pulse	0 55	2 76	8 74

The calculated rate-constants for both the initial rapid loss and the subsequent slow decomposition of C_2H_5X molecules are shown in Table 1

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Space Group and Unit Cell of the *m*-Bromobenzoate of Photoisopyrocalciferol

THE unit cell and space group of the *m*-bromobenzoate of photoisopyrocalciferol have been determined, and an X-ray diffraction investigation with three-dimensional data is in progress Dauben and Fonken[1] have postulated a structure for photoisopyrocalciferol based on chemical and spectroscopical investigations The *m*-bromobenzoate has been prepared with a chemical analysis of C 72 23 per cent, H 8 17 per cent, and Br 13 58 per cent compared with the calculated values of C 72 52 per cent, H 8 17 per cent, and Br 13 79 per cent for the $C_{35}H_{47}O_2Br$ molecule The crystals melt over the range 93 5–95° C The crystals are colourless, flat needles with the long dimension parallel to the b axis The a axis is perpendicular to the flat sides of the needles Weissenberg photographs of the type hnl and $hk0$ have been taken showing systematic absences only of the type $0k0$ with k odd The usual intensity relationships for the monoclinic system are observed The space group $P2_1$ is the only one consistent with the above observations and compatible with the asymmetrical molecules The following cell dimensions have been determined from films calibrated with quartz powder patterns $a = 21\ 13$ Å., $b = 7\ 39$ Å , $c = 10\ 35$ Å , $\beta = 92°\ 14'$

The density calculated from the above cell dimensions assuming two molecules a cell of 1 19 gm /c c compares favourably with the density of 1 17 gm /c c determined by flotation methods

A grant from the Research Corporation for equipment has made this preliminary work possible and is gratefully acknowledged

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Alkali Metal Titanium Oxide Bronzes

THE crystallographic relationships between $K_2Ti_2O_5$, $Na_2Ti_3O_7$ and $Na_2Ti_6O_{13}$, the structures of which have been determined by us[1], led to the belief that oxide bronzes with host lattices of formula TiO_2 might be induced to form under reducing conditions

The reduction in hydrogen of coarsely crystalline $Na_2Ti_3O_7$ for two days at 950° C , followed by extraction with hot sulphuric and hydrofluoric acids, led

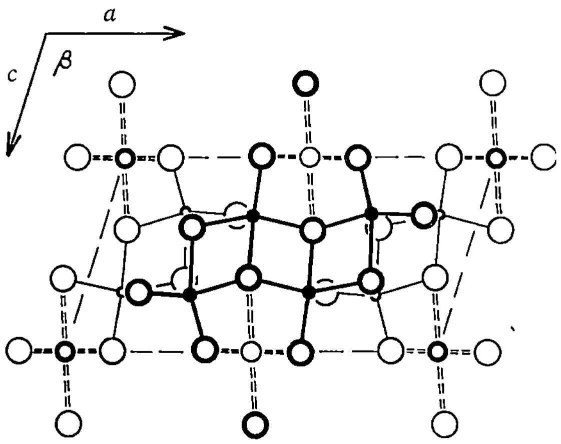

Fig 1 The structure of Na_xTiO_2 seen in projection on to (010) The smaller circles are titanium, the intermediate ones are sodium, and the largest oxygen The heavier outlined atoms are at the level $y = 0$, the lighter at $y = \frac{1}{2}$ The sodium–oxygen bonds are drawn as broken lines, and the unit cell is shown in outline

to the isolation of opaque, blue-black electrically conducting crystals with the empirical formula Na_xTiO_2 (x approximately equal to 0 2) The symmetry is monoclinic, and the unit cell dimensions are $a = 12\ 146$, $b = 3\ 862$, $c = 6\ 451$ Å., $\beta = 106\ 85°$, space group $C2/m$ The structure, deduced by trial and error and refined by Fourier methods as well as by a least squares treatment, is shown as a projection in Fig 1 The titanium atoms each have a distorted octahedral grouping with oxygen, and the octahedra themselves share edges to form a double sheet which, in Fig 1, is parallel to the a axis These sheets are joined together by corners and this results in a new structure of formula TiO_2 The Ti—O bonds vary between 1 7 and 2 2 Å with an average of 1 97 Å, and the oxygen–oxygen distances range from 2 4 to 3 2 Å, average 2 76 Å

This host lattice contains within it interstitial positions which are occupied, in part only—and at random—by sodium ions each of which has four bonds to oxygen of 2 5 Å and four longer ones 3 0 Å Ideally speaking these positions would be cubic, but the distortions introduced by the irregular Ti—O octahedra force the sodium to adopt a virtually rectangular planar configuration

In a discussion of the crystal chemistry of the vanadium oxides, the possibility of structural homologues with the general formula $Me_{2n}O_{5n-2}$ was pointed out[2], V_6O_{13} ($n = 3$) and V_2O_5 being the only members known at that time It is now interesting to note that the host lattice of this sodium titanium bronze, while having a different metal ion, nevertheless has the structure of the end member of the series, $n = 2$

Blue fibrous needles of K_xTiO_2 (x approximately 0 13) were formed by the reduction of $K_2Ti_2O_5$ under identical conditions The tetragonal unit cell dimensions were $a = 10\ 172$, $c = 2\ 846$ Å, the indices showing pseudo body centring None of the needles proved to be a single crystal, and a further detailed investigation by us is not contemplated The structure of this substance is undoubtedly related to that of the mineral hollandite which, however, is truly body-centred tetragonal or monoclinic[3] This structural type, previously considered to be capable of occurring as chemical compounds with bronze-like characteristics[4], is one containing continuous linear interstices, or tunnels, which enclose variable numbers of ions such as barium, potassium, or lead, all of which are considerably larger than sodium The host cation may be one or other of the quadrivalent elements manganese, as in hollandite itself, or titanium[5] The minor symmetry anomaly which alone distinguishes it from these published data possibly could involve order and disorder of the potassium ions in the tunnels

One other titanium bronze, La_xTiO_3 ($0\ 7 < x < 1\ 0$), has been recorded, and this is isomorphous with the cubic tungsten bronzes with the perovskite structure[6] Thus it bears little relationship to either of the two briefly described here

We are indebted to Mr E S Pilkington for the chemical analyses

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BIOCHEMISTRY

Formation of 2,3-Dihydroxybenzoic Acid in Fermentation Liquors during the Submerged Culture Production of Lysergic Acid α-Hydroxyethylamide by *Claviceps paspali* Stevens and Hall

A STRAIN of *Claviceps paspali* Stevens and Hall has previously been shown to be capable of producing lysergic acid α-hydroxyethylamide in submerged fermentation[1,2]. Examination of chromatograms (solvent n-butanol/acetic acid/water, 4 1 5) of culture fluids at the end of the fermentation period revealed the presence of a mobile (R_F 0 83) spot showing bright blue fluorescence, not giving the Ehrlich–Van Urk reaction for lysergic acid derivatives but giving a blue-violet colour with ferric chloride

The substance responsible for this spot could be extracted from the culture medium acidified with concentrated sulphuric acid with two volumes of ether and was obtained crystalline after transfer to water at pH 4, re-extraction with ether at pH 1, washing the ether extract repeatedly with water and evaporating to dryness It was recrystallized from water The yields were of the order of 200–350 mgm. from 1 litre of culture medium, showing that the substance is a metabolite of major importance in the lysergic acid fermentation by *Claviceps paspali* Stevens and Hall It gave a strong blue-violet colour with ferric chloride which turned red on alkalinization with sodium hydroxide It analysed for $C_7H_6O_4$ (C, 54 65, H, 3 92 per cent, calc C, 54 52, H, 4 01 per cent) and was identified as 2,3-dihydroxybenzoic acid by comparison with an authentic sample by chromatographic behaviour in three solvent systems as well as by its ultra-violet and infra-red spectrum, its melting point (200° C), mixed melting point (no depression) and its first dissociation constant (found $1 1 \times 10^{-3}$)

2,3-Dihydroxybenzoic acid is also formed from l-tryptophan by washed mycelium of *Claviceps paspali* Stevens and Hall in replacement cultures and clearly is one of its breakdown products in this micro-organism To our knowledge, 2,3-dihydroxybenzoic acid has not been identified as a product of tryptophan degradation in any other tryptophan derivative synthesizing micro-organism It has, however, been shown to be formed from salicylic acid in *Aspergillus niger*[3], and in the rat[4], and to occur in Nature in the buds of *Populus balsamifera*[5] and as a glucoside in the leaves of *Vinca minor*[6].

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Identification of an Amylobarbitone Metabolite in Human Urine

Amylobarbitone (5-ethyl-5-(3-methylbutyl) barbituric acid) fed to dogs in doses of 45 mgm. per kgm has been shown to produce 5-ethyl-5-(3-hydroxy-3-methylbutyl) barbituric acid as the main metabolite, about 67 per cent of the original dose being excreted in this form[1]

We have recently identified this metabolite in the urine of a woman who had died 30 hr after ingesting an unknown quantity of 'Tuinal' (equal parts of amylobarbitone and quinalbarbitone)

The metabolite was obtained by saturating the urine with ammonium sulphate, acidifying with hydrochloric acid, and continuously extracting with ether for 8 hr A 0 5 *N* ammonium hydroxide extract of the ether was acidified with hydrochloric acid, saturated with ammonium sulphate, and re-extracted with a large volume of ether The ether was evaporated, and the residue dissolved in a small volume of alcohol, which was spotted on Whatman No 1 chromatography paper The paper was developed in *n*-butanol/0 880 ammonia (3 1) for 4 hr

After exposing the paper to ammonia vapour, four absorbing spots were detected with R_F's 0 65, 0 18, 0 08 and 0 respectively using ultra-violet light (2537 Å). A permanent record was obtained by contact printing the ammonia-treated paper chromatogram using Ilford 50 reflex paper and the same ultra-violet source[2] (Fig 1)

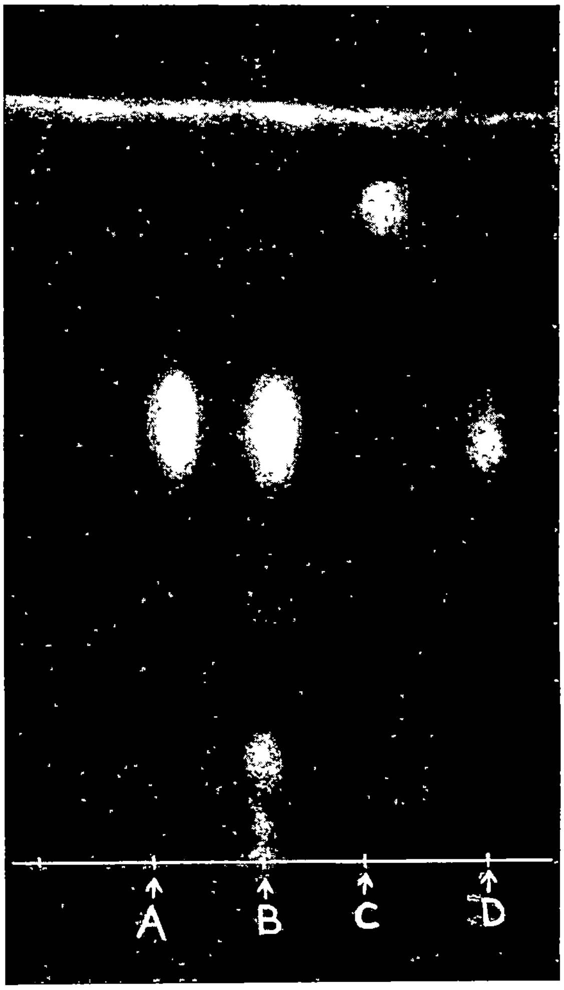

Fig 1 Chromatogram developed in butanol–ammonia *A*, 5-Ethyl-5-(3-hydroxy-3-methylbutyl) barbituric acid, *B*, total urine extract, *C*, amylobarbitone, *D*, eluted R_F 0 65 metabolite Ultra-violet transmission photograph after exposing chromatogram to ammonia vapour

The highest of the spots had the same R_F as a synthetic sample of 5-ethyl-5-(3-hydroxy-3-methylbutyl) barbituric acid run adjacently None of the spots corresponded with amylobarbitone The substances with R_F's 0 65 and 0 18 gave purple colours after spraying with cobalt chloride (1 per cent in acetone) and exposing to ammonia vapour[3] and were the only ones showing greatly increased absorption after ammonia vapour treatment, indicating an intact pyrimidine ring

The R_F 0 65 urine metabolite was eluted with alcohol acidified with hydrochloric acid, and the X-ray

Table 1 *d*-Values of 5-ethyl-5-(3-hydroxy-3-methylbutyl) Barbituric Acid

d-Value	Intensity	*d*-Value	Intensity
9 82	20	4 225	10
8 865	80	3 525	40
6 32	20	3 425	10
6 1	20	3 27	20
5 71	70	2 925	20
5 45	100	2 795	30
5 37	50	2 71	20
4 99	10	2 67	10
4 925	30	2 56	20
4 79	10	2 525	20
4 55	100	2 175	20
4 275	20		

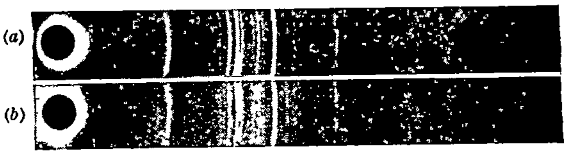

Fig 2 X-ray diffraction patterns of 5-ethyl-5-(3-hydroxy-3-methylbutyl) barbituric acid 2*a*, Synthetic, 2*b*, urine metabolite R_F 0 65

diffraction pattern compared with that of the synthetic material, using copper $K\alpha$ radiation Patterns were identical (Fig 2*a* and *b*) The *d*-values calculated from this pattern are given in Table 1

The nature and origin of the other absorbing spots are being investigated and will be reported later

It is of interest that no unchanged amylobarbitone or quinalbarbitone was detected in this urine

We are indebted to Eli Lilly and Co , Ltd , for a gift of the synthetic 3-hydroxy amylobarbitone

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Chromatography and Nucleotide Sequences of Deoxyribonucleic Acid synthesized in *Salmonella typhimurium* after Irradiation with Ultra-Violet

IT has been shown[1,2] that ultra-violet irradiation produces in some kinds of bacteria, for example, *Escherichia coli* B/r and *Salmonella typhimurium*, a temporary inhibition of deoxyribonucleic acid (DNA) synthesis The synthesis is resumed after 30–60 min and proceeds at a rate similar to the control, but the surviving bacteria are growing only in mass, having lost their ability to form colonies One of the possible explanations for such behaviour is that DNA synthesized after temporary inhibition is different from the normal one That this might be so is supported by Kanazir *et al* [3], who found that whereas DNA from non-irradiated *Sal typhimurium* could reduce the damage to an irradiated culture, that from an irradiated culture could not, and by Hudnik and Glisin[4], who showed that the thermal denaturation pattern followed by viscosity and ultra-violet absorption measurement was different in the two DNA's

Ord and Stocken[5] using the usual analytical procedures were unable to distinguish between DNA of thymus from normal and irradiated animals, but a difference could be established by chromatography on 'Ecteola' columns according to the method of Bendich *et al* [6] The same method was therefore used for characterization of DNA from normal and irradiated *Sal typhimurium*

The growth of bacteria and the irradiation conditions were the same as described in ref 3 At zero time one-half of the bacteria were irradiated with an LD_{50} dose of ultra-violet light delivered from Hanovia mercury vapour lamp and phosphorus-32 (1 μc /ml culture) added 120 min later At this time there is reason to suppose that the bacteria are synthesizing DNA at the same rate as their controls Phosphorus-32 was added to the control bacteria 90 min. from zero time and both irradiated and control samples were gathered 90 min after the addition of phosphorus-32 They were then washed and DNA isolated according to the method of Zamenhof[7] using 15 per cent 'Dupanol' for the lysis of the cells Ribonucleic acid (RNA) was removed by digestion with ribonuclease and ethoxyethanol precipitation as described by Kirby[8] The possible contamination with RNA was checked by measuring radioactivity in the supernatant after digestion of the DNA with alkali No radioactivity could be detected in this fraction The protein content was less than 0 5 per cent when tested according to Lowry[9]

Chromatography on 'Ecteola' was performed as described by Ord and Stocken[5] Two mixing chambers in series were used for gradient elution and the elution pattern followed by measurement of optical density at 260 mμ The control and irradiated samples of DNA were eluted in parallel from the common supply of solvent The fractions were pooled and analysed for radioactivity in a liquid Geiger counter and for DNA by the method of Ceriotti[10]

Nucleotide sequences were estimated according to the method of Burton and Petersen[11] on unlabelled DNA samples prepared in the same way as described here

If the amount of ultra-violet absorbing material eluted in the main fractions in the control and irradiated DNA are compared (Table 1) it can be seen that more is eluted in the irradiated DNA in fractions I*A*, *B* and III and less in fractions II and IV, as compared with the control Although small, the differences are consistent in both experiments

Table 1 CHROMATOGRAPHY ON 'ECTEOLA' OF DNA FROM NORMAL AND ULTRA-VIOLET IRRADIATED *Salmonella typhimurium*

Fraction	E_{260} as percentage of control Exp 1	 Exp 2
1 *A*	119	127
1 *B*	104	105
2	90	79
3	103	104
4	86	89

Fig 1 represents the specific activities of pooled sub-fractions plotted against the composition of the eluant It can be seen from the elution pattern that the radioactive fractions of the irradiated DNA start with the elution at a lower concentration of sodium chloride, and that the specific activities of the fractions eluted with sodium chloride gradient are higher in the irradiated DNA It is difficult to see why this is so since both control and irradiated bacteria synthesize practically the same amount of DNA in the same time It is possible that although the net synthesis is roughly the same in the two cases, the DNA formed in the irradiated bacteria is less stable[12] and the turnover correspondingly greater There are smaller differences in the subsequent fractions, and at the end of ammonia

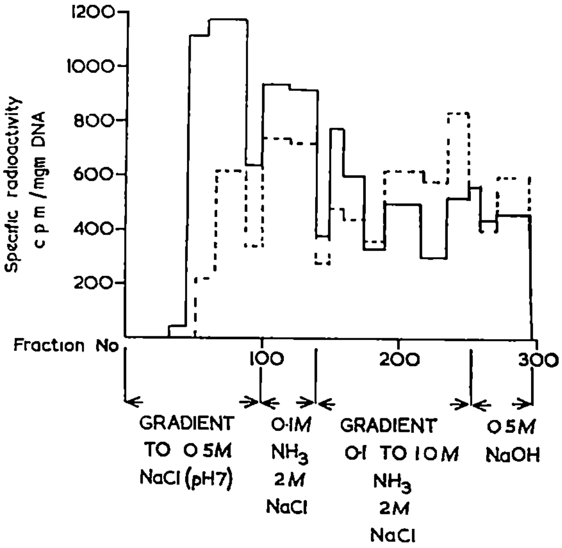

Fig 1 Specific activities of DNA fractions (*Salmonella typhimurium*) eluted from 'Ecteola' – –, Control, —, irradiated

gradient and in the sodium hydroxide sub-fractions the specific activity of the irradiated DNA is lower than that of the control

The results of the estimation of frequencies of some nucleotide sequences are summarized in Table 2 No difference was found in the frequency of the following sequences C, T, T_3p_2, T_4p_3, T_5p_4, CT_5p_5, T_3Cp_3 and C_2Tp_2 But some of the nucleotides containing more cytosine appear to be more frequent in the irradiated DNA while some nucleotides with more thymine seem to be more frequent in the control It will be noted that the TpC/CpT ratio is changed from 1 33 to 1 16 and this adds further support for the idea that irradiation has modified the structure of the DNA (cf Burton[13]) Relevant to this are the observations of Beukers and Berends[14], who found a dimerization of thymine which had been exposed to ultraviolet irradiation *in vitro*, and of Wacker *et al* [15], who noted a change in 2^{14}C-thymine of bacterial DNA irradiated *in vivo*

Table 2 NUCLEOTIDE SEQUENCES OF DNA FROM NORMAL AND IRRADIATED *Salmonella typhimurium*

Product*	Pyrimidine (moles/100 gm atoms of phosphorus) Control DNA	Irradiated DNA
T_2P	2 62 ± 0 06	2 4 ± 0 1
TpC	3 10 ± 0 09	2 98 ± 0 06
T_2Cp_2	2 32 ± 0 10	2 12 ± 0 07
T_4Cp_4	0 75 ± 0 02	0 52 ± 0 07
C_2p	3 54 ± 0 06	3 65 ± 0 07
C_2p_2	0 77 ± 0 04	0 94 ± 0 07
CpT	2 33 ± 0 02	2 57 ± 0 06
$C_2T_2p_3$	0 97 ± 0 04	1 17 ± 0 08
T	5 2 ± 0 06	5 2 ± 0 1
C	5 80 ± 0 02	5 8 ± 0 1
T_3p_2	0 90 ± 0 1	0 87 ± 0 07
T_4p_3	0 41 ± 0 04	0 39 ± 0 03
T_5p_4	0 20 ± 0 03	0 19 ± 0 02
T_3Cp_3	1 11 ± 0 07	1 11 ± 0 11
T_5Cp_5	0 40 ± 0 08	0 35 ± 0 02
C_2Tp_2	2 41 ± 0 06	2 48 ± 0 14

* Nomenclature according to Burton and Petersen (ref 11)

We are grateful to Dr J Lascelles and Dr R Tucker for help with the irradiation and growth of the organism and to Dr K Burton and Dr M. Lunt for advice on the determination of nucleotide sequences We wish to acknowledge financial support from the Department of Scientific and Industrial Research and the International Atomic Agency, and to one of us (T H -P), from the Institute of Nuclear Sciences, Belgrade

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Determination of Argininosuccinic Acid by Ion-Exchange Chromatography

RECENTLY, an unknown amino-acid excreted in the urine by two mentally defective children[1] was identified as argininosuccinic acid (ASA)[2] This is unstable in weakly acid aqueous solutions and cyclizes[3] More recently Westall[4] has shown that under these conditions two separate types of anhydride (*B* and *C*) are formed, rapidly at high but more slowly at room temperatures

For the original metabolic studies of ASA in the mentally defective children[5], the gradient elution fraction-collecting method of Moore and Stein[6] employing the cation exchange resin 'Dowex 50' of 4 per cent cross-linking was used Under these conditions ASA emerged cleanly from the column as two separate peaks which we now know corresponded to the two anhydride forms *B* and *C* The rate of chromatographic development of these columns is slow (6–8 ml /hr) and ASA is subjected to a *p*H of between 3 and 4 for a period of about 60–70 hr at 30–50° C This time is presumably long enough for the ASA to be completely converted to the two anhydride forms which emerged from the columns in the position on the flow sheet between the peaks of leucine and tyrosine

More recently we have attempted to measure ASA using an automatic self-recording amino-acid analyser[7] which has been built in this Department This method used the 'Amberlite' cation-exchange resin *CG*–120 of 8 per cent cross-linking and the columns are operated at a flow-rate of 30 ml /hr ASA is not eluted from the 150 cm column with the *p*H 3 25 buffer at 30°, but after changing to the *p*H 4 25 buffer at 55° a small amount of free ASA emerges after the passage of the new buffer front and this is followed by a slowly rising baseline and the emergence of several partially separated peaks immediately after the phenylalanine position Pure samples of either of the *B* and *C* anhydrides when treated similarly are stable and give each time a single peak at 5 and 45 ml , respectively, after the centre of the phenylalanine peak There is no sign of any reconversion to ASA and the baseline is unaltered We believe, therefore, that after only 10 hr at 30° with the *p*H 3 25 buffer the original ASA is not fully converted to the anhydride forms; conditions are then changed to *p*H 4 25 at 55°, but before a new equilibrium is attained a mixture of ASA and its anhydrides is eluted from the column

On the 50 cm column used for the estimation of basic amino-acids in biological fluids, operated at *p*H 4 19 and 30°, free ASA emerges at 25 ml and the anhydrides *B* and *C* at 50 and 60 ml respectively About 5 per cent of anhydride was found in the analysis of a sample of ASA on the 50 cm column, but this amount could well have been present in the original sample itself It is therefore possible to estimate ASA in pure solution on the 50 cm column but this is of little practical use since the presence of the acidic and neutral amino-acids, which are usually present in biological fluids, grossly interfere at the beginning of the run between 10 and 40 ml

In order to overcome these difficulties we have made use of the fact that ASA in solution can be readily converted to the anhydride forms by standing for 48 hr at *p*H 2 and the anhydrides are stable providing the *p*H does not rise above 5 Provided that the cyclization is complete then the formed anhydrides can be determined on the 50 cm column where they emerge conveniently between the large peak of combined acidic and neutral amino-acids and the peak given by phenylalanine and tyrosine at about 80 ml In a test of the experimental conditions by analysis carried out on the 50 cm column, 1 μmole of a sample of ASA gave, after standing at *p*H 2 for 24 hr at 20°, 0 04 μmole of ASA, 0 04 μmole of *B* and 0·69 μmole of *C* After increasing the time of standing to 48 hr the values were 0 01 μmole of ASA, 0 04 μmole of *B* and 0 71 μmole of *C*

Provisionally we are applying a correction factor to compensate for the 75 per cent recovery of ASA as the anhydrides

In practice, samples of urine and deproteinized plasma are brought to *p*H 2 by the dropwise addition of 2 *N* hydrochloric acid and are allowed to stand at room temperature for 48 hr prior to analysis.

Miles Laboratories Inc and the Ames Co , Ltd , provided a generous grant for the construction of the automatic amino-acid analyser which was built in the Medical Unit by Mr C. E Glover and Mr. G Marshall

One of us (D C C) is indebted to the Drummond Trust for a senior fellowship

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Chromatographical Separation of Carbohydrate O-Isopropylidene Derivatives

DURING investigations on fluorocarbohydrates, it became necessary to examine the purity of small amounts of *O*-isopropylidene derivatives Hitherto, for such compounds in the carbohydrate series, there has been no reliable chromatographical method of separation from fully protected or nearly related hydroxylated compounds We have found, however, that separations can be achieved rapidly and easily by reversed-phase, ascending chromatography on cellulose acetate strips (20 × 4 5 cm , as used for electrophoresis and obtainable from Oxo, Ltd , Queen Street Place, London, E C 4)

Strips were washed thoroughly in methanol and dried in air Compounds (50–800 μgm in ethanol) were applied as spots to the washed strip which were then developed by ascending chromatography with a mixture of methanol and water (6/4 v/v). The separation was achieved in 2 hr at 2–5° C

After drying for 20–25 min in air at room temperature, compounds can be detected in two ways

(*a*) Developed chromatograms are immersed in a solution[1] of 2,4-dinitrophenylhydrazine (0 4 per cent w/v) in 2 *N* hydrochloric acid Carbonyl, *O*-isopropylidene and *O*-benzylidene compounds appear as deep yellow zones which are further intensified when the chromatogram is washed briefly in aqueous sodium hydroxide (10 per cent w/v)

(*b*) Alternatively, the developed chromatograms are floated on the surface of a solution[2] (1 per cent w/v) of potassium permanganate in 1 *N* sulphuric acid for 2 min and immersed for a further minute The chromatogram is then washed in running water, when the components appear as sharply defined purple-brown spots, which turn brown on standing This is sensitive to about 150 μgm of such compounds as 1,2 5,6-di-*O*-isopropylidene-D-glucose

Table 1

Substance	*R_F*	No. of free hydroxyl groups
1,2 4,5-di-*O*-isopropylidene-3-mesyl-D-fructose	0 15	0
1,2-*O*-benzylideneglycerol	0 23	1
Methyl *O*-isopropylidene-DL-glycerate	0 28	0
1,2 3,4-di-*O*-isopropylidene-6-tosyl-D-galactose	0 32	0
Methyl 2,3-*O*-isopropylidene-αβ-D-ribofuranoside	0 48	1
1,2 5,6-di-*O*-isopropylidene-D-glucose	0 52	1
1,2 3,4-di-*O*-isopropylidene-D-galactose	0 55	1
1,2-*O*-isopropylideneglycerol	0 65	1
Methyl DL-glycerate	0 66	2
(±)-butan-1,3-diol	0 73	2
1,2-*O*-isopropylidene-D-glucofuranose	0 80	3
2,3,4,6-tetra-*O*-methyl-D-glucose	0 90	1
Methyl 2,3,6-tri-*O*-methyl-αβ-D-glucoside	0 98	2
3-*O*-methyl-D-glucose	0 98	4

As seen from the list of R_F values in Table 1, the method in general can be used to distinguish fully protected sugars from those with one or more free hydroxyl groups In general, unsubstituted sugars move at the solvent front

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Isolation of Histones(?) from *Staphylococcus aureus*

ANY theories that try to give an explanation of the function of histones have had in the past to take account of the reported absence of histones from bacteria[1,2] If histones are solely concerned with cell differentiation or 'gene suppression' in multicellular organisms, then one might expect them to be absent from bacteria and protozoa[3,4] But if, on the other hand, they have an even more fundamental function, related to the function of deoxyribonucleic acid (DNA) in all cells, then they should also be present in unicellular organisms

In the present investigation an attempt was made to extract basic proteins from bacteria, following a modification of the procedure routinely used in our laboratory for the isolation of histones from cell nuclei[5]

The bacteria, *Staphylococcus aureus*, were washed with 4 per cent acetic acid, and then defatted and dried by washing several times with ethanol and then with ether This procedure denatures many of the non-basic proteins, rendering them insoluble on extraction with acid The acid-soluble proteins were extracted by grinding the dried bacteria with glass powder in the presence of 0 1 *N* sulphuric acid, and then centrifuging Large amounts of non-protein material extracted were removed by dialysis against 0 1 *N* sulphuric acid, and the proteins were precipitated from the dialysed extract by the addition of seven volumes of ethanol, and dried with ethanol followed by ether

Some of the extracted material was subjected to starch-gel electrophoresis in the presence of 4 *M* urea

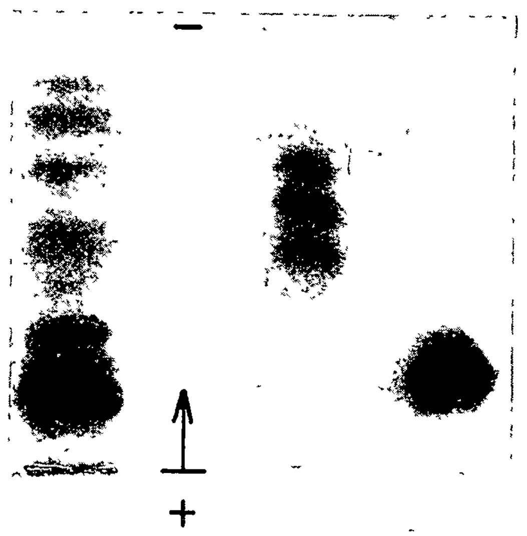

Fig 1 Starch-gel electrophoresis of proteins extracted from *Staphylococcus aureus* with 0 1 *N* sulphuric acid (*a*) total protein, (*b*) basic protein fraction, (*c*) non-basic fraction

using a modification of the technique developed by Neelin and Neelin for use with histones[6] The gel, rolled 1 mm thick on a glass plate and coated afterwards with paraffin wax, was prepared with 0 014 *M* acetate buffer containing the urea, giving a *p*H within the gel of 6 After migration, the gel was stained with amido black 10*B* and showed the presence of at least eight components (Fig. 1). The direction of migration of the slower components is reversed if the *p*H of the gel is increased above 6 5

Those components which were strongly basic, like histones, with isoelectric points well above *p*H 7 were separated from the others by using carboxymethyl-cellulose The protein mixture, dissolved in bicarbonate buffer *p*H 7, was applied to a very short carboxymethyl-cellulose column previously washed with buffer Under these conditions the non-basic components run through the column while the more basic components are retained The column was washed with water, and then the basic proteins eluted with about 1 ml of 0 1 *N* hydrochloric acid

Starch-gel electrophoresis of these two fractions, recovered from the eluates by ethanol precipitation, shows the complete separation of the faster components from the remainder, which, although extracted by acid, do not have isoelectric points above *p*H 7 (Fig 1)

Amino-acid analysis of both fractions, by acid hydrolysis followed by paper chromatography (two successive runs in one direction using an *n*-butanol, acetic acid, water system and detection of the spots by ninhydrin reagent), indicated that the faster, more basic fraction was very similar to unfractionated ox-thymus histone[7] It contained particularly large amounts of lysine, arginine, alanine, leucine and proline with relatively small quantities of acidic amino-acids, histidine and tyrosine, while the slower, less basic fraction was quite different It is concluded from the above results, from ultra-violet absorption and from solubility characteristics that the proteins in the faster electrophoretic fraction are very similar indeed to histones

Thus it is clear that these bacteria contain basic proteins like the histones found in the higher forms of life Comparable results have been obtained with *Micrococcus lysodeikticus* These basic proteins may also occur in the DNA-protein fraction previously isolated by Vendrely *et al* from *Escherichia coli*[8].

It is not yet possible to say whether all the basic protein components demonstrated here have come from association with the DNA or the ribonucleic acid of the bacteria But somewhat similar components have recently been shown to occur in ribosomes prepared from *Escherichia coli*[9].

We wish to thank Dr J F Wilkinson for providing us with liberal supplies of bacteria

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Detection and Distribution of Enzymes for oxidizing Thiocysteamine

The intermediate role of the persulphide analogues (R—SSH) of cysteine and cysteamine in the biological conversion of cystine and cystamine into the corresponding sulphinates and thiosulphonates has been suggested in ref 1. This view has been emphasized by the detection of an enzyme, or enzyme system, able to convert thiocysteamine (the persulphide analogue of cysteamine NH_2—CH_2—CH_2—SSH) into thiotaurine (R—SO_2SH) and hypotaurine (R—SO_2H) in the presence of air[2] Careful study of this reaction merits attention since it may throw light on the first steps of the metabolic pathway of cystine and cystamine.

In our earlier work[2] thiotaurine and hypotaurine were detected in the incubation mixtures by using labelled substrate and by submitting the final deproteinizate to chromato-radioautography or chromato-scanning. Although providing good results, these methods are time-consuming, expensive, and cannot be used as routine procedures for the isolation of the enzyme. Thus we have sought simpler methods for obtaining activity values Since thiotaurine is the main product of the enzymic oxidation of thiocysteamine, methods have been tried for determining this compound. The cyanolysis method[3] and ion-exchange chromatography[4,5] seemed simple enough and were adapted to the determination of thiotaurine in our incubates The results obtained with these two methods have been compared with those obtained by scanning radiochromatograms

The enzyme was extracted from pig kidney by fractionation between 20 and 60 per cent ammonium sulphate[2] Thiocysteamine was produced in the incubates by adding cysteamine hydrochloride and elementary sulphur[2]. The experimental conditions are reported in Tables 1 and 2 The distribution of the enzyme has been investigated by comparing the amount of thiotaurine produced under standard con-

Table 1. TIME-COURSE OF THE ENZYMIC CONVERSION OF THIOCYSTEAMINE INTO THIOTAURINE, AS DETERMINED BY THREE DIFFERENT METHODS

Enzyme: 32 mgm. of proteins of 20–60 per cent ammonium sulphate saturation fraction of pig kidney homogenate dissolved in 2 ml. water. Thiocysteamine: 50 μmoles containing 8 μc. of sulphur-35. Phosphate buffer pH 7·4: 0·6 ml., 0·1 M. Final volume, 3 ml.; Temperature, 38°. Values reported in μmoles of thiotaurine produced

Time of incubation (hr.)	Cyanolysis	Ion-exchange chromatography	Chromato-radio scanning
2	2·3	1·9	2·3
4	2·5	2·1	2·8
6	2·9	3·3	3·0

Table 2. DISTRIBUTION OF THIOCYSTEAMINE OXIDIZING ENYZME

Values in μmoles of thiotaurine produced after 2 hr. under standard conditions by using the cyanolysis procedure. Conditions were the same as described in Table 1, with the exception that the enzyme was substituted with 2 ml. of a homogenate of 1 part of the organ with 5 parts of phosphate buffer, and that thiocysteamine was not labelled

	Kidney	Liver	Muscle	Heart
Rat	1·35	1·72	0·91	0·85
Rabbit	1·23	0·50	0·23	0·25
Pig	1·76	2·56	—	0·48
Cattle	2·07	0·90	0·50	0·88
Horse	4·88	1·21	0·71	0·90

ditions by the same amount of homogenate of various organs. Details are reported in ref. 2.

The cyanolysis technique was that described by Sorbo[3]. Various controls have been performed and it was found that interfering substances were removed by the addition of 'Dowex 50'. The cyanolysis determination was performed as follows. The final incubation mixture (3 ml.) was deproteinized with 0·3 ml. 100 per cent trichloracetic acid and added to 0·3 gm. of 'Dowex 50 ×4', 200–400 mesh, in the H$^+$ form. After shaking and centrifuging, an aliquot of the supernatant was added with 2 ml. 1 N ammonia and 1·5 ml. of 0·1 M potassium cyanide. After 30 min. in a boiling water-bath, the solution was cooled, added to 1 ml. ferric nitrate reagent[3] and read at 470 mμ.

Ion-exchange determination of thiotaurine was performed on the trichloracetic filtrate, without the addition of the resin, following the procedure of Stein[5]. The eluant was 0·1 M citric acid in 0·2 M sodium chloride at 37°. By using this solution, thiotaurine is eluted together with taurine, far from other disturbing amino-acids[4]. Since our enzyme preparation was free from taurine, the peak which developed with ninhydrin in the area of thiotaurine was calculated as pure thiotaurine.

The procedure for scanning uni-dimensional radiochromatograms has been described in ref. 2. We have observed that part of the radioactivity is recovered in the trichloracetic precipitate at the end of the incubation. This is probably due to exchange of thiocysteamine with thiols and disulphides of proteins[6]. For that reason values for thiotaurine have been calculated by comparing the radioactivity of the area occupied by thiotaurine in the chromatograms with the radioactivity of a known amount of the substrate. In our earlier papers radioactivity of thiotaurine was compared with the radioactivity distributed in the whole chromatogram. The yield of thiotaurine, by using the present procedure, is consequently lower than that reported in ref. 2 and is more accurate.

As shown in Table 1, the procedure for cyanolysis gives results which are comparable with those obtained by ion-exchange and by chromato-radioscanning. This finding permits the use of the very simple procedure of cyanolysis for the detection of the enzyme. Table 2 shows the first results of the study of the distribution of the enzyme in various organs and animals. These preliminary results suggest a wide distribution of the enzyme and a general importance of the reaction. Moreover, they indicate that the horse kidney is the best source for the extraction of the enzyme.

This work has been aided by a grant of the Consiglio Nazionale delle Ricerche.

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Presence of Haptoglobin in Synovial Fluid

ELECTROPHORETICALLY classified α- and β-glycoproteins have been shown to be normal constituents of synovial fluid[1]. Evidence based on immunoelectrophoresis and Cohn fractionation of human traumatic effusions also suggests the presence of most of the serum proteins in synovial fluid[2,3]. Individual biologically active serum proteins have also been found, namely, some enzymes[4], siderophilin[3], cærulo-plasmin[3], and the thyroxine-binding protein[5]. The accepted blood-coagulating factors, however, were found to be absent from normal bovine fluid[6].

In an experiment using starch-gel electrophoresis, the patterns obtained with bovine serum and synovial fluid were found to be identical. Electrophoretic zones obtained for both were albumin, α-globulin, 4β-globulins, a slow α-globulin and γ-globulin[7]. Since the hæmoglobin-binding protein, haptoglobin, migrates electrophoretically as an α-globulin, it seemed likely that this glycoprotein might be a normal constituent of bovine synovial fluid. Its presence in the normal fluid was further suggested by its relatively low molecular weight (85,000).

Since haptoglobin complexes hæmoglobin[8], its presence is readily ascertained by determining the electrophoretic migration of the haptoglobin–hæmoglobin complex. Thus, at a pH of 7·0–7·5 the complex migrates anodically while free hæmoglobin is immobile. The amount of haptoglobin may then be expressed in terms of the hæmoglobin-binding capacity.

In the present work the electrophoretic procedure was that described in detail by Zak *et al.*[9] using a dilute agar medium. A 0·25 per cent solution of agar (certified pure) in 0·05μ veronal–citrate buffer of pH 7·3 (10·3 gm. sodium barbital and 2·1 gm. citric acid per litre) was transferred to an 8·3 cm. × 10·2 cm. glass basin containing a sheet of parchment paper (No. 72 vegetable parchment powder paper, Upjohn Co.). After electrophoresis for 45–50 min. at 250 V. and 40–60 m.amp., the agar plate was dried under an infra-red lamp. The dried parchment paper was stained with a 1 : 1 mixture of benzidine (saturated solution in glacial acetic acid) and hydrogen peroxide (3 per cent solution). The hæmoglobin-containing bands gave a typical blue reaction.

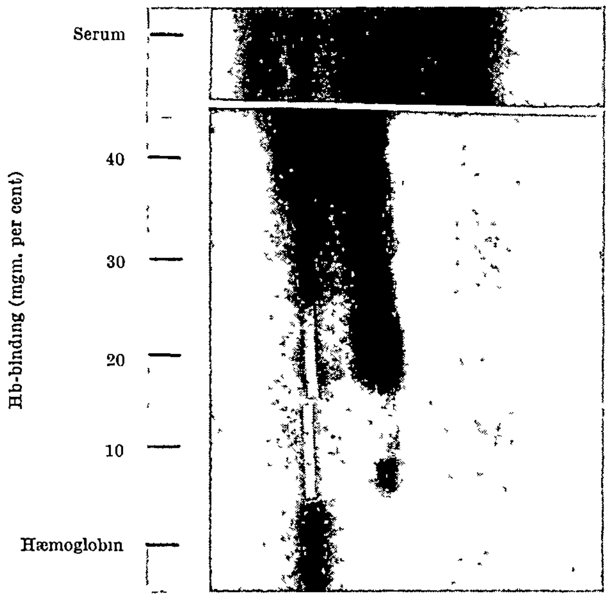

Fig. 1. Electrophoretic determination of hæmoglobin-binding capacity in human serum

Graded series of bovine sera and concentrated synovial fluids were prepared so as to contain 5, 10, 20, 30 and 40 mgm. per cent of hæmoglobin. For these a standard solution (5 gm. per cent) was previously diluted to contain 50, 100, 200, 300 and 400 mgm. per cent. Then 0·01 ml. aliquots of the diluted standard solutions were mixed with 0·1 ml. portions of serum or concentrated (1 : 10) synovial fluid. Bovine synovial fluids (5 ml.) were first incubated with hyaluronidase prior to concentration by lyophilization. Small strips (0·5 mm. × 8 mm.) of Whatman No. 3 *MM* filter paper were soaked in the samples and then were placed on the agar gel approximately 1·5 in. from the cathodic end.

In a preliminary experiment, human serum was used (Fig. 1) to establish the applicability of this electrophoretic technique to the determination of haptoglobin. Fig. 2 demonstrates the presence of haptoglobin in bovine synovial fluid. Free hæmoglobin remained at the origin while the haptoglobin-bound hæmoglobin migrated between the α- and β-fractions. The average hæmoglobin-binding capacity of seven samples of concentrated synovial fluid was approximately 10 mgm. per cent. When based on the total protein content of synovial fluid (average 1 gm. per cent) the binding-capacity was 1 mgm. of hæmoglobin per gm. of total protein. Bovine serum gave a range of 10–20 mgm. per cent. However, it was impossible to collect bovine serum from the slaughter-house without some degree of hæmolysis. Determinations of the content of hæmoglobin showed the presence of 5–10 mgm. per cent in the serum. Therefore, the actual hæmoglobin-binding capacity for serum averaged 20 mgm. per cent for 7 animals or 2·5 mgm. of hæmoglobin per gm. of total protein.

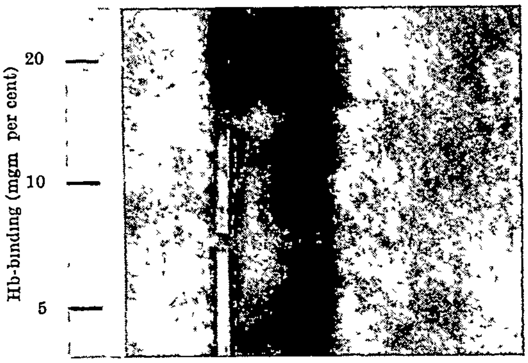

Fig. 2. Electrophoretic demonstration of haptoglobin in normal bovine synovial fluid

On the basis of the preceding results, it is apparent that the glycoprotein, haptoglobin, is a normal constituent of bovine synovial fluid. The hæmoglobin-binding capacity of synovial fluid, expressed in mgm. hæmoglobin/gm. total protein, is approximately 40 per cent of the binding capacity of the serum.

This work was supported by grants from the U.S. Public Health Service (*A*-532) and the Michigan Chapter, Arthritis and Rheumatism Foundation.

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PHYSIOLOGY

Hormonal Control of Oxidized Flavour in Milk

IN two recent papers the importance of natural copper in producing oxidized flavour in milk has been stressed[1,2]. The pronounced effect of adding copper salts to induce oxidized flavour, as well as the inhibiting effect of a great many compounds with copper-binding abilities, has been recognized for a long time. Contamination with copper occurs easily in metal equipment with which the milk has been in intimate contact[3]. Contribution from equipment was considered to be the main origin of copper in milk, and thus the importance of natural copper for development of oxidized flavour may have been overlooked. High copper values are found in carefully sampled milk[1,4], and particularly high values in milk from the early lactation period[1]. Elevated content of natural copper in this period seems to indicate a hormonal regulation of copper metabolism. The copper-catalysed oxidation in milk may be enhanced or depressed, and factors of this kind will combine to give the final result. The addition of copper sulphate to milk is a method used to evaluate these factors, or the so-called susceptibility, and is of practical importance. The relative effect of natural copper contents is lessened. Indications of hormonal influence on oxidized flavour in milk are reported here.

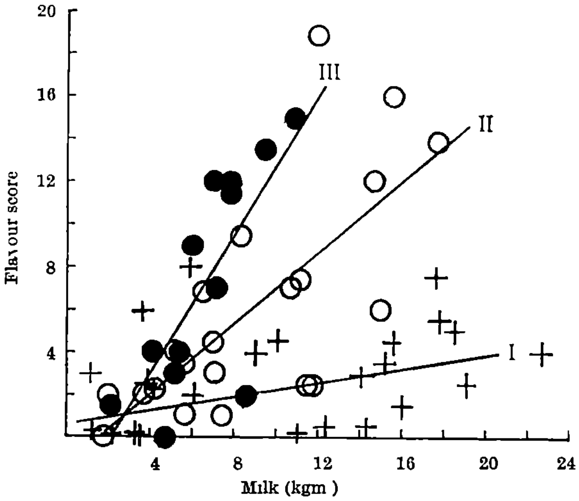

Fig. 1. Flavour score versus milk yield. Groups I, II and III are cows with 4 or more, 2 and 3, and 1 gestations, respectively. Coefficients of correlation are 0·34, 0·70 and 0·74. Scores of group I are significantly lower than those of groups II and III

The results are obtained by scoring milk from the College farm herd, sampled from each cow during milking. The samples were left overnight in the cold store to be judged next day by a single taster. Oxidized flavour scores were magnified by adding copper sulphate to the milk samples. Thus, sufficient grading was attained, and this made statistical treatment possible. Good correlations were obtained for flavour score versus milk yield on the same day, especially when the cows of the herd were divided into groups due to the number of gestations they had had. The interrelations given in Fig. 1 for a scoring in September are retained for all parts of the year and lactation period. Very recent freshened cows may, however, disturb the regression, particularly cows in their first lactation period. The regression coefficient in the latter case has a negative value in the first part of the lactation period.

The liability of a certain farm to produce milk with oxidized flavour must then, besides frequency of recent calvings, also depend on age-distribution of the animals. The correlation coefficient between the sum of monthly flavour scorings of untreated milk last year and the average age of animals in 23 herds in the district was found to be − 0·52, with regression coefficient significantly deviating from zero. Milk sampled from farms was drawn at point of delivery.

When a group of young cows was followed through a 5-wk period, 8 cows showed heat. The average score throughout the 3-wk sex cycle could then be calculated and plotted. The curve showed its summit at, or near, the day of heat (Fig. 2).

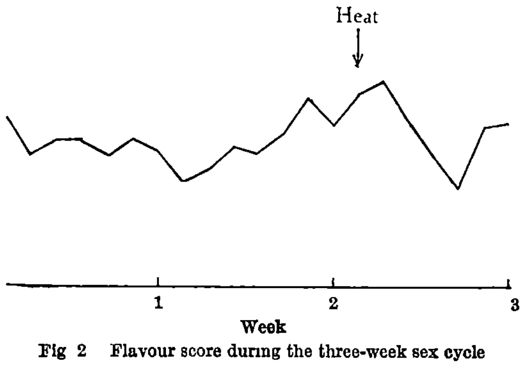

Fig. 2. Flavour score during the three-week sex cycle

Not only natural copper but also pro- and antioxidants may contribute to the noted differentiation in Fig. 1 so far as they are controlled by the same mechanism.

Physical state, like size of fat globules, organizing and complexing compounds (important for excretion of copper in milk), or tocopherols, are factors likely to interfere with oxidation and may be factors influenced by hormonal activity.

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Thyroid Activity at Higher Ambient Temperatures

THYROID activity is increased in animals exposed to cold with release of thyroid hormone[1] and both the output from, and the uptake of, iodine-131 by the thyroid is augmented[2,3], but the blood-level of protein-bound iodine[4] and the epinephrine-potentiating effect of thyroxine[5] are decreased. Work on the evolution of thermoregulation reveals that birds and baby mammals suffer from defective thermoregulation[6,7] and there is increased activity of the adrenergic system on exposure to high ambient temperature[8,9]. In the present investigations, thyroidal activity of birds and baby rabbits has been investigated after exposing them to high ambient temperature.

Baby rabbits (weighing about 300 gm.) and pigeons (weighing about 275 gm.), maintained at an average temperature of 32° C., served as controls, and they were exposed to 45° C. and 48° C. respectively in constant-temperature chambers for 2·5 hr., the standard error of temperature variation being within ± 0·03° C. Protein-bound iodine (PBI) level of serum was estimated[10] and a histological study of the thyroid was carried out. After exposure to heat, the PBI-level increased in blood in both groups. Histological work on the thyroid showed an increase in acinar cell height (140 per cent) with an overall release of the acinar colloid in pigeons exposed to heat, while in the exposed baby rabbits there is no significant increase in acinar cell height, although there is a slight release of the colloid (Table 1).

On exposure to cold there is an excessive release of thyroid hormone, but the serum PBI-level falls due to greater utilization of the hormone in the cold[11,12]. In the present work, the high concentration of serum PBI, associated with histological evidence of release of thyroid hormone, might indicate a decrease in peripheral utilization of the latter, although this point has not yet been verified experimentally. Thyroid acinar cell height in pigeons exposed to heat is 140 per cent of the control, while in the baby rabbits there is no difference, indicating increased thyrotrophic hormone secretion by the anterior pituitary in pigeons, probably as a result of the stimulation of hypothalamus. In the baby rabbits a small depletion of thyroid colloid with unaffected acinar cell height indicates little release of thyrotrophic hormone. This difference in behaviour might be due to the fact

Table 1 NORMAL PIGEONS AND BABY RABBITS EXPOSED TO WARM ENVIRONMENT

Animals, with number in parentheses		Ambient temperature	Serum PBI in μgm per 100 ml ± standard deviation	Increase (per cent)	Thyroid acinar cell height in μ ± standard deviation	Increase (per cent)	Colloid
Pigeon	(15)	32° C	4 37 ± 1 02	143	3 31 ± 0 42	140	Overall release
Pigeon	(15)	48° C	6 23 ± 0 90		4 62 ± 0 63		
Rabbit	(12)	32° C	6 32 ± 1 1	24	4 40 ± 0 60	101*	Release, but comparatively less
Rabbit	(12)	45° C	7 84 ± 1 0		4 46 ± 0 51		

* Not statistically significant.

that these baby rabbits have passed the stenothermic phase of thermoregulation and the small release of thyroid hormone is probably a direct temperature effect unlike that in pigeons[9] The cause of the increased serum PBI concentration and its physiological effect on tissue respiration are being investigated

We thank M S Das for his interest and advice

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Effect of Quinones on the Uptake of Oxygen by Red Cells

IT is well recognized that many oxidizing agents will convert hæmoglobin to methæmoglobin Among these are the quinones[1] Vitamin K_3, however, could have an additional effect through its role in electron transport[2], although it is difficult to envisage a complete electron chain since mature red cells possess no cytochromes

In this work human erythrocytes were separated from plasma and the other formed blood elements and incubated in a Warburg manometric apparatus in the usual way[3]. Vitamin K_3 (0 2 mgm /flask), 1 4-naphthoquinone (0 2 mgm /flask), which has a similar structure but no known physiological role, and dicoumarol (0 3 mgm /flask), an analogue of vitamin K_3 and also a potent inhibitor of vitamin K_3 reductase[2], were added from the side-arm at the beginning of the experiment as indicated

Red cells have a predominantly glycolytic metabolism The small amount of oxygen uptake (Fig 1) is probably accounted for by the auto-oxidation of hæmoglobin to methæmoglobin Normally the latter so formed is reduced by reduced diphosphopyridine nucleotide (DPNH) derived from the oxidation of 3-phosphoglyceraldehyde, the reaction being catalysed by methæmoglobin reductase As a result of this reduction mechanism only 1–2 per cent of the total hæmoglobin is normally present as methæmoglobin. Following the addition of vitamin K_3 or 1 4-naphthoquinone there was a rapid oxidation of hæmoglobin to methæmoglobin within 2 min and a corresponding oxygen uptake Thereafter the rate of oxygen utilization fell but was still greater than in the absence of the quinones. After 3 hr incubation 45 per cent of the hæmoglobin was present as methæmoglobin compared with 1·7 per cent in the absence of quinones Dicoumarol has no effect on oxygen uptake nor does it lead to the formation of methæmoglobin

In the absence of glucose the rate of uptake of oxygen fell after 1 hr and the level of methæmoglobin in the presence of vitamin K_3 or 1 : 4-naphthoquinone was 58 per cent after 3 hr This may be attributed to the gradual depletion of endogenous glucose, which makes available a supply of DPNH and TPNH for methæmoglobin reduction Similar results were obtained using lysed red cells Under these conditions uptake of oxygen is much reduced, possibly due to configurational changes in the proteins or co-factor dilution

Elsewhere[3] we have shown that compounds which lead to the production of methæmoglobin in the cell stimulate both uptake of oxygen and the amount of glucose oxidized via the pentose pathway with the production of TPNH, which then reduces methæmoglobin There is at present some controversy as to whether DPNH and TPNH are linked to the same or different enzymes

Since vitamin K_3 and 1 4-naphthoquinone had identical effects on oxygen uptake and led to the formation of excess methæmoglobin in the cells while dicoumarol did not depress oxygen uptake, it would

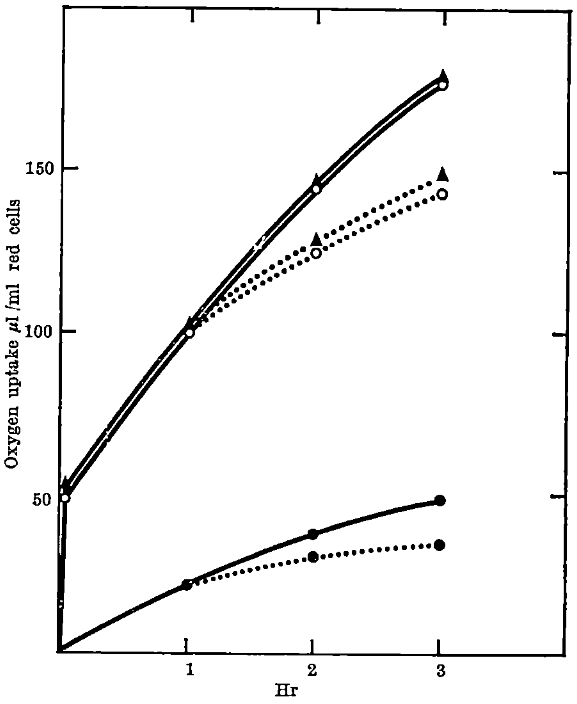

Fig 1 Effect of quinones in presence and absence of glucose on oxygen uptake of 1 ml of packed red cells in isotonic buffer *p*H 7 4 at 37°
●—●, red cells + glucose only , ● · ●, red cells without glucose added , ○—○, red cells + 1 4-naphthoquinone + glucose , ○ · ○, red cells + 1 4-naphthoquinone without glucose added , ▲—▲, red cells + vitamin K_3 + glucose , ▲ ▲, red cells + vitamin K_3 without glucose added

seem that the effect of vitamin K_3 in stimulating oxygen uptake by the red cells is a result of the oxidation of hæmoglobin.

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Plasma Albumin Catabolism in Experimental Nephrosis

GLOMERULAR filtration and tubular re-absorption of plasma albumin occur as part of the normal function of the mammalian kidney[1] The fate of the re-absorbed protein is less certain Oliver *et al* [2] have presented evidence that enzyme systems capable of catabolizing certain of the plasma proteins exist in the renal tubule cell, suggesting that re-absorbed protein is broken down in transit through the cell However, we have demonstrated that in the normal rat[3] and dog[4], bilateral nephrectomy depresses the breakdown of plasma albumin to only a limited extent The amount of plasma albumin contained in the renal tubules of rats with experimental nephrosis is much greater than normal, and Goodman and Baxter[5] maintain that this is due to enhanced glomerular filtration and subsequent tubular re-absorption of greater than normal quantities of plasma protein. We have reasoned that if the kidney has any important role in plasma albumin catabolism it should be demonstrable under these conditions of exaggerated albumin reabsorption

Rats were made nephrotic by 5–10 daily subcutaneous injections of the aminonucleoside of puromycin[6]. Such rats had plasma albumin concentrations of 8–15 mgm /ml., and they excreted 100–250 mgm of albumin daily in their urine With 8–10 injections most rats had lipæmic sera, œdema, and ascites With 5–7 injections the serum was often clear, there was less œdema, and only occasional ascites. Animals were used within 15 days of the start of injections Rat plasma albumin was iodinated with iodine monochloride labelled with iodine-131 (ref 7) and 'screened', according to McFarlane[8], for 2–4 days before use All rats received 0 3–0 8 μc. of screened iodoalbumin intravenously immediately after nephrectomy or sham nephrectomy Urine was collected quantitatively when available After 8 or 24 hr rats were killed and ground in a meat grinder, and the non-protein radioactivity extracted from the ground rat tissue with trichloroacetic acid The methods used have been described previously[3]

The results are summarized in Table 1 Normal rats broke down 24 per cent of the injected iodo-albumin in 24 hr Following nephrectomy the rate of breakdown in the non-nephrotic rats averaged 20 per cent This is a significant change, and carries a P value of 0 01–0 005 In previous experiments with screened iodo-albumin[3] we found depression in breakdown following nephrectomy of 0–15 per cent, and experiments with perfused kidney[3] indicated that up to 15 per cent of the albumin turnover in the body may occur in kidneys.

Table 1 EFFECT OF BILATERAL NEPHRECTOMY ON IODOALBUMIN BREAKDOWN IN NORMAL AND NEPHROTIC RATS

Duration of exp (hr.)	Condition of animal	Bilateral nephrectomy	No of rats	Iodoalbumin breakdown Injected radioactivity (per cent) Range	Average
24	Nephrotic	–	22	16–29	23
		+	21	4–15	11
24	Normal	–	16	20–28	24
		+	12	16–26	20
8	Nephrotic	–	13	8–15	12 5
		+	13	2 5–9 0	4 6

Nephrotic rats broke down 23 per cent of the injected iodoalbumin in 24 hr and about 14 per cent in 8 hr On removal of the kidneys breakdown was markedly reduced, averaging 11 and 5 per cent in 24 and 8 hr respectively In nephrotic rats the turnover of plasma albumin is very rapid (estimated half-life 4–12 hr) This is due primarily to the urinary excretion of albumin, which in a day amounts to several times that of the circulating plasma albumin 30–80 per cent of the injected radioactive iodoalbumin was excreted as such in the urine within 24 hr

It should be stressed that the breakdown of radioactive iodoalbumin recorded in Table 1 cannot be directly equated with the rates of breakdown of the total exchangeable plasma albumin In rats without proteinuria the breakdown of iodoalbumin reflects primarily the catabolism of plasma albumin, and during the period of these experiments iodoalbumin breakdown is approximately proportional to the rate of albumin catabolism On the other hand, in animals with extensive proteinuria, labelled iodoalbumin is removed very rapidly from the circulation and the specific activity of circulating albumin drops to within 10–20 per cent of the initial value within 24 hr. In the experiments described here, the nephrotic rats retained from less than 20 to 50 per cent of the injected radioactivity in the exchangeable albumin pool On the other hand, in animals without proteinuria (including nephrectomized nephrotics) 70–80 per cent of the injected radioactivity was retained. Thus the effect of removal of kidney on the rate of plasma albumin breakdown in the nephrotic rat cannot be readily estimated in quantitative terms from our results. To evaluate quantitatively the effect of nephrectomy on the rate of albumin breakdown the turnover kinetics of albumin in animals with and without kidneys must be evaluated For this purpose the size of the vascular and extravascular albumin pool, the rates of albumin excretion, breakdown, and synthesis, as well as the rates of exchange of albumin between the vascular and extravascular pools must be determined Most of these parameters are being measured in work now in progress in our laboratory

In spite of the limitations indicated above, our results indicate that albumin catabolism is greatly reduced following nephrectomy in nephrotic rats In these animals the initial fractional rate of iodoalbumin degradation, and thus of plasma albumin breakdown, is as high as in normal rats On nephrectomy the initial fractional breakdown of iodoalbumin is less in the nephrotic rat than in the normal (with or without kidneys) and much less than in the nephrotic with kidneys. This indicates that the rate of albumin catabolism in nephrotic rats without kidneys is much less than in all the other groups

It stands to reason that if the only change following nephrectomy were the elimination of albumin loss in the urine, the fractional rate of iodoalbumin breakdown should increase. This would be so, since the radioactivity of the circulating albumin pools remains high, and thus the specific activity of the breakdown products would be, on the contrary, higher than in rats the iodoalbumin of which is rapidly depleted by proteinuria. Our results show the fraction broken down is greatly decreased.

The best explanation of our findings is provided by the assumption that in the nephrotic rats, kidneys are a major site of plasma albumin breakdown. Thus, the results strongly suggest that in amino-nucleoside nephrotic rats with extensive proteinuria, removal of kidneys markedly decreases plasma albumin breakdown, and that a major, and possibly predominant, part of plasma albumin breakdown in this type of acutely nephrotic animal occurs in the kidney.

This work was supported by grants from the National Heart Institute, U.S. Public Health Service, and the American Heart Association.

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Inhibitory Properties of the Catecholamines

FOR some years considerable attention has been focused on the inhibitory action of γ-aminobutyric acid (GABA). One of the first systems used to demonstrate this property involved inhibition of the spontaneous discharge of the slowly adapting stretch receptor neurone of the crayfish[1,2]. This bio-assay procedure has been used to investigate the inhibitory characteristics of many other compounds[2–4], but none reported to date has proved more active than GABA. A number of aliphatic amino- and guanidino-acids have, however, shown activity approaching that of GABA, and pre-requisites for activity appear to be the presence in the molecule of an acidic and a suitably separated basic function. Such general characteristics are to be found among the catecholamines. The inhibitory characteristics of these substances were therefore investigated.

Pacifastacus leniusculus (Dana) was the crayfish used and the method was essentially that described by Florey[1] and by Elliott and Florey[5]. The commercially obtained compounds were made up in 'crayfish saline'[6] at *p*H 6·5–7 and the 'threshold blocking concentration' was determined; this was the concentration which would cause a transient (*c*. 20 sec.) block of the spontaneous discharge. The activity of any compound was expressed in arbitrary units, defined as the reciprocal of the concentration, in m.moles/litre, which produces a threshold block when applied to the stretch receptor neurone.

Results comparing the inhibitory activity of GABA and the catecholamines are given in Table 1. 3-Hydroxytyramine showed a much higher order of activity than GABA or the other catecholamines on every crayfish preparation used. Although there was great variability in the response of the different preparations to the substances tested, it was repeatedly shown that a given substance tested under two code numbers on a given preparation gave concordant results.

Table 1. INHIBITORY ACTIVITY OF GABA AND SOME CATECHOLAMINES ON THE CRAYFISH STRETCH RECEPTOR NEURONE

Substance	No. of preparations	Activity in units defined in text (mean)	Activity in units defined in text (range)
GABA	18	80	15–206
3-hydroxytyramine hydrogen chloride	15	6,250	566–41,580
L-noradrenaline bitartrate, water	7	110	5–468
L-adrenaline bitartrate	5	65	< 4·5–185

Our results on L-noradrenaline bitartrate and L-adrenaline bitartrate, indicating an activity comparable with that of GABA, are in conflict with those of Elliott and Florey[5], who reported that these compounds were inactive at a concentration of 1 mgm./ml. (less than 0·3 unit in each case). These workers used crayfish of the species *Orconectes virilis*, and did not specify the number of trials. The results we have obtained with many aliphatic amino- and guanidino-acids agree remarkably well with those of Edwards and Kuffler[3], who used both *O. virilis* and *Procambarus alleni*; it therefore seems unlikely that the difference in species of crayfish used can account for the difference in results between ourselves and Elliott and Florey.

The ability of various agents to block the inhibitory effects of 3-hydroxytyramine and of GABA was significantly different. Picrotoxin (10^{-3} *M*) blocked about 60 per cent of the activity of GABA, but was only slightly effective against 3-hydroxytyramine. Chlorpromazine ($1·5 \times 10^{-4}$ *M*) and dibenzylene (8×10^{-4} *M*), on the other hand, were ineffective against GABA but blocked 3-hydroxytyramine almost completely. These results suggest that more than one receptor site on the stretch receptor neurone is involved.

The significance of these results remains to be assessed by work on other systems. The high activity of 3-hydroxytyramine lends support to the many investigators who have suggested that it may be an effector substance in its own right as well as a precursor of adrenaline and noradrenaline.

The work was supported by Federal Mental Health grants No. 609–5–128 and No. 609–5–130 to E. G. and P. L. McGeer; and by a National Research Council of Canada grant to H. McLennan.

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Acquired Tolerance to Homografts produced by Homologous Spleen Cell Injection in Adult Mice

THE dosage of skin homografts has been shown to be a factor in their survival. While small homografts show a prolonged survival-time when compared with medium-sized grafts, the application of large or near total body size homografts results in increased survival time[1,2]. Billingham and Sparrow also showed that intravenous injection of living homologous dissociated epidermal cells caused a two- to three-fold increase in the survival of skin homografts which were afterwards placed on rabbit hosts[3]. Because of our success in producing acquired tolerance to outcross skin in adult mice using parent–F_1 hybrid parabiosis[4,5], the following experiment was designed to study the effects of dosage of homologous antigen on the future recipients of skin homografts in adults, reasoning along lines that the success of parabiosis was primarily a dosage phenomenon.

Groups of adult *C3H* female mice were injected intravenously with a spleen cell suspension obtained from either $(Ce \times C3H)F_1$ hybrid, $(A \times C3H)F_1$ hybrid, *A*, or $(C_{57}(BL) \times C3H)F_1$ hybrid mice. The contents of three donor spleens were suspended in 1 ml. of Ringer's solution such that each ml. contained 300 to 340 million nucleated cells. Injections were made intravenously through a 30-gauge needle placed in the tail vein. One week later a 2·5 × 3·5-cm. full thickness, homologous, abdominal skin-graft taken from donors corresponding to the strain of injected spleen cells was placed on the back of host animals. Each graft was rotated 180° to facilitate identification of viability and checked daily for gross signs of rejection.

Table 1 demonstrates the differences in graft behaviour between the experimental groups. $(C3H \times Ce)F_1$ hybrid skin, accepted by *C3H* hosts at 30–48 days of age, is not accepted at 120–130 days of age unless receiving spleen cells when 30–48 days old. In the case of *C3H* mice injected with $(A \times C3H)F_1$ spleen and grafted with $(A \times C3H)F_1$ skin, the final behaviour of the skin homograft depended on the dosage of spleen injected one week before grafting. Seven of ten grafts placed, following an injection of 300–340 million cells, showed no signs of destruction except in two cases where there was shrinkage to approximately 50 per cent of the original size and a less dense hair-distribution than the other 5 successful homografts (Fig. 1). These latter homografts have been viable for four and a half months. In the other 3 instances the homografts were rejected following a prolonged survival. In the strain combinations, $A \longrightarrow C3H$ and $(C_{57}(BL) \times C3H)F_1 \longrightarrow C3H$, the groups receiving intravenous spleen displayed prolonged survival of grafts but no permanent tolerance.

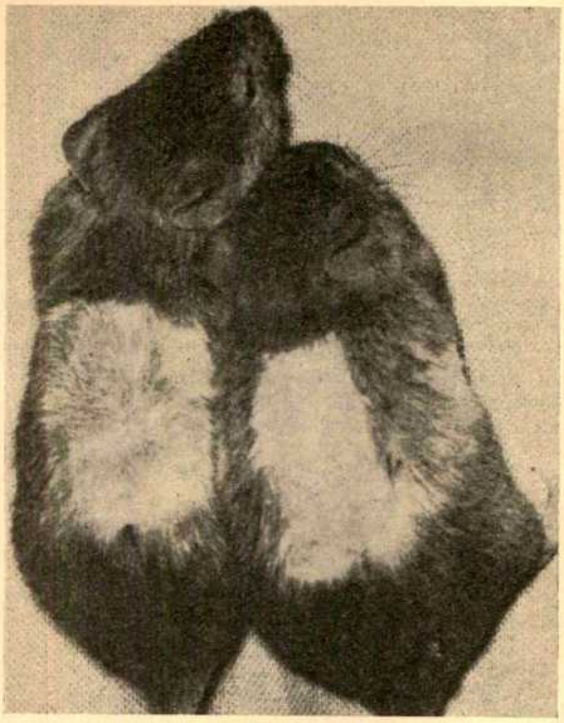

Fig. 1. *C3H* female mice bearing $(A \times C3H)F_1$ female skin-graft of 4·5 months duration. Animal on the left is one of five showing complete tolerance. Animal on the right is one of two, the graft of which is 50 per cent of the original size of grafted abdominal skin

In inbred-F_1 hybrid parabiosis of adult mice a cellular exchange between partners can result in tolerance of the inbred animal to the outcross tissue of its F_1 hybrid partner. The success in provoking a tolerant state depends on the genetic disparity between inbred and F_1 hybrid[4,5]. It would seem logical, in addition, that the dosage afforded by linking the animals is an important factor in the success of this method. The results obtained in this experiment confirm those described by Billingham and Sparrow[3] on the effect of intravenous injection of cells on graft survival. Our results indicate that high doses of homologous cells alter the subsequent capacity to react against these cells, analogous to the demonstration of immunological paralysis to pneumococcal polysaccharide by Felton[6]. The dosage effect was best demonstrated when strong histocompatibility differences were minimized by the use of hybrid

Table 1

No. of animals	Host strain	Strain donor spleen*	No. of cells injected	Strain donor graft	Host age in days when grafted	Mean survival time of grafts (days)	No. tolerant
10	*C3H* ♀			$(C3H \times Ce)F_1$ ♀	37–55	24·8†	8
6	*C3H* ♀			$(C3H \times Ce)F_1$ ♀	120–130	18·2	0
14	*C3H* ♀	$(C3H \times Ce)F_1$ ♀	$0·2–0·3 \times 10^8$	$(C3H \times Ce)F_1$ ♀	120–130		14
10	*C3H* ♀			$A \times C3H\ F_1$ ♀	37–55	12·5	0
6	*C3H* ♀	$(A \times C3H)F_1$ ♀	$1·5–1·7 \times 10^8$	$A \times C3H\ F_1$ ♀	37–55	23·4	0
10	*C3H* ♀	$(A \times C3H)F_1$ ♀	$3·0–3·4 \times 10^8$	$A \times C3H\ F_1$ ♀	37–55	19·7‡	7
14	*C3H* ♀			*A* ♀	37–55	8·9	0
6	*C3H* ♀	*A* ♀	$3·0–3·4 \times 10^8$	*A* ♀	37–55	16·1	0
6	*C3H* ♀			$(C_{57}(BL) \times \mathrm{C3H})F_1$ ♀	37–55	8·7	0
6	*C3H* ♀	$(C_{57}(BL) \times C3H)F_1$ ♀	$3·0–3·4 \times 10^8$	$(C_{57}(BL) \times C3H)F_1$ ♀	37–55	13·6	0

* Host animals were between 30–48 days of age when injected with homologous spleen.

† Mean survival time of two rejected homografts.

‡ Mean survival time of three of ten rejected homografts.

animals. A spectrum of results was obtained depending on the genetic disparity between these hybrid to pure line grafts. $(C3H \times Ce)F_1$ skin on $C3H$ took with minimal doses of spleen in adults. The strains Ce and $C3H$ have the same H–2 locus. A large dose of spleen was required to allow the acceptance of homografts from strains differing unilaterally at the H–2 locus with $(A \times C3H)F_1$ and $C3H$. In the combinations $(C_{57}(BL) \times C3H)F_1 \longrightarrow C3H$ and $A \longrightarrow C3H$, a unilateral and bilateral H–2 locus difference respectively, even this large dose of spleen cells was insufficient to produce tolerance.

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Effect of Diethylstilbœstrol on the Serum γ-Globulin in the Guinea Pig

IT has been reported previously that the active œstrogens, both natural and synthetic, are strong stimulants of the phagocytic activity of the reticulo-endothelial system[1-3]. It has also been reported that cortisone depresses reticulo-endothelial phagocytosis, lowers the total and differential white cell blood counts, and reduces the serum γ-globulin to about half the normal level[4].

The present work was carried out to ascertain whether diethylstilbœstrol, which is a strong stimulant of the reticulo-endothelial phagocytes, raises the level of γ-globulin in the serum.

Thirty-six male guinea pigs of 450–660 gm. body-weight were used in these experiments. Sixteen of the animals were used as controls of which 9 were given no treatment and 7 received 1·5 ml. arachis oil intraperitoneally daily for 6 days. The remaining 20 animals each received 0·5 mgm. diethylstilbœstrol in 1·5 ml. arachis oil intraperitoneally once daily for 6 days. The serum γ-globulin was estimated in all the animals; in the arachis oil controls and in those given diethylstilbœstrol this was carried out on the seventh day after treatment commenced.

The procedure was as follows: From each animal 2 ml. of blood was withdrawn by cardiac puncture, placed in a centrifuge tube and allowed to clot. The specimen was then centrifuged at 3,000 r.p.m. (r.c.f. 1,533*g*) for 20 min. and the supernatant serum removed by pipette. The serum proteins were then separated by paper electrophoresis, using a vertical tank, a barbitone buffer of *p*H 8·6, and Whatman filter paper No. 3 *MM*. Each separation was carried out on 0·01 ml. of serum using a current of 240 V. for 12 hr. The paper strips were dried, then stained with light green for 15 min. and excess stain removed with acetic acid until a clear background was obtained.

The relative distribution and the density of the serum protein bands were recorded using an 'Elphor' densitometer, and from the readings a graph was constructed on 1 mm. paper. The area enclosed by the curve representing the total serum protein was cut out and weighed; from this the area enclosed by the γ-globulin curve was then cut out and weighed separately.

Table 1. EFFECT OF DIETHYLSTILBŒSTROL ON THE SERUM γ-GLOBULIN LEVEL IN THE GUINEA PIG

No. of animal	Body-weight (gm.)	Weight of graph	
		Area of total serum protein (gm.)	Area of γ-globulin (gm.)
Untreated controls			
1	660	0·93	0·090
2	570	0·72	0·045
3	640	0·82	0·068
4	630	0·79	0·060
5	615	0·84	0·068
6	600	0·73	0·063
7	630	0·83	0·076
8	625	0·82	0·058
9	620	0·85	0·070
Mean ± *S.D.*	621 ± 25·3	0·81 ± 0·063	0·069 ± 0·013
Arachis oil controls			
10	630	0·87	0·073
11	645	0·81	0·075
12	610	0·74	0·060
13	600	0·76	0·066
14	590	0·79	0·052
15	600	0·81	0·058
16	595	0·73	0·049
Mean ± *S.D.*	610 ± 17·3	0·79 ± 0·048	0·062 ± 0·010
Animals which received 0·5 mgm. diethylstilbœstrol daily for 6 days			
17	625	1·086	0·230
18	590	0·929	0·173
19	610	1·022	0·184
20	630	1·055	0·213
21	580	1·012	0·182
22	640	1·044	0·203
23	650	1·031	0·221
24	600	0·954	0·184
25	625	1·052	0·215
26	600	1·011	0·197
27	600	0·965	0·188
28	450	0·851	0·146
29	510	0·894	0·159
30	560	0·922	0·166
31	640	1·023	0·184
32	510	0·925	0·166
33	620	1·084	0·215
34	600	1·057	0·206
35	640	1·089	0·207
36	480	0·915	0·165
Mean ± *S.D.*	588 ± 57·2	0·996 ± 0·071	0·191 ± 0·024

The results are shown in Table 1. It will be noted that in the untreated controls the mean weight of the graph area of total serum protein is 0·81 gm. and that of the γ-globulin is 0·069 gm. The arachis oil controls show little if any variation from the results in the untreated controls. In the animals which received treatment with diethylstilbœstrol the mean weight of the graph area of total serum protein is increased to 0·996 gm. and that of the γ-globulin to 0·191 gm. These results represent an elevation of the total serum protein-level of 23 per cent above that of the control groups. This increase is composed principally of serum γ-globulin, which in fact has risen 177 per cent above normal, whereas the remaining serum proteins as a group have increased only 9 per cent. From this evidence it appears that when the reticulo-endothelial system is stimulated by diethylstilbœstrol the serum γ-globulin is increased to about 2¾ times the normal level.

Since the development of antibodies is considered to be closely related to the serum γ-globulin, it seems likely that the great increase of the latter should be accompanied by a corresponding rise in the antibody-forming potential of the animal. This has now been confirmed by further researches which are at present being prepared for publication.

In these investigations we gratefully acknowledge financial assistance from the Tobacco Manufacturers' Standing Committee and the Medical Research Council.

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HISTOLOGY

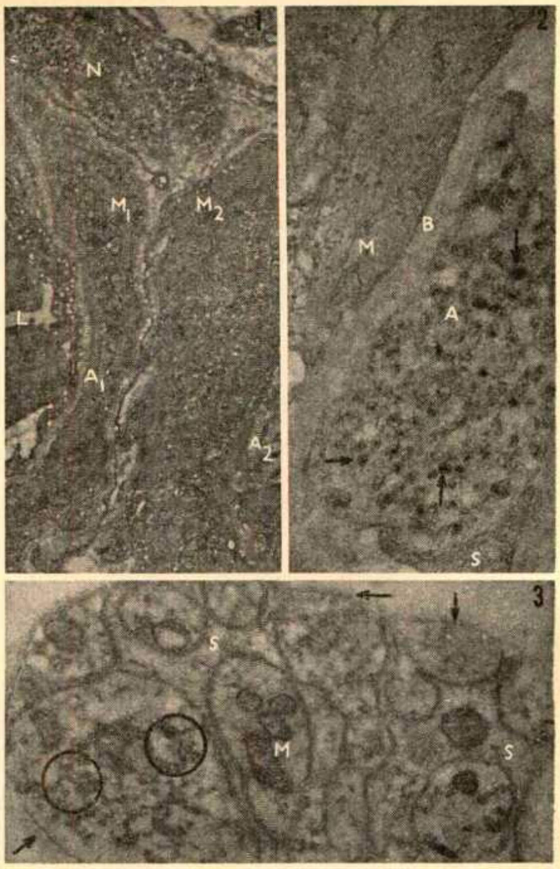
Fine Structure of the Arteriolar Nerves in the Guinea Pig Pancreas

DURING the course of an investigation into the effects of cobalt chloride on the endocrine pancreas of the guinea pig (Esterhuizen and Lever, in the press), our attention was arrested by certain fine structural features—which we believe have not hitherto been described—in the nerve fibres associated with the pancreatic arterioles.

According to Mitchell[1] the nerve supply to the pancreas is a mixture of post-ganglionic sympathetic fibres from the cœliac plexus and some vagal fibres. These nerves reach the gland by way of a peri-arterial plexus in company with the vessels of supply, and for the most part their subsequent distribution is alongside the branching arterial tree between the acini and ducts of the exocrine tissue and within the islets of Langerhans. It is likely that the pancreatic innervation is almost entirely vasomotor, although Lacy[2] has reported, and we also have seen, the occasional axon in direct contact with an endocrine cell.

One may commonly observe groups of unmyelinated axons lying together with a variable amount of connective tissue in the peri-vascular spaces around the small arteries (Fig. 1) in any part of the pancreas. At a fine structural level the well-known axon–Schwann cell relationship[3,4] can be seen in most of the nerve fibres present. That is to say, several axons are enwrapped by the plasma membrane of the same Schwann cell (Fig. 3), each being suspended by mesaxonal invaginations of this membrane. There is nevertheless a number of nerve fibres which are incompletely shrouded by Schwann plasma membrane, and, but for a thin surface covering of basement membrane, these axons could be described as naked over their exposed areas (Fig. 3).

Naked axons of this sort are seen frequently in close approximation to the muscular layer of the arterioles in the guinea pig pancreas. In electron micrographs the plasma membranes of opposing axon and smooth muscle cell are separated by a space which may either contain a single layer of a material having the appearance of basement membrane (Fig. 2), or be lined by this material both on its axonal and muscular sides. This space was found to range from 100 to 400 mμ in width. Although closer neuro-vascular approximation has not yet been observed, the high concentration of micro-vesicles and mitochondria[5] (Figs. 2 and 3) in many of the naked axons would suggest that in these situations nerve fibres are at, or near, their points of termination. Perhaps the synaptic arrangements of peri-vascular nerves are similar to those noted by Fawcett and Selby[6] at nerve endings in the turtle atrium in which contact between pre- and post-synaptic elements is not as intimate as at nerve terminals in many other situations.



Electron micrographs of specimens taken from guinea pig pancreas at intervals following intravenous injection of cobalt chloride (25 mgm./kgm. body-weight). Figs. 1 and 2, 24 hr.; Fig. 3, 2 hr. after injection

Fig. 1. Unmyelinated nerves (N) within a peri-vascular space around two small arteries A_1, A_2; lumen of first artery (L); smooth muscle cells M_1 and M_2 in walls of vessels. Note dense granular material in axons (N) (× 11,250)

Fig. 2. Naked axon (A) separated by a space containing 'basement membrane' (B) from the plasma membrane of a smooth muscle cell (M). Most of the micro-vesicles in the terminal axoplasm contain dense granular material (↑). Note Schwann cytoplasm (S) (× 40,500)

Fig. 3. Peri-arterial axons invested by Schwann cytoplasm (S). Note areas (↑) where axons are covered only by basement membrane. Concentrations of mitochondria (M) and micro-vesicles are common in these axons. In the encircled areas several micro-vesicles are seen to contain dense granular specks (× 27,000)

The micro-vesicles, found in high concentration within the terminal axoplasm of these pancreatic peri-vascular nerves, have a diameter of 350 (±50) Å. In the normal animal, although the occasional one of these vesicles was seen to contain a dense granular material, the majority of them are without visible content in electron micrographs. However, in guinea pigs examined at intervals up to five days after injection of cobalt chloride, a high proportion (at 24 hr.—the majority) of the micro-vesicles exhibit a filling of this dense material (Figs. 1 and 2). These facts might suggest: (1) that cobalt had some specific and local action on the peri-vascular nerve terminals; or (2) that filling of a large proportion of the terminal vesicles may be a local manifestation of the response to a non-specific but stressful action of cobalt As the neurones in question are vasomotor and presumably sympathetic post-ganglionic in nature, they can be expected to elaborate noradrenaline or a comparable substance In tissues such as the adrenal medulla[7,8] and the carotid body[9], work has already indicated that the catecholamine transmitter agent is visualized in electron micrographs as a dense granular substance parcelled up within vesicles before release from the cell. It is therefore conceivable that the osmiophile granular material seen within some of the micro-vesicles at the termination of these arteriolar nerves in the pancreas (Fig 2) may be visible evidence of the presence of such a transmitter substance

One of us (J D L) acknowledges a Royal Society equipment grant

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HÆMATOLOGY

Relationships between Red Blood Cell Potassium Concentrations, Medial Corpuscular Fragility and Hæmoglobin Type in Merino and Southdown Sheep

WITHIN any breed of sheep there are animals which have red blood cells containing a high concentration (c 80 m equiv /litre of red blood cells) or a low concentration (c 20 m equiv /litre of red blood cells) of potassium[1] The two types are simply inherited, the latter being dominant[2]

Low-potassium Merino sheep characteristically have a very low concentration of potassium in their erythrocytes (c 12 m equiv /litre of red blood cells) and this is associated with a very low gene frequency for high potassium concentration (approx 0 07)[3] This breed appears to be particularly well adapted to the semi-arid regions of Australia This could be associated with the observation that low-potassium sheep consume less water and void less urine than high-potassium animals when both types are kept under controlled conditions[4]

Although potassium concentrations in red blood cells remained remarkably constant during long periods in sheep kept under controlled conditions[5] it has been shown recently[6] that four-fold increases in the concentration of potassium in the erythrocytes of Merino sheep in the field can occur, and that this is associated with a marked lowering of the hæmatocrit value and red blood cell count The increase in potassium concentration appears to be correlated with the severity of the anæmia and has only been recorded so far in low-potassium animals The nature of this correlation is being investigated at present

If a hæmolytic process associated with the swelling of the red blood cells is involved, there could be differences in the median corpuscular fragility between low- and high-potassium cells This preliminary point has now been investigated in Merino and Southdown sheep of known hæmoglobin type[7] and red blood cell potassium type Median corpuscular fragility determinations were carried out using the method described by Dacie[8]

Initially, the median corpuscular fragility of the red blood cells of 28 Merino ewes (23 low potassium, 5 high potassium) was examined on three occasions and a significant ($P < 0$ 1 per cent) difference between low potassium (median corpuscular fragility = 0 695) and high potassium (median corpuscular frequency = 0 625) animals was found The between animal variation was greater than the within animal variation ($P < 0$ 1 per cent), and animals with the lowest concentrations of potassium in their red blood cells within the low potassium group appeared to have the highest median corpuscular frequency values within the group

This initial observation was followed up with an experiment in which 21 Merino sheep were selected and divided into three groups—low potassium (7 animals with red blood cell potassium ion concentrations below 17 0 m equiv /litre), high potassium (9 animals with more than 17 0 m equiv /litre), high potassium (5 animals) The median corpuscular fragility was determined on each animal on 10 separate occasions The results are given in Table 1

A significant difference in median corpuscular fragility between low potassium and high potassium animals was again demonstrated in this second experiment ($P < 1$ 0 per cent), but although the difference between the high low potassium and low potassium group was in the direction expected from the high potassium results it was not significant In the high potassium group one of the animals was of the type classified as *Keβ*[5]—a type not common in British breeds—and the remaining four animals were of the type classified as *Keγ*—the normal high potassium type in British breeds of sheep This *Keβ* animal has a significantly lower median corpuscular fragility ($P < 5$ 0 per cent) than the remaining *Keγ* type animals

A similar examination of the red blood cells of 7 high potassium and 8 low potassium Southdown sheep has also been carried out and a significant difference ($P < 1$ 0 per cent) between the median corpuscular fragility of the two cell types has been found

Although no obvious correlation between median corpuscular fragility and hæmoglobin type was observed there was a correlation between hæmoglobin

Table 1 HÆMOGLOBIN TYPE, RED BLOOD CELL POTASSIUM TYPE AND MEDIAN CORPUSCULAR FRAGILITY IN MERINO SHEEP

	Hæmoglobin type	No of animals	Mean median corpuscular fragility ± *S E*	Mean median corpuscular fragility ± *S E*	Mean median corpuscular fragility ± *S E*
High potassium	*AB*	3	0 637 ± 0 019	0 647 ± 0 015	0 647 ± 0 015
	B	2	0 661 ± 0 024		
High low potassium	*A*	6	0 703 ± 0 014	0 691 ± 0 011	0 706 ± 0 008
	AB	2	0 685 ± 0 024		
	B	1	0 700		
Low low potassium	*A*	1	0 698	0 716 ± 0 013	
	AB	5	0 721 ± 0 015		
	B	1	0 706		

Levels of significance for differences in median corpuscular fragility between high potassium and low potassium, $P < 1$ 0 per cent, high potassium and high low potassium, $P < 5$ 0 per cent, high potassium and low low potassium, $P < 1$ 0 per cent, and between high low potassium and low low potassium, not significant Effect of hæmoglobin type not significant, variation within groups of animals less than variation between groups of animals, $P < 0$ 1 per cent

Table 2 HÆMOGLOBIN TYPE AND MEAN RED BLOOD CELL POTASSIUM CONCENTRATIONS IN MERINO AND SOUTHDOWN SHEEP

Hæmoglobin type	Low potassium Mean red blood cell potassium concentration in m equiv /litre					High potassium Mean red blood cell potassium concentration in m equiv /litre				
	Merino			Southdown		Merino		Southdown		
A	(10) 21 7 ± 1 52	*	*					(1) 92 3	†	*
AB	(17) 16 6 ± 1 17	N S		(4) 18 5 ± 2 41	N S	(2) 79 9 ± 3 41	N S	(3) 80 1 ± 2 78	N S	
B	(13) 14 7 ± 1 34			(6) 14 1 ± 1 97		(6) 76 2 ± 1 97		(3) 72 3 ± 2 78		

* $P < 1 0$ per cent, † $P < 5 0$ per cent, N S , not significant No of animals in parenthesis

type and mean red blood cell potassium concentrations

All animals in a small experimental flock of Merino sheep which had been selected originally on the basis of red blood cell potassium concentrations were therefore examined along with a small flock of Southdowns The results are shown in Table 2

Low potassium (*A*) Merino sheep had a significantly higher mean red blood cell potassium concentration than low potassium (*AB*) animals ($P < 1 0$ per cent) or low potassium (*B*) animals ($P < 1 0$ per cent) Sufficient animals in the three hæmoglobin phenotypes were only available for the low potassium Merino, but other results show a similar trend—a trend which had been noted previously in relation to whole blood potassium concentrations[9]

The correlations between median corpuscular fragility and potassium type, and between potassium type and hæmoglobin type, are of interest in relation to the possible adaptive significance that the gene pairs controlling potassium and hæmoglobin types may have in some Australian environments[11,12]

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Human, Cow and Rabbit Native Fibrin Powders as Anticoagulants

THE anticoagulant action of fibrin-coated surfaces on native blood and plasma has been demonstrated recently[1,2] Since the fibrin-coating process is quite laborious[3], we tested the anti-coagulant effect of a fibrin surface by simply placing native plasma in contact with fibrin powder. It was realized that the contact of native plasma with surfaces foreign to blood known to initiate coagulation, such as glass, would have to be counteracted by a large enough fibrin surface In a preceding investigation by Copley and Cliffton[4] so-called fraction I fibrin powder, prepared from a Cohn fraction I of bovine plasma by the addition of thrombin, was found to have an anticoagulant action similar to that observed in glass tubes coated with bovine fraction I fibrin Since the bovine fibrinogen (Armour) contains 40–50 per cent sodium citrate, it was not clear whether the latter was responsible for the anticoagulant effect of the fraction I fibrin powder Therefore, work has been done on native plasma fibrin powder, prepared from native plasma without the addition of anticoagulants or coagulants

Blood was drawn from the antecubital vein of healthy human subjects, the jugular vein of cows, or the rabbit's heart and immediately centrifuged for 10 min at 5,000 r p m at 0° C The undiluted native plasma thus obtained was transferred to a glass tube and allowed to coagulate at room temperature, and the clot removed with a glass rod and incubated at 37° C. in a Petri dish to speed up retraction The serum exuding from the clot was absorbed by filter paper every 10 min for 1 hr , by which time clot retraction appeared to be complete The clot was then transferred to another Petri dish and was allowed to dry in the incubator for about 21 hr after which the fibrin was ground to a fine powder 80 mgm of the powder were mixed with 1 ml of freshly prepared native plasma in a glass tube which was shaken by flicking it several times to ensure a thorough contact of the plasma with the powder. The tubes were incubated in a water-bath at 37° C. and observed for coagulation As a rule, the plasma was kept in contact with the powder for 1 hr and then was separated from the fibrin particles by low-speed centrifugation Such so-called 'fibrin-contact plasma' was then further observed for clotting in glass tubes at 37° C In all cases, regardless of the species used. coagulation never was found to occur for 7 or more hours Thereafter, the plasma was stored in a refrigerator at 4° C to counteract bacterial growth, and coagulation times were taken on successive days for periods up to several months The initial contact of 1 hr. of rabbit native plasma with cow native fibrin powder did not result in coagulation for 98 days

Table 1. EFFECT OF THROMBIN ON HUMAN, COW AND RABBIT FIBRIN-CONTACT PLASMA
(5 N I H units thrombin per 0 1 ml plasma)

Hours of contact with fibrin powder	Gelation times (sec) of fibrin contact plasma at 37° C Human native plasma exposed to human native fibrin	Rabbit native plasma exposed to rabbit native fibrin	Rabbit native plasma exposed to cow native fibrin
1	4 1	10 8	7 3
3	4 6	11 2	8 4
26]	4 9	11 0	8 9

Whenever coagulation times were taken, the fibrin-contact plasma was tested for its coagulability by mixing 0 1 ml with 0 1 ml thrombin solution, containing 5 N I H (National Institutes of Health) units, and timing the appearance of gel formation As seen from Table 1, the thrombin coagulability of the fibrin-contact plasma does not change significantly when the native plasma is in contact with the fibrin for 1–26 hr Human native plasma exposed to autologous native fibrin gives the shortest clotting or gelation times on the addition of thrombin, while this is not always the case with rabbit native plasma exposed to autologous native fibrin which exhibits longer clotting times As can be seen, rabbit plasma exposed to cow native fibrin gives shorter clotting times with this thrombin test The possibility of species-specific reactions and their variations is under investigation Frequently we observed that ageing of fibrin-contact plasma was associated with a lengthening of the thrombin clotting times However, ageing had a similar effect on oxalate plasma of the same blood withdrawal used as a control

Fibrin-contact plasma, prepared by using native fibrin from the three species, exhibited anticoagulant action This was tested by mixing 2 vol of fibrin-contact plasma with 1 vol of native plasma, resulting in markedly prolonged clotting times or in no coagulation Such mixtures always showed coagulation with the above thrombin test When 2 vol of native plasma were mixed with 1 vol of fibrin-contact plasma, coagulation occurred, and the clotting times were only slightly longer than the controls of native plasma

In contrast to fraction I fibrin powder, plasma exposed to native fibrin powder often exhibited decreases in fibrinogen content following incubation of 1 or more hr regardless of the species used for the native fibrin Such decreases amounted to 44 per cent for 2 hr of contact when compared with the control of oxalate plasma from the same blood It will have to be established whether the fibrinogen lost from the fibrin-contact plasma has been adsorbed to the native fibrin or whether it has been converted into fibrin The use of native fibrin appears to differ also in another way from that of fraction I fibrin By using the latter in both native plasma and whole blood, coagulation could be prevented; however, when native whole blood was exposed to native fibrin such an anticoagulant effect did not always result Our findings appear to show that the use of fraction I fibrin introduces possible artefacts in contrast to that of native fibrin The latter may well prove to have a more physiological anticoagulant action

Our findings suggest that if the proposed endo-endothelial fibrin film[1,2,5] did exist, it might have an anticoagulant action This might well aid in maintaining the fluidity of blood in the circulation Outside some promising practical applications, our results necessitate alteration of certain views, generally believed to be responsible for the fluidity of circulating blood and the origin of thrombosis

We are grateful to Dr R G Macfarlane, Radcliffe Infirmary, Oxford, for helpful discussions, and to Mr F A Glover, of the National Institute for Research in Dairying, University of Reading, for the preparation of cow native plasma fibrin

The work was aided by grant *H*–4793 from the National Institutes of Health, Bethesda, Md

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PATHOLOGY

Persistence of Motility in Bone-Marrow Cells from the Cadaver

A READILY available source of large quantities of bone marrow would be valuable for replacement therapy in such cases as radiation accidents Cadaveric marrow may be suitable for this purpose as the cells are believed to remain viable for several hours after death[1], although no criteria of viability are given. The present work was undertaken to determine the persistence of viability of marrow cells by investigating their motility *in vitro* after somatic death

Sternal marrow (0 1–0 2 ml) was obtained by normal puncture technique from fifty human subjects at various times after death Bodies were refrigerated about 2 hr after death The range of age was 33–89 although the great majority were more than 50 years old and the ratio of males to females was approximately 2 1 An occasional sample was taken within 2 hr of death, increasing difficulty was often experienced the longer after death the attempt was made

Most leucocytes of the bone marrow exhibit some degree of motility although this is greatest in the neutrophil polymorphonuclear[2], and the present results are based for the most part on the motility of this cell Motility was considered to be present if a cell was observed to move a distance of approximately its own diameter or more during a 5-min search of the preparation

For examination of the specimen a drop of marrow was expelled on to a chemically cleaned glass slide, a coverslip applied and the edge sealed with soft paraffin The remaining marrow was mixed with bovine albumin (5 per cent) in physiological saline and used to prepare stained films for differential counting[3] This is important because for practical purposes polymorphs represent the only motile cells of the marrow and any notable reduction of their number clearly reduces the chances of observing motility The sealed preparation, on a warm stage at 37° C , was examined for motility with the 1/6 objective A 5-min search usually entailed the examination of about twelve different fields

In a preliminary examination of living subjects 12 out of 14 samples from routine diagnostic sternal marrow biopsies showed motility Of the 2 negatives one was a case of lymphocytic leukæmia, the other a case of myelosclerosis

In the post-mortem investigation, 50 results were assessed as satisfactory for analysis (Table 1) Cases were excluded from analysis because of myeloproliferative or aplastic disorders, failure of refrigeration of the body or unavailability of temperature records within 24 hr of death The presence of pyrexia before death, that is a temperature of more than 37° C at some stage during the terminal 24 hr , reduced motility and such cases are recorded separately in Table 1 Thirty-four of the fifty cases showed motility This was invariably present less than 5 hr after death and up to 20 hr in apyrexial subjects and absent in all subjects after 40 hr Oscillation of the intra-leucocytic granules frequently persisted after detectable motility had ceased McLeod and McLeod[4] believe that such oscillation is a criterion of the viability of leucocytes Whitby and Britton[2] while by inference agreeing that cells exhibiting these properties are living, state that the oscillation is a preliminary to cellular death and the present investigation supports this view Monocytic and lymphocytic motility exists[2,5,6], but was considered unimportant because of their small numbers and sluggish movement

Table 1

		Hours after death	
		0–20	> 20
a	Motile	18	3
	Non-motile	0	5
b	Motile	9	4
	Non-motile	3	8

a, Apyrexial, *b*, pyrexial

Cellular viability in the bone marrow of the refrigerated cadaver persists for 20 hr. or more after death. A shortening of the period of motility by ante-mortem pyrexia is in accord with Jeanneret's[7] finding that in pyrexial human subjects degeneration of cells in the stained film of post-mortem marrow occurs earlier than in the apyrexial subject. It is uncertain whether this phenomenon is attributable to a common factor, for example toxæmia, which causes pyrexia and hastens cellular death, or to the pyrexia alone increasing the metabolic rate of the cell with quicker exhaustion of its energy potential.

The clinical inference from these leucocyte motility investigations is that cells may remain transplantable longer than hitherto supposed. The technique for studying the motility is a simple one which should be useful in other ways, as for example in the examination of possible cytotoxic effects of anti-leucocytic sera.

I wish to thank Dr. J. F. Wilson for the idea from which this work developed as well as Prof. J. S. Young, Dr. R. C. Nairn and Mr. W. Brass for their interest and advice.

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Effect of Phagocytosis on the Lysis of *Staphylococcus aureus* (Type 81) by Bacteriophage

CERTAIN investigations on animals have suggested the efficacy of treatment of bacterial infections with specific bacteriophage. Dubos *et al.*[1] demonstrated the therapeutic effect of *Shigella dysenteriae* phage in experimental infections in mice. Morton and Engley[2] showed a prophylactic, as well as therapeutic, effect in mice infected with *Shigella dysenteriae*. Recently, Muir and Blakemore[3] protected 70 per cent of mice lethally infected with *Staphylococcus aureus* type 80/81 with high-titred specific phage given simultaneously with the bacteria.

Work on the use of bacteriophage *in vivo* is necessarily complicated by a variety of factors, one of which is the role of phagocytes. This report will present evidence to indicate that *S. aureus* is phagocytized *in vitro* by rabbit monocytes and polymorphonuclear leucocytes, and that bacteria released from the phagocytes are susceptible to subsequent lysis by specific bacteriophage.

Staphylococcus aureus, propagating strain 81, was grown and maintained on tryptacase soybean broth (TSB). Type 81 staphylococcal bacteriophage was propagated on *S. aureus* strain 81 in the same broth, filtered through UF fritted glass filters and was stored at 4° C. Polymorphonuclear leucocytes (PMN) were obtained from normal rabbits by injecting 100 ml. of sterile 0·85 per cent sodium chloride intraperitoneally, followed by a similar injection 18 hr. later. Rabbits were killed about 3 hr. after the second injection either by bleeding or by a blow on the back of the head and the peritoneal cavities were opened aseptically. Peritoneal fluid was removed into sterile 0·85 per cent saline containing about 10γ of heparin per ml. Monocytes (MN) were obtained from rabbits given 100 ml. of sterile heavy mineral oil intraperitoneally and killed three days later. Both MN and PMN were washed three times with sterile Hanks's solution and collected after centrifugation at 1,000 r.p.m. for 5 min. at 4° C. By these methods, about 75 per cent of the collected cell population were of the desired type.

In the experiments reported here, 5 ml. of an 18 hr. culture of *S. aureus* (about 5 × 10^{10} bacteria/ml.) were mixed with an equal volume of either PMN or MN suspended in medium 199 to contain 2 × 10^{6} cells/ml. These mixtures were incubated in a roller drum revolving at 15 r.p.h. at 37° C for 45 min. Following incubation, the mixtures were immediately chilled in an ice-bath and all subsequent washings of the cells were carried out at 4° C. The original cell bacteria mixtures were washed five times with Hanks's solution and the cells resuspended at 1 × 10^{6} cells per ml. in medium 199. One of these suspensions was taken as the zero time sample. The tubes were tightly stoppered and returned to the roller drum. At intervals one tube was removed from the roller drum and washed as described. The final cell pellet was lysed in 1·5 ml. of sterile distilled water, releasing intracellular bacteria, and isotonicity was restored with an equal volume of sterile 1·6 per cent sodium chloride. Ten-fold dilutions were made in TSB in 1·0-ml. amounts. To one-half of each dilution 0·5 ml. of phage type 81 (RTD = 10^{-4}) was added. The same volume of TSB was added to the other half of each dilution. These mixtures were incubated at 37° C for 10 min. and were poured on to fresh tryptacase soybean agar plates. All plates were incubated at 37° C overnight and colony counts carried out the following day.

The colonies present on each control (*C*) plate and each test or phage-treated plate (*T*) at each time-interval and at each dilution were counted. In three experiments with PMN and two with MN from five different rabbits, the control colony count at each time-interval reflected the ten-fold dilutions made. The colony counts given in Table 1 (PMN) and Table 2 (MN) represent geometric means of the three ten-fold dilutions using cells from five rabbits at each time interval. The *T*/*C* ratios were calculated from the geometric means at each time-interval. The *T*/*C* ratio for both PMN and MN reached its peak at about the same time the intracellular bacterial count was lowest. The number of bacteria not lysed by the phage was quite constant within the time-limits of the experiments, while the number of intracellular bacteria not exposed to phage decreased steadily for about 1·5 hr. in PMN, then increased. MN, on the other hand, kept the number of viable intracellular

Table 1. *T*/*C* RATIOS AND GEOMETRIC MEANS OF COLONY COUNTS IN PMN FROM THREE RABBITS

Hr.	Colony count *T**	Colony count *C*†	*T*/*C*
0	2	475	4·2 × 10^{-3}
1	1·5	69	2·2 × 10^{-2}
1·5	1·4	25	5·6 × 10^{-2}
2	1·6	46	3·5 × 10^{-2}
2·5	2·6	61	4·2 × 10^{-2}

*T**, Phage treated, *C*†, no phage treatment.

Table 2. *T*/*C* RATIOS AND GEOMETRIC MEANS OF COLONY COUNTS IN MN FROM TWO RABBITS

Hr.	Colony count *T**	Colony count *C*†	*T*/*C*
0	1	74	1·4 × 10^{-2}
1	1·5	13	1·1 × 10^{-1}
1·5	1·1	8	1·4 × 10^{-1}
2	1·1	7	1·6 × 10^{-1}
2·5	1·2	7	1·7 × 10^{-1}

*T**, Phage treated, *C*†, no phage treatment.

bacteria at a constant low level for at least 1 hr longer Microscopic examination revealed no viable phagocytes in either system after 24 hr

Bacteria which were not lysed by the phage after passage through either PMN or MN were later plated and incubated at 37° C for 18 hr When these bacteria were exposed to phage 81, complete lysis occurred This indicates that after passage through either PMN or MN, *S aureus* was unaffected in its susceptibility to lysis by phage 81

The meaning of the *T/C* ratio is perhaps best explained by the *T* and *C* columns in Tables 1 and 2 The number of viable bacteria in the phage-treated (*T*) columns was nearly constant in five different experiments If phagocytosis caused an increase in the number of phage-resistant bacteria, the *T/C* ratio should approach unity when the control values fell However, the approach of the *T/C* ratio toward unity was entirely due to a decrease in control counts and not to an increase in test counts. Thus the *T/C* ratio also becomes a measure of the bactericidal activity of the phagocytes and the higher the *T/C* ratio, the greater the bactericidal activity

Our results appear to be in partial agreement with the findings of other workers[4,6] Cohn and Morse[4] found that *S aureus* was not readily phagocytized in normal rabbit serum unless the multiplicity was ten bacteria/leucocyte However, *S aureus* was killed intra-cellularly only in the presence of specific immune serum Mackaness[6] reported similar findings using rabbit macrophages (monocytes) Kapral and Shayegani[5] demonstrated phagocytosis by rabbit MN and PMN at a multiplicity of 25 or 50 1, but reported no inactivation of intracellular bacteria Our results suggest that the intracellular inactivation of *S aureus* was accomplished by both PMN and MN to the point where the number of extracellular bacteria reached a critical value[5], and the cells became overwhelmed by bacteria The increase in viable intracellular bacteria probably does not represent multiplication, but may be due to additional phagocytosis[5]

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RADIOBIOLOGY

Induction of Tumours in Mice with Tritiated Thymidine

We wish to record preliminary results of an experiment, in which we are investigating the possibility of inducing tumours in mice with tritiated thymidine The β-particles from tritium incorporated into deoxyribonucleic acid (DNA) from ^{3}H-thymidine produce an irradiation which primarily affects the cell nucleus, since about 90 per cent of the energy of these particles is dissipated within the nucleus This procedure, therefore, enables one to distinguish in long-range experiments the results of nuclear damage from that caused by irradiation of whole cells and tissues, and to study the importance of nuclear damage in radiation carcinogenesis It is desirable to do this, since there are many indications that alterations in deoxyribonucleic acid are involved in carcinogenesis in general. It is also commonly assumed that the tumour-inducing properties of ionizing radiations are mediated through changes in the nucleus, but there is no proof of this assumption

Table 1 INCIDENCE OF TUMOURS IN *CAF*$_1$ MICE AFTER A SINGLE INTRAPERITONEAL INJECTION OF ^{3}H-THYMIDINE

Groups	Dose-level	No of animals	Age at time of injection (months)	Age at present (months)	No of deaths	No of animals with tumours
1	1 0 μc /gm	100	new-born (1-day)	17	8	0
2	1 0 μc /gm	100	2	19	23	8
3	1 0 μc /gm	98	6–7	23–24	34	16
4	Control	170	new-born (1 day)	17	9	2
5	Control	99	3–4	20–21	21	4

Our experiment has now been in progress for 17 months It involves more groups of animals than those mentioned here The mortality among the experimental animals has been remarkably low and the capacity of tritiated thymidine to induce tumours under these conditions has not been spectacular This is of some interest in itself Nevertheless, although the total number of tumours is small, there is evidence that the death-rate from tumours is significantly higher among the exposed than among the control animals

Three groups of male and female *CAF*$_1$ mice received a single intraperitoneal injection of ^{3}H-thymidine, with a specific activity of 360 mc /m mole (New England Nuclear Corporation, Boston, Mass), at a dose-level of 1 0 μc /gm of body-weight The groups differed from one another in age at the time of injection as follows (Table 1) Group 1 consisted of new-born animals less than 24 hr old, the animals of Group 2 were 2 months old and those of Group 3 6–7 months old Groups 4 and 5 served as controls Group 4 consisted of new-born animals and those of Group 5 were 3–4 months old The controls were injected either with non-radioactive thymidine or with saline solution. The animals were permitted to live out their life-span. The diagnosis was verified in each instance by histological examination

The following types of malignant tumours have been observed among the experimental animals 6, thymic lymphoma, 9, non-thymic, disseminated lymphoma, 2, salivary gland tumour, 2, carcinoma of the skin, 5, carcinoma of the lung, 1, carcinoma of the liver (hepatocellular) The tumours were distributed equally among males and females The six tumours among the control animals were 5 non-thymic lymphomas and 1 carcinoma of the lung In animals without tumours, death could be attributed to a variety of causes, including pneumonia and intestinal infections It should be mentioned that no lung adenomata have been observed so far, either in the control or in the experimental animals

These results indicate that, at this dose-level, tritiated thymidine is a carcinogen in mice. This conclusion is supported by a similar finding in another group of 100 animals in the same experiment that were 12–14 months old at the time of injection Of these animals, 84 are now dead and 43 of them have died with tumours Although we do not at the moment have age-specific controls for this group of animals (and hence we have not listed them in Table 1), a tumour incidence of more than 50 per cent is considerably higher than that ordinarily seen in control animals of this strain and age in our Laboratory

No results are available as yet in another part of this experiment, in which we are studying the comparative effectiveness in the induction of tumours by ^{14}C-thymidine with its more penetrating β-rays.

The observations on induction of tumours by tritium-labelled thymidine are being reported now not only because they are of interest in connexion with the question of nuclear damage in radiation carcinogenesis, but also because they lend weight to the evidence that tritiated thymidine can produce significant changes in mammalian cells. Such changes have included short-term inhibition of growth[1,2], developmental and chromosome abnormalities[3,4], as well as cytogenic aberrations as shown by an increased frequency of mutations in *Drosophila*[5]. The observations in the present experiment also tend to support the opinion of Oliver and Lajtha[6], who have given warning of the hazards involved in using tritium as deoxyribonucleic acid label in man. They pointed out the possibility of mutagenic effects of the radiation on those cells that have a long life-span and several mitotic cycles.

This work was carried out under the auspices of the U.S. Atomic Energy Commission and supported by U.S.A.E.C. contract No. *AT* (II-I)-809.

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Mouse Lymphoma Cells with Different Radiosensitivities

FISCHER[1] devised a medium in which murine leukæmia cells (*L* 5178 *Y*) can be grown in free culture starting from a single cell. These cells have retained their strain specificity and they will grow only in *DBA*/2 mice where they give rise to ascites and solid tumours. A logarithmically growing culture was obtained 24 hr. after transfer of the ascites cells.

We have exposed cultures to 220 kVp X-rays and observed that cells changed their radiosensitivity on being maintained continuously in culture. Fig. 1 shows the effect of irradiation on cultures that had been started from cells obtained six to ten days earlier from a mouse. Cells that had been kept going for six months continuously in culture—transfer to new medium being made twice a week—were very much more radiosensitive due to the appearance of a radiosensitive mutant. Sensitive strains were isolated by cloning from a single cell[2] and their response to radiation is shown in Fig. 2.

The effect of radiomimetic alkylating agents on these lymphoma cells has been described[3] and their response to radiation is similar. With doses up to the LD_{90}, most of the cells divide at least once and degenerate after the second day when they have grown to double their volume as measured with a Coulter cell counter. Before lysing, 10–20 per cent of the cells grow into giants having ten to fifty times the normal volume. With higher doses of radiation (that is, greater than 500 r. for the resistant strain and 200 r. for the sensitive strain) an increasing proportion of cells degenerate without having undergone division. Cells that have retained their capacity for indefinite multiplication give rise to the new population that grows up from the cultures. The fraction of 'survivors' can be calculated by extrapolation[3] and the dose response curves shown in Fig. 3 have been obtained in this way. If radiation induces a delay in mitosis which extends over several hours then the number of survivors obtained by the extrapolation method would be an underestimate. While this is improbable for reasons which we have given[3], the method was checked by determining the dilution from which an irradiated culture grows up and which must therefore contain at least one viable cell—the cloning efficiency from one cell being between 70–80 per cent[2]. The number of 'survivors' computed in this way agreed with doses up to 300 r. with the values obtained by the extrapolation method.

We have indications that mutants with varying degrees of radiosensitivity arise during growth in culture, but we have not found any strain more resistant than that carried by passage through animals. The sensitive strain divides in culture about 25 per cent more rapidly than the insensitive (compare control curves in Figs. 1 and 2) and this presumably gives them a proliferative advantage so that they establish themselves *in vitro* on continued culture. The sensitive cells have retained their host specificity for *DBA*/2 mice, but they grow less rapidly in animals than the resistant and give rise more frequently to solid tumours.

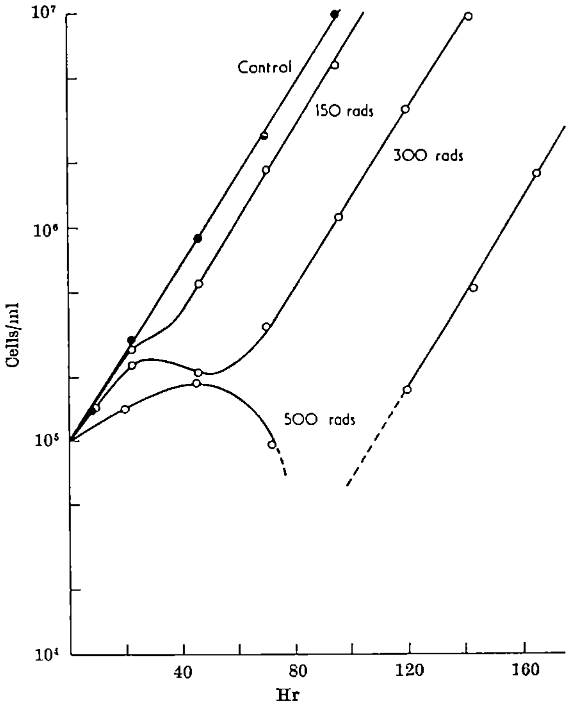

Fig. 1. Effect of irradiation with 220 kVp. X-rays (900 r./min.) on lymphoma cells in a culture which was fully aerated. The culture was diluted before reaching 10^7 cells/ml. and counted in a hæmocytometer

Cells transferred to culture from the ascites fluid of a mouse ten days earlier, referred to as a resistant strain

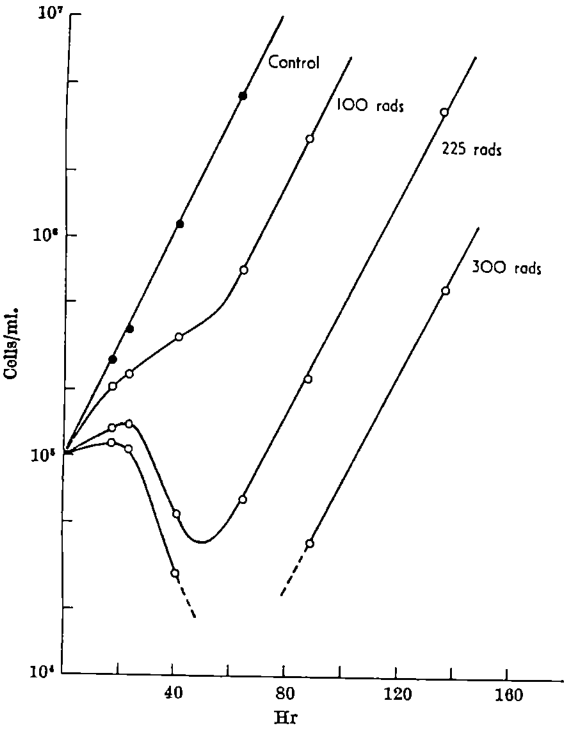

Fig 2 Effect of irradiation with 220 kVp X-rays (900 r /min) on lymphoma cells in a culture which was fully aerated The culture was diluted before reaching 10^7 cells/ml and counted in a hæmocytometer

A cloned derivative from cells continuously maintained in culture for six months, referred to as sensitive strain

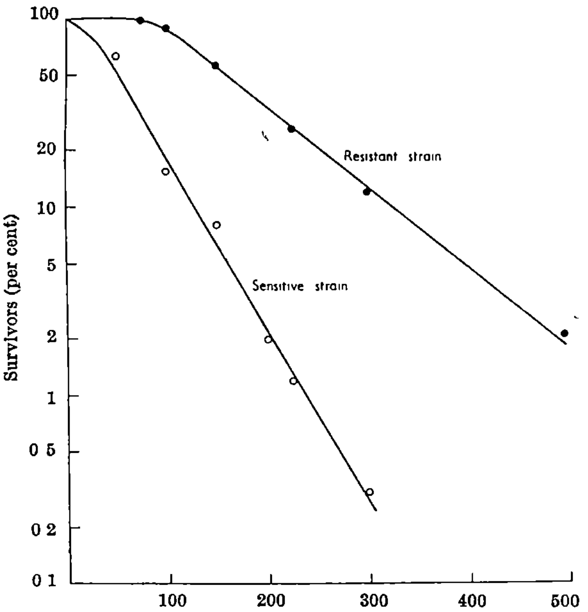

Fig 3 Fraction of cells capable of normal growth as a function of radiation dose

The average volume, as measured with a Coulter counter, of the sensitive cells is some 30 per cent greater than that of the resistant cells but the content of deoxyribonucleic acid, determined by Burton's method, is the same for both In neither type of cell, when taken from culture or from animals, could any viruses be seen in the electron microscope

These experiments demonstrate that the radiosensitivity of mammalian cells *in vitro* can change, presumably by a mutation, and suggest that deductions concerning the radiosensitivity of cells *in vivo* from *in vitro* studies are liable to serious error. Fischer[4] has shown a similar phenomenon with regard to resistance to folic acid antagonists

This work was supported by a grant from the International Atomic Energy Agency and by grants to the Chester Beatty Research Institute (Institute of Cancer Research Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U S Public Health Service

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Effects of Iodoacetic Acid on the Incorporation of Phosphorus-32 into the Deoxyribonucleic Acid of some Radiosensitive Organs in Rats exposed to X-Rays

It is now known that iodoacetic acid is able to sensitize mice[1,2] and rats[3] to ionizing radiations as well as cultured mammalian cells[4] and bacteria (Bridges, B A , personal communication).

In a recent paper[5] we presented evidence that iodoacetic acid markedly inhibits the uptake of phosphorus-32 into the deoxyribonucleic acid (DNA) of some radiosensitive organs and of the regenerating liver in the rat This effect is similar to that shown by ionizing radiations that inhibit the incorporation of ^{32}P as well as of many labelled precursors, into DNA of dividing irradiated cells[6] Considering this common interference with DNA synthesis, we have undertaken work on the effect of the combined treatment with iodoacetic acid and X-rays on ^{32}P uptake into DNA of rat thymus, spleen and intestine

Laboratory bred male albino rats (70–90 gm) of Wistar Glaxo strain were used Lots of 16 animals, subdivided into 4 groups of 4, were used in each experiment, and treated according to the schedule reported in Tables 1 and 2

Iodoacetic acid at a dose of 40 mgm /kgm was administered intravenously This dose, as already indicated in a previous paper[5], is definitely sub-lethal X-irradiation was performed with a Picker instrument operating at 220 kV , 15 m amp , with an added filtration of 0 5 mm copper and 1 mm aluminium, at a dose rate of 26 r /min , for a total dose of 150 r

When the animals were subjected to a combined treatment, irradiation was performed 30 min after the administration of iodoacetic acid

At a dose of 25 μc /rat ^{32}P was injected intramuscularly immediately after irradiation Non-irradiated rats were given ^{32}P 36 min after the iodoacetate treatment The animals were killed with ether 2 hr after the injection of ^{32}P, and the thymus, spleen and 15 cm of the small intestine, just distal

Table 1. Corrected Specific Activity of DNA* extracted from the Spleen, the Intestine and the Thymus of Normal and Treated Animals 2 hr. After the Administration of 25 μc ^{32}P/Rat (Mean ± Standard Error of the Mean)†

	Controls	Iodoacetic acid (40 mgm./kgm.)	X-rays (150 r.)	Iodoacetic acid (40 mgm./kgm.) X-rays (150 r.)
Spleen	4·44 ± 0·76	(21 per cent)‡ 3·52 ± 0·63	(46 per cent) 2·40 ± 0·34	(56 per cent) 1·94 ± 0·36
Intestine	2·16 ± 0·19	(12 per cent) 1·90 ± 0·30	(5 per cent) 2·05 ± 0·16	(24 per cent) 1·64 ± 0·16
Thymus	3·64 ± 0·36	(23 per cent) 2·79 ± 0·35	(28 per cent) 2·61 ± 0·27 $t = 2·606$,	(50 per cent) 1·81 ± 0·14 $0·05 > P > 0·02$

* Corrected specific activity of DNA =

$$\frac{\text{specific activity DNA (c.p.m./}\mu\text{gm. phosphorus)}}{\text{specific activity of blood orthophosphate (c.p.m./}\mu\text{gm. phosphorus)}} \times 100$$

† Each figure is an average of five values obtained from the pooled organs of four animals.

‡ Figures in brackets represent percentage of inhibition.

to the pylorus, were rapidly removed. The organs of the four animals of each group were pooled for the extraction of DNA. All successive steps have been described in our previous paper[5].

Table 1 contains the values of the corrected specific activity of DNA extracted from thymus, spleen and intestine of the experimental animals. These values show an inhibiting effect following treatment with iodoacetic acid, as already described. The results, moreover, confirm the well-known inhibitory effect of radiation on ^{32}P uptake into DNA from lymphoid organs, whereas the effect is not yet detectable for the intestine at the dose-level employed. Finally they show that after combined treatment, the inhibitory effect nearly corresponds to the sum of the effects obtained by single treatments.

Experiments in which ^{32}P was injected 4 hr. after irradiation have also been carried out. The results are reported in Table 2. In this case we considered only thymus and intestine. These experiments were undertaken because the inhibition by irradiation of the incorporation of labelled precursors into DNA of radiosensitive organs reaches higher values after this time interval[7].

Table 2. Corrected Specific Activity of DNA extracted from the Intestine and the Thymus of Normal and Treated Animals 2 hr. After the Administration of 25 μc ^{32}P/Rat (X-ray Treatment 4 hr. Before ^{32}P)

	Controls	Iodoacetic acid (40 mgm./kgm.)	X-rays (150 r.)	Iodoacetic acid (40 mgm./kgm.) X-rays (150 r.)
Intestine	2·43*	(12 per cent)† 2·14	(46 per cent) 1·31	(49 per cent) 1·24
Thymus	3·09	(9 per cent) 2·82	(63 per cent) 1·13	(73 per cent) 0·82

* Each figure is an average of three values obtained from the pooled organs of four animals.

† Figures in brackets represent percentage of inhibition.

It will be noted that the effect of irradiation is much more marked at 4 hr. than at 2 hr., as expected, whereas the effect of iodoacetate is clearly lower. The response to the combined treatment in this case also nearly corresponds to the sum of the effects obtained after single treatments.

In conclusion the results reported in the present paper seem to indicate that (a), the combined treatment with iodoacetate and X-rays, at the doses used in our experiments, causes an inhibition of ^{32}P uptake into DNA corresponding nearly to sum of the inhibitions obtained by single treatments, (b), the effect of iodoacetate decreases significantly a few hr. after the administration of this substance, whereas at the same time interval the irradiation effect increases.

A possible interpretation of the results could be that the effect of iodoacetate on DNA synthesis combining with the effect of radiation may increase the total cellular damage in radiosensitive tissues.

In discussing the present results it must be taken into account that, with the doses of X-rays used in our experiments, it is possible that the reduction in DNA synthesis is the result of temporary mitotic delay and not of cell-death, whereas the sensitizing effect of iodoacetic acid has been established for cell-death. Another point to be considered is that the effect of iodoacetic acid seems to be reversible in a short time. It is not yet known whether the increased inhibition of DNA synthesis, induced by iodoacetic acid in combination with low doses of X-rays, can really affect the final cellular damage in radiosensitive tissues.

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Protection by Inorganic Condensed Phosphates against the Lethal Effects of X-rays

A radioprotective effect of adenosine triphosphate has been described by Langendorff *et al.*[1], but the inorganic condensed phosphates do not appear to have been studied previously in this connexion. We have found that sodium pyrophosphate, the linear tripolyphosphate and the cyclic trimeta- and tetrametaphosphates markedly reduce the mortality in mice due to X-rays when injected intraperitoneally in neutral solution, shortly before exposure.

Female mice, weighing 20–25 gm., of *R* stock (a cross between *PCT* female and *C3H* × 101 male) were used at the age of 14 weeks. In a typical experiment 50 mice, randomized into groups of 5, were irradiated in partitioned boxes as follows: 250 kV., 15 m.amp., H.V.L. 1·2 mm. copper, distance to underside of box 60 cm., average dose-rate 73·6 r./min. Ten mice were used on each of 4 protective treatments, with 10 saline-treated controls. All 5 treatments were represented in each group during irradiation and the subsequent period of observation. Survival at 30 days was taken as the measure of protective effect. In a series of experiments on this pattern 49 saline-treated control animals died after 1,025 r., out of a total of 53 used. Thus the overall control mortality was 92 per cent, and this can be used for statistical tests with the tables of Fisher and Yates[2], but the mouse population may change its radiosensitivity and a safer though more severe test, using only the simultaneous controls, is made possible by Fisher's exact 2 × 2 contingency function as tabulated by Mainland, Herrera and Sutcliffe[3]. The results shown in Table 1 were submitted to both tests as indicated.

Since trimetaphosphate at 15 m.moles/kgm. protected 100 per cent against 1,025 r. a further experiment was done to estimate to what level it raised the radiation LD_{50}, and to compare it in this respect with *S*,β-aminoethylisothiuronium bromide hydrobromide (AET) at 1 m.mole/kgm., the highest safe dose for *R* mice. Both were given intraperitoneally 10 min.

Table 1. PROTECTIVE EFFECTS OF INORGANIC CONDENSED PHOSPHATES AGAINST 1,025 r. OF X-RAYS

Compound	Dose (m.moles/kgm.)	Time of injection (min. before irradiation)	No. of mice surviving / No. used: Exp.	No. of mice surviving / No. used: Controls (saline)
$Na_4P_2O_7·10H_2O$	0.75*	10	6/10†	1/10
,,	0.50	10	2/10	1/10
$Na_5P_3O_{10}·6H_2O$	1.00*	10	14/20‡	0/20
,,	0.75	10	3/10	0/10
,,	1.00	30	8/10‡	0/10
,,	1.00	60	2/10	0/10
$(NaPO_3)_3·6H_2O$	15*	10	10/10‡	1/10
,,	9	10	1/10	
$(NaPO_3)_4·4H_2O$	2.4*	10	8/10‡	

* These doses resulted in no deaths ascribable to toxicity. At higher dose-levels some deaths occurred shortly after irradiation, as a result of potentiation of toxicity by X-rays.

† Significant ($P < 0.005$) assuming 8 per cent control mortality (ref. 2).

‡ Also significant ($P < 0.01$) by 2 × 2 contingency test using only internal controls (ref. 3).

Table 2. PROTECTIVE EFFECTS OF TRIMETAPHOSPHATE AND AET

X-ray dose (r.)	No. of mice surviving at 30 days out of: 4 given trimetaphosphate	4 given AET	2 given saline
1,050	3	4	0
1,100	2	4	0
1,150	3	4	0
1,200	0	4	0
1,250	1	4	0
1,300	1	0	0
1,350	0	3	0

before irradiation. The results are given in Table 2. From these the normal-logarithmic maximum-likelihood estimate of the LD_{50} (by the iteration procedure of Cornfield and Mantel[4]) is 1,133 (1,059–1,213) r. for trimetaphosphate and 1,325 (1,265–1,387) r. for AET. The figures in brackets are 5 per cent fiducial limits.

Thus trimetaphosphate is significantly less protective than AET, but its lower fiducial limit, 1,059 r., is well above the LD_{50} of uninjected mice of this strain, which, though not measured simultaneously, is known to be about 860 r. There are several conceivable reasons for the protective activity of condensed phosphates, which we are investigating.

We wish to thank Mrs. D. M. R. Lowman for able technical assistance, Mr. M. J. Corp for irradiations and Dr. D. R. Peck, of Albright and Wilson, Ltd., for his interest and for generous gifts of pure chemicals.

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BIOLOGY

'Bound' Gibberellin in Mature Runner Bean Seeds

RADLEY[1] has mentioned the possibility that gibberellins occur in the tissues of higher plants in both 'free' (readily extractable) and 'bound' forms. By analogy with plant-growth regulating substances of the auxin type, the hypothetical 'bound' gibberellins might be liberated by the hydrolysis of proteins of the plants being investigated. This communication describes one of several experiments in which more 'gibberellin-like' activity was detected in hydrolysed than in non-hydrolysed extracts. Radley[1] has observed that the level of readily extractable gibberellin of the seeds of the runner bean (*Phaseolus multiflorus*) falls as the seeds mature. One could visualize that the gibberellin is becoming 'bound' in some way as maturation proceeds, and because of this possibility seeds of the runner bean were chosen for investigation.

Mature runner bean seeds, mean length 28 mm., were divided into three samples, each of 120 gm. Sample *A* was homogenized in 500 ml. of a mixture of equal parts of acetone and water. (A mixture of acetone and water has been used as a primary solvent for the extraction of gibberellins from plant tissue by several workers, for example Phinney *et al.*[2], Bunsow *et al.*[3], Lang[4], Lona[5], Murakami[6], and Nickell[7].) The extract was filtered through cheese cloth, centrifuged, and the acetone evaporated *in vacuo* to leave an aqueous residue which was adjusted to 500 ml. with distilled water.

Sample *B* was homogenized in 500 ml. of phosphate buffer, *p*H 6.2, made by dissolving 136 gm. of potassium dihydrogen orthophosphate and 24 gm. of potassium hydroxide per litre of water. (This buffer has been used by Borrow *et al.*[8] as a solvent at one stage in the isolation of gibberellic acid from fungal cultures, and by Radley[1] as a solvent at one stage in experiments demonstrating the presence of gibberellin-like substances in higher plants, but it was not used as a primary extracting solvent.) The extract was filtered and centrifuged.

Sample *C* was homogenized in 500 ml. of phosphate buffer, *p*H 6.2, and the extract filtered and centrifuged. 4.0 gm. of crude ficin (L. Light and Co., Ltd.) and 6.0 mgm. of cysteine were added to the extract which was then stored at 30° C. for 12 hr. At the end of this time the extract was again centrifuged.

The three extracts were purified further in the same way: each was adjusted to *p*H 2.8–3.0 using *N* hydrochloric acid, and extracted three times with 400 ml. of ethyl acetate. The lower (aqueous) phase was discarded and the bulked ethyl acetate-rich extract reduced in volume to 50 ml. *in vacuo* at a temperature not exceeding 30° C. The extract so obtained was adjusted to *p*H 2.8–3.0 using *N* hydrochloric acid, and extracted three times with an equal volume of ethyl acetate. The bulked ethyl acetate-rich extract was reduced in volume and placed as a line across a 23-cm.-wide strip of acid-washed Whatman No. 3 chromatography paper. The chromatogram was run using a descending technique, and as a solvent the upper phase of a mixture of *n*-butanol/1.5 *N* ammonium hydroxide (3 : 1). Gibberellin A_1 (which is known to occur in the runner bean[9]) and gibberellic acid run with an R_F in the region 0.2–0.3 in this solvent system (see, for example, refs. 2, 10). When the solvent front had moved a convenient distance down the paper, some 25 cm. from the start line, each chromatogram was allowed to dry at room temperature and cut into transverse strips, each strip being in width one-tenth of the distance between the start line and the solvent front. Each strip was eluted with approximately 30 ml. of acetone in an apparatus designed by Canny[11]. The acetone was evaporated, and the dry residue stored in a deep freeze ready for assay.

Dwarf pea seedlings (var. Meteor) were used as assay plants in a technique essentially similar to that already described[10]. At the time of assay each residue was taken up in 0.8 ml. of 30 per cent ethanol.

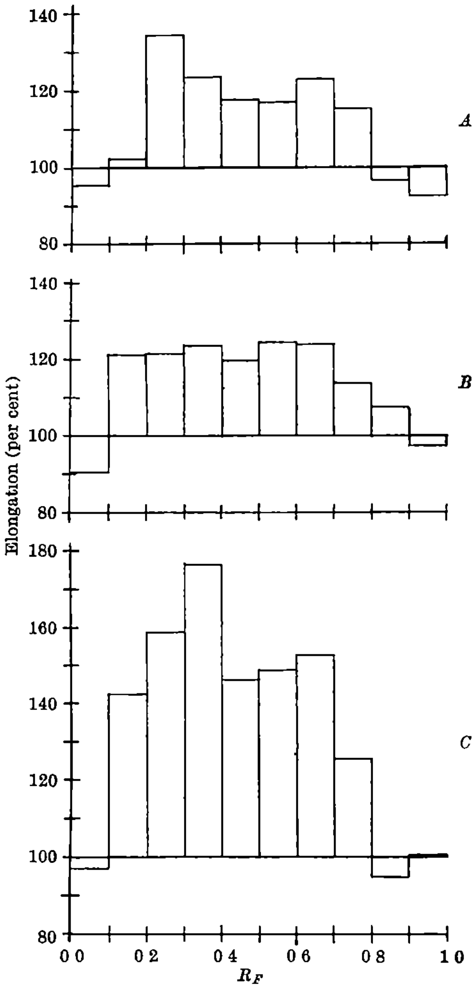

Fig 1 Percentage elongation of pea seedlings treated with eluates from chromatograms of bean extracts *A*, Extract made in acetone/water, *B*, extract made in phosphate buffer, *C*, extract made in phosphate buffer and hydrolysed with ficin

and a 0 004 ml droplet of this solution was placed on each assay plant A small puncture was made in the stem of each plant to aid penetration Ten plants were used for each solution being assayed Eluates from corresponding R_F values of the chromatograms of the three extracts were assayed in the same batch of assay plants

Fig 1 shows the mean elongation of the measured internodes of the treated plants expressed as a percentage of the corresponding internodes of the controls It will be seen that there is considerably more activity in the hydrolysed than in either of the non-hydrolysed extracts Activity is in each case spread over much of the chromatogram, but two zones are in evidence—one at R_F 0 2–0 4, the other at 0 6–0 7 By comparing the responses brought about by the extracts with those induced by known amounts of gibberellic acid, the total activities of the extracts were estimated as *A*, 0 04, *B*, 0 04, and *C*, 0 23 μgm of 'gibberellic acid equivalents' per gram fresh weight of extracted material That is, there is about six times as much activity in the hydrolysed extract. The gibberellins detected here may very well differ in their biological activities from the activity of gibberellic acid, so that the above figures should not be taken to indicate the actual levels of gibberellin in the tissue

Other extracts of mature runner beans have shown increased activity due to enzyme hydrolysis, while extracts of young beans have in general shown no such increase Negligible activity was detected in an extract subjected to alkaline hydrolysis In all cases buffer has proved to be at least as effective an extracting solvent as a mixture of acetone and water

I am indebted to Dr M J Canny for his interest and advice during the course of these experiments

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Gibberellic Acid and Chlorophyll Content of Leaves of Meteor Peas

The present investigation was to determine whether the effect of gibberellic acid in causing chlorosis was due to an interference in chlorophyll formation or to some secondary effect[1] *Pisum sativum* var. Meteor were grown in the field in a block arrangement, and alternate rows were treated with gibberellic acid (*Y F* 4961, Plant Protection, Ltd) Gibberellic acid diluted to 100 p p m with water was sprayed on the upper surface of the plant 10 days after emergence

Three days after spraying slight chlorosis of the third leaves was apparent Chlorosis increased in intensity in leaves expanding afterwards, but no change in colour was observed in the first and second leaves The internodes from the second upwards showed a 180–250 per cent increase in length over the controls Measurement of the length and breadth of a random sample of leaves of control and treated plants was made 7 and 14 days after spraying The total number of cells[2], chlorophyll content[3], and the fresh and dry weight of a finite area of leaf were measured for disks of known area punched from a similar position 8 and 15 days after spraying Measurements were also made of individual palisade cells in a similar region from which the disks were punched, and the depth of the sections measured to find the thickness of leaf in this area

Measurements of the leaf dimensions showed that those of treated plants, with the exception of the oldest leaf, were longer than those of the controls, but of similar breadth Palisade cells in treated material tended to be broader and shorter in length The results given in Table 1 show that total chlorophyll tended to be slightly less in treated leaves expanded after spraying However, chlorophyll content expressed in terms of dry weight, fresh weight, or weight/cell shows no such decrease Cell counts show that treatment with gibberellic acid resulted in a significant decrease in the number of cells per unit leaf area in leaves not fully expanded at the time of spraying. This decrease is related to the decreased thickness of the leaf

Table 1

Leaf No	Total chlorophyll (mgm /90 mm ²)		Chlorophyll gm /100 gm fresh weight		Chlorophyll gm /100 gm dry weight		Chlorophyll per cell, μgm × 10⁻⁵		Ratio chlorophyll a/b		Leaf thickness (μ)		No of cells/ 90 mm ² × 10⁶	
	Control	Gibberellic acid	Control	Gibberellic acid	Control	Gibberellic acid	Control	Gibberellic acid	Control	Gibberellic acid	Control	Gibberellic acid	Control	Gibberellic acid
	8 days after spraying													
1	128 ± 21	129 ± 23	0 14 ± 0 02	0 14 ± 0 03	1 4 ± 0 2	1 4 ± 0 3	5 9	6 2	2 6	2 5	388 ± 53	398 ± 24	2 16	2 10
2	123 ± 7	115 ± 9	0 14 ± 0 01	0 12 ± 0 01	1 4 ± 0 1	1 4 ± 0 2	5 6	5 8	2 7	2 7	385 ± 25	354 ± 28	2 18	1 96
3	101 ± 14	94 ± 11	0 13 ± 0 01	0 14 ± 0 03	1 2 ± 0 1	1 4 ± 0 2	3 9	4 5	3 2	3 3	369 ± 43	256 ± 32	2 62	2 11
4	63 ± 3	59 ± 11	0 12 ± 0 01	0 14 ± 0 02	0 82 ± 0 2	1 1 ± 0 2	1 8	2 1	2 8	3 3	209 ± 37	145 ± 10	3 73	2 85
	15 days after spraying													
2	106 ± 3	99 ± 5	0 098 ± 0 01	0 12 ± 0 01	0 94 ± 0 05	0 89 ± 0 06	4 4	5 0	2 6	2 7	—	—	2 40	1 85
3	100 ± 25	81 ± 29	0 12 ± 0 02	0 12 ± 0 02	0 91 ± 0 07	0 91 ± 0 1	3 6	4 2	2 9	2 7	288 ± 48	246 ± 20	2 77	1 94
4	72 ± 11	61 ± 14	0 12 ± 0 01	0 13 ± 0 01	0 93 ± 0 06	0 98 ± 0 1	2 3	4 0	3 2	3 1	262 ± 35	169 ± 32	2 49	1 55
5	80 ± 18	57 ± 11	0 15 ± 0 02	0 16 ± 0 02	1 2 ± 0 3	1 1 ± 0 1	2 3	3 0	2 9	2 8	185 ± 39	140 ± 15	3 46	1 90

It can be concluded from the above results that the appearance of chlorosis in material treated with gibberellic acid is not due to lower chlorophyll content per cell but is apparently an artefact arising from the effect of gibberellic acid on leaf expansion This results in the treated material having leaves of greater surface area which are thinner than the controls and thus having a shorter optical path for absorption of light Similar results were obtained in two successive seasons, the hot bright summer of 1959, and the cooler wet summer of 1960 Our results are in agreement with those of Wolf and Haber[4] In their case with wheat seedlings an influence of nutritional factors on the chlorophyll content became apparent in older plants, this was not observed with the meteor pea plants grown in the field

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Effect of Auxin on the Osmotic Value in Pea Stem Sections

The osmotic concentration in pea stem pieces was found to be increased by indole-3-acetic acid (IAA) when they were smeared with lanolin paste of IAA and kept at constant humidity[1] Treatment with auxin solution was avoided in this case in order to exclude the possibilities of a secondary osmotic change due to water absorption and leaching of solutes from cells In the experiment to be reported here, however, auxin solution was used in order to observe an osmotic change immediately following the auxin action

Excised pieces, 10 mm long, from etiolated stems of Alaska peas were weighed and floated on 10 and 200 mgm /litre solutions of IAA and on water at 25 5° C Samples were withdrawn at intervals, blotted with filter paper, and the freezing point depression of tissue juice was determined to the nearest 0 01° C [1], using a thermojunction The freezing point of the juice was around − 0 56° C before the tissue was introduced into the solutions Changes of freezing point in the solutions are shown in Fig 1

The osmotic concentration at first increased, following exposure to IAA, and then decreased rapidly after 30 min or so In the water control, on the other hand, no temporary increase in osmotic concentration was observed Experiments not presented here showed that phenylacetic acid and acetic acid in the molar concentration equivalent to 200 mgm /litre of IAA did not cause a rise in osmotic concentration as IAA did

No significant difference in the rate of water absorption was found between the two auxin-levels in the first 30 min, even though the osmotic values at the two levels differed greatly Water permeability may have been a limiting factor

The drop in osmotic concentration may be ascribed to (1) dilution due to water absorption (which is calculable) and (2) leaching of solutes from cells to outer solution The more rapid drop in the presence of IAA may probably be ascribed to an increased permeability[2-6]

It has been reported that the osmotic concentration is increased by IAA[7,8] It is, however, worthy of note

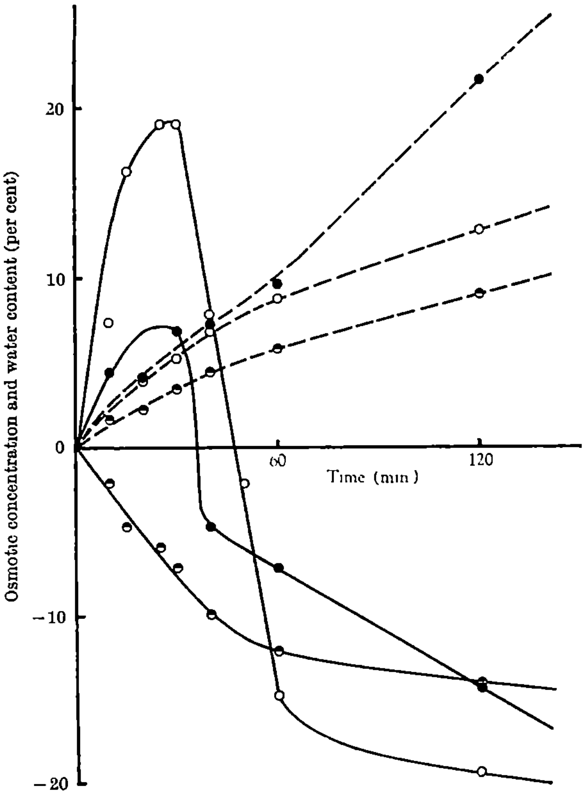

Fig 1 Water absorption (- - -) and osmotic concentration (—) of pea stem sections treated with water (◐), and 10 mgm (●), and 200 mgm (○), of indole-3-acetic acid per litre

that the osmotic effect appears in such a short time as shown here In order to see how rapidly the cell wall extensibility is affected by auxin, longitudinal tension of 2 5 gm was applied to stem pieces soaked for various periods in 200 mgm /litre solution of IAA By this method[9,10], an increase in extensibility became observable in 15 min of auxin action, but not in 10 min Hence the osmotic change occurs earlier than the change in cell wall extensibility

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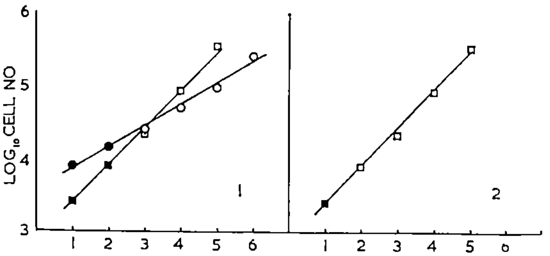
Determination of Cell Numbers in Leaf Primordia

Sunderland and Brown[1] have pointed out that there are two major problems involved in the determination of the number of cells in leaf primordia (1) numerous macerating fluids[2] tend to disintegrate the relatively delicate cell wall system of leaf primordia and (2) owing to the minute size of leaf primordia and their relatively small number of cells, fragmentary losses are a serious source of error when counting by hæmocytometer slide after dilution

A method is suggested here which mainly overcomes these difficulties and has been used successfully for the second to the sixth primordia of tomato (*Lycopersicon esculentum*) and tobacco (*Nicotiana tabaccum*) stem apices It has greater ease and rapidity of counting than the squash method originally used by Sunderland and Brown[1] and can be directly integrated with values obtained by other techniques (for example chromic acid) for larger leaflets[3]

Primordia were excised under a dissecting microscope using a small splinter from a safety razor blade gripped in a holder Immediately after excision the primordia were placed in acetic acid/ethanol, 1 1 (v/v), for at least 10 min at room temperature and then treated with *N* hydrochloric acid for 8–15 min at 60° C in an oven, depending on the size of the primordia[1] The primordia were immersed in the liquid in wells constructed by placing on a slide up to three rings cut in lengths of 3 mm from polyvinyl chloride tubing (10 and 15 mm diameter) with a razor During the treatments the slide rested on the rim of a watch glass, which stood in a Petri dish containing the same liquid as the slide in order to reduce evaporation of the liquid[1] After the removal of each reagent by applying filter paper strips to the base of the outside of the well, the polyvinyl chloride rings were removed with a pair of forceps without disturbing the primordia The primordia were then removed from the slide under the dissecting microscope, using a fine glass rod or a filter paper strip, and transferred to 10 × 0 6 cm (approx) specimen tubes containing a precisely known volume —between 0 3 ml and 1 0 ml —of 0 1 *M* ethylenediaminetetraacetic acid in 5 per cent (v/v) aqueous butanol mixture at *p*H 8 (ref 4) using ammonia solution to adjust the *p*H The tubes were tightly stoppered and the primordia in the ethylenediamine tetraacetic acid/butanol mixture were incubated overnight at 30° C The primordia were then macerated gently with a small syringe (1 0 ml) to which a finely drawn out piece of glass capillary tubing was attached with surgical polythene tubing After maceration, 0 1 ml of 1 per cent (w/v) malachite green in water was added to each tube to produce a minimum of 0 4 ml for the smallest primordia, for larger primordia the volume was made up to 1 or 2 ml and the volume of the stain increased proportionally The suspension was again drawn a few times through the syringe to ensure uniform distribution of cells and 0 2 ml was transferred to a hæmocytometer slide (Fuchs–Rosenthal, depth, 0 2 mm area, 1/16 mm^2) and counted Duplicate estimations on separate aliquots were made for each primordium



Figs 1 and 2 Tomato, ○, hæmocytometer, ●, squash, tobacco, □, hæmocytometer, ■, squash

Preliminary results obtained with this method for the second or third and older primordia of tomato and tobacco plants aged 8 weeks in November and grown in water culture are shown in Figs 1 and 2, in relation to those obtained for the first and second primordia by the squash method of Sunderland and Brown[1] For the particular purpose of these comparisons plants about 8 weeks old were selected when the youngest primordium visible on the apex (numbered 1) had attained what was judged to be almost the maximum size before the next was due to appear In Fig 2, all primordia except the first were measured by the hæmocytometer method

The numbers of cells counted for each aliquot of 0 2 ml ranged between 60 and 600 according to primordial position and species The standard error of the means of 18 pairs of counts was ± 3 7 and the mean difference between pairs did not differ significantly from zero These counts were about 0 5 or 1 per cent of the total cell number for the smaller primordia (Nos 3 or 2) and 0 1 per cent of the total for the sixth primordium The extension of counts to more mature leaves using chromic acid maceration[3] showed a linear relationship for log cell number up to about the eighth or ninth leaf in both species

We thank G M Clarke for statistical treatment and the British Council for an Overseas Scholarship to one of us (L A W)

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Potential Productivity of Marrow Stem and Thousand-headed Kale

SEED of marrow stem kale was sown in August and the plants transplanted to permanent quarters in October Approximately half of the plants flowered in the following spring The non-flowering plants continued their vegetative growth and were sampled by the 'perfect plant method' in the following October Dry-matter production (excluding roots) was equivalent to 9·7 (from an August 4 sowing) and 7·6 (from an August 19 sowing) tons per acre If we neglect the weight of the plants at transplanting time this represents a complete year's production of dry matter Plants from later August and September sowings, transplanted in April, produced by October 8·6 and 6·2 tons of dry matter (excluding roots) per acre Comparable production figures for thousand-headed kale from these later sowings were 7·7 and 6·2 tons These figures represent a production of dry-matter two or three times as great as that obtained when the kales are sown in the traditional manner in April, and much in excess of that obtained with other agricultural crops They may be compared with the annual dry matter production of 7·6 for the evergreen *Pinus*[1], 3·2 for birch[2], and 1·6–5·6 tons per acre for natural herbaceous vegetation[3]

The high productivity of the kales undoubtedly results from their early development of a full leaf cover, the autumn-transplanted marrow stem kale having a mean leaf area index of 0·4 by early May and one of approximately 2·0 by the end of that month, which compares with a leaf-area index of 0·06 at that date for a commercial crop sown in April as is customary, and which yielded 3·3 tons of dry matter per acre

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Cytochrome c Oxidase, the Terminal Oxidase

Table 1 THE EFFECT OF CARBON MONOXIDE/OXYGEN AND NITROGEN/OXYGEN GAS MIXTURES ON THE RESPIRATION OF *Iris* RHIZOME AND BAKER'S YEAST

Organisms	Inhibitor	Per cent inhibition as compared with controls in an atmosphere of 95 per cent nitrogen/5 per cent oxygen
Rhizome tissue	95 per cent carbon monoxide/5 per cent oxygen in dark	60
	95 per cent carbon monoxide/5 per cent oxygen in light	0
Baker's yeast	95 per cent carbon monoxide/5 per cent oxygen in dark	60
	95 per cent carbon monoxide/5 per cent oxygen in light	0

were split lengthways, rinsed and used immediately The uptake of oxygen of the 'half-cores' was measured in Warburg manometers at 10° in a damp atmosphere of 95 per cent carbon monoxide/5 per cent oxygen or 95 per cent nitrogen/5 per cent oxygen both in the light (250-W mercury vapour lamp) and in the dark A suspension of baker's yeast in 0·1 *M* phosphate buffer, *p*H 6·0, was also used as a comparison and the dark carbon monoxide inhibition was 60 per cent in both cases and fully reversed in the light (Table 1)

It is generally accepted that cytochrome *c* oxidase is the terminal oxidase of baker's yeast and the fact that a similar light-reversed carbon monoxide inhibition of 60 per cent was obtained with *Iris* is evidence that a similar state of affairs exists in this tissue These results would support the widely held view that cytochrome *c* oxidase forms the main terminal oxidase even in mature plant tissues[2]

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GERONTOLOGY

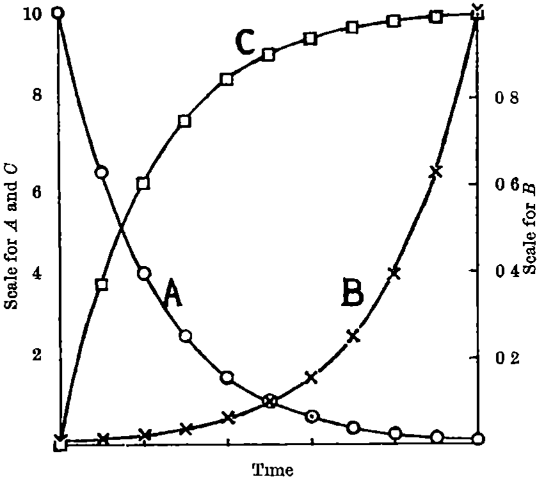

Fig 1 Graphs against time of (*A*) specific growth-rate in arbitrary units, (*B*) its reciprocal, (*C*) its negative (deficit below its maximal value)

looking into the basis for Minot's view on the rate of senescence Most people probably believe that his relation implies that the state of senescence is proportional to the reciprocal of the specific growth-rate and accept it on this understanding. However, if the reciprocal of the values of the specific growth-rate in Fig 1,*A* are plotted on a suitable scale (Fig 1,*B*) the curve is a climbing exponential, convex and not concave to the time axis, the rate increasing most slowly at first and progressively more rapidly afterwards ; this is in keeping with the specific death-rate and other criteria, as well as with intuitive judgments Minot's relation really assumes that the state of senescence is proportional to the negative of the specific growth-rate (Fig 1,*C*) or more precisely to its deficit below an initial maximal value, that is, to $(k-x)$ rather than to k/x. This has much less appeal on the score of biological rationale, and so there is neither a very strong argument, nor much evidence, in favour of his relation, with its grotesque paradox should greatly clarify the situation and but is maximal between sixty and seventy years of age, and slower in the very old Consequently the specific growth-rate increases again in old age There is direct evidence of this in the rate of cell division in some tissues[5]. Other properties also reverse their trend in later life, and if this is true of senescence, also, it may provide some slight justification for the popular jest about second childhood[5]

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MICROBIOLOGY

Physical Conditions as Important Factors for the Regeneration of Naked Yeast Protoplasts

In our previous papers[1] we have discussed the growth and regeneration of naked yeast protoplasts of *Saccharomyces cerevisiae* obtained by means of cell wall autolysis In the former experiments the growth and regeneration took place on solid synthetic media The frequency of regeneration was low on these media, while nearly all the protoplasts were capable of growth, in fact only about 1 per cent regenerated into new yeast cells (in a complicated manner formerly described).

Studying the factors influencing regeneration, we have found that practically all the protoplasts are capable of regeneration under suitable conditions We were successful in finding conditions under which the protoplasts regeneration is nearly 100 per cent

Protoplasts do not regenerate in liquid media at all The frequency of regenerations is very low on the surface of solid agar media and the chemical composi-

[illegible] *Iris pseudacorus* L.

WHILE it is generally agreed that cytochrome *c* oxidase is the terminal oxidase of embryonic plant tissues, some workers doubt if it has this role in older plant tissues as well[1] It is of interest that a mature tissue such as the rhizome of *Iris pseudacorus* L , obtained from a Lancashire marsh, should contain a cytochrome system which might reasonably be claimed to account for the oxygen uptake of the tissue The rhizome tissue used was cut from within the endodermis of the current year's growth, 1 0 cm or farther from the meristematic region The cells were typical parenchymatous cells except that the tissue also contained some vascular cells

The presence of a cytochrome system was apparent from the absorption spectra of slices of reduced tissue when examined with a direct-vision spectroscope The positions of the maxima of the absorption bands in the visible region corresponded approximately with the $(a + a_3)_a$, b_a and c_a cytochrome bands of a reduced yeast suspension with which they were compared, except that the b_a and c_a bands of the *Iris* tissue were not distinctly separated

Cores of tissue were used for respiration experiments These cores 1 cm diameter and 2 5 cm long

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WE owe to Minot[1] a startling paradox [illegible] view that the rate of senescence is most rapid at the beginning of life and slowest at the end to quote the fourth of the laws by which he summarized his conclusions, 'Senescence is at its maximum in the very young stages and the rate of senescence diminishes with age" The justification for his view was that the intrinsic or specific growth-rate of the tissues decreases most rapidly at the outset and progressively more slowly afterwards, following a roughly exponential die-away curve (Fig 1,*A*) It seemed a reasonable assumption that the senescent state is related to the loss of the power of growth and so the paradox has been widely accepted, notwithstanding the intuitive conviction that the process of senescence is not significant until after the prime of life The postulated initial, rapid stages of senescence have to be shrugged off as 'insensible'

However, there are properties relevant to senescence which can be measured and which are not found to increase most rapidly at the outset the specific death-rate, for example, is minimal not at the outset but in late childhood , it then begins to increase slowly, with progressive acceleration[2], and not most rapidly at first It is therefore worth

Even the reciprocal relationship between senescence and growth-rate is only acceptable with a certain stipulation In man increase in height and weight ceases around the age of thirty At this early age, therefore, when he is manifestly in his prime, the reciprocal of the specific growth-rate tends to infinity Soon after this time actual shrinkage begins, and the specific growth-rate becomes negative Consequently, its reciprocal now swings sharply to $-\infty$ and then climbs to finite but permanently negative values To convert this into biological sense it is necessary to assume that the relevant growth-rate is not mere increase in size but that of growth in the broadest sense, including maintenance and repair—perhaps in fact anabolism *in toto*[3] Growth in this sense remains positive throughout life, though eventually unable to keep pace with catabolism It does not imply an infinite rate of senescence at any stage, therefore With this modification, moreover, senescence is not maximal at the age of thirty, there is no discontinuity at that age, anabolism continuing a smooth decline

There is a further point which deserves attention The rate of shrinkage in man, as shown, for example, by Quetelet's data[4], does not increase to the end

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tion of the media does not influence appreciably the frequency of regenerations But nearly 100 per cent regeneration can be achieved 24 hr after inoculation by cultivation of protoplasts inside solid media containing gelatin (see Table 1) (On the surface of agar media regeneration does not take place before 5 days)

It is seen from Table 1 that the concentration of gelatin is important Regeneration is not accomplished in gelatin with concentration less than 15 per

Table 1

Medium	Mode of cultivation	Concentration of gelatin (per cent) 0	5	10	15	20	30	40
Synthetic	On the surface of gelatin	0	0	0	0*	0*	0*	0*
	Inside gelatin	0	0	0	Reg + –	Reg	Reg	Reg
Synthetic + 30 per cent of gelatin hydrolysate	On the surface of gelatin	0	0	0	0*	0*	0*	0*
	Inside gelatin	0	0	0	Reg + –	Reg	Reg	Reg

* Only the protoplasts which have penetrated into gelatin regenerate

0, No regeneration, *Reg* + –, sporadic regeneration, Reg , nearly all the protoplasts regenerate cent (at a temperature of 22° C) Protoplasts growing on the surface of gelatin of any concentration do not regenerate, but protoplasts have proteolytic activity and can penetrate into the gel where they can regenerate The contact of a protoplast with gelatin gel is not sufficient for regeneration, protoplasts have to be surrounded by the gel Further, we can see from Table 1 that regeneration does not take place in media containing 30 per cent of hydrolysate of the gelatin, unless a gel of unhydrolysed gelatin of proper concentration is used Thus, regeneration is not influenced by the chemical composition of gelatin Regeneration is not, likewise, accomplished in liquid media with albumin of different concentrations

It may be concluded, therefore, that nearly all the yeast protoplasts have not only the ability to grow but also capacity for regeneration, and that physical conditions, that is, surrounding of protoplasts by the gel of certain consistency, are important for regeneration The explanation of the mechanism of this process will be considered later

These results, as well as the micro-cinematographic registration of the whole process, seem to disprove the opinion of Eddy and Williamson[2] that in naked yeast protoplasts there is not an autoregeneration, but a formation of cells by budding of spores accidentally present in the suspension of protoplasts

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VIROLOGY

Transmission of Clover Phyllody Virus by *Aphrodes bicinctus* (Schrank) in North America

Phyllody, a disease of clovers, occurs in eastern North America and appears to be similar or identical to the disease present in England, continental Europe

six healthy plants later showed signs of virus infection (chlorosis, bronzing and stunting) and five out of the six plants developed phyllody symptoms The remaining plant failed to flower

Three adults of *A bicinctus*, collected at Lavaltrie, Quebec, from a field of clover where phyllody was naturally present, were allowed to feed on disease-free ladino clover for one week to determine if they carried the virus They were then allowed to feed for nine days on ladino clover which was infected with phyllody virus, after which they were transferred to virus-free ladino clover plants at irregular intervals until the last insect died. The leafhoppers were not viruliferous when captured but became infective after feeding on diseased plants They transmitted the virus to each of four healthy test plants on which they were serially transferred These plants later displayed typical leaf symptoms Three out of the four clover plants developed phyllody

These experiments demonstrate that *A bicinctus* is capable of transmitting the virus that causes clover phyllody in North America A search for additional vectors of the virus in eastern Canada is in progress

This is believed to be the first report of transmission of the virus causing clover phyllody by a leafhopper in North America

Grateful acknowledgment is extended to Dr W R Richards, Entomology Research Institute, Ottawa, for identification of *Aphrodes bicinctus* (Schrank)

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Movement of Tobacco Mosaic Virus through Epidermis of *Nicotiana glutinosa* Leaves

were taken 3, 3½, 20 and 21 hr. after inoculation. The experiment was repeated several times. No local lesions appeared on the stripped area even 48 hr. after stripping, whereas about 150 local lesions appeared 23–26 hr. after inoculation on the rest of the leaf. On the other hand, bio-assays of areas devoid of epidermis revealed that virus was always present even after only 3 hr. In another experiment, portions of the epidermis of detached leaves were stripped off and 20 hr. later the stripped areas were then inoculated in the usual manner. No local lesions appeared on the stripped areas.

Stripping of the upper epidermis is very difficult, presumably because the epidermal layer is tightly connected to the underlying palisade cells. The strips were examined under the low magnification of a compound microscope and appeared to consist of the epidermal cells plus a thin portion of the palisade layer. The palisade layer was therefore rather heavily damaged in the stripping process.

It appears that in detached *N. glutinosa* leaves tobacco mosaic virus penetrates through epidermal strips into the palisade in less than 3 hr., but that for some reason under my conditions, the presence of epidermis is required for necrotic lesion formation.

This work was done while I was a research assistant at the University of California and was supported in part by a grant No. *E*-3415 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

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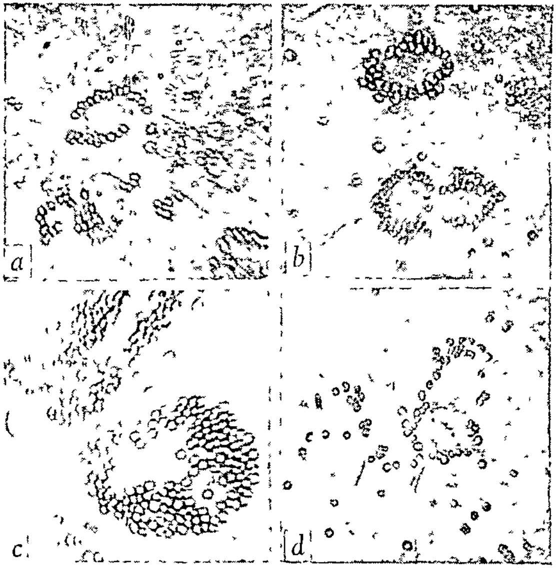

Fig. 1. Utilization of sheep red blood cells in the hæmadsorption test for myxoviruses

(Japan 305), *B* (Lee and *B*54), and mumps virus. Controls consisted of normal uninoculated cells and infected cells to which a 1 : 10 dilution of specific rabbit virus antiserum or normal rabbit serum was added 10 min. prior to the addition of sheep red blood cells. In all cases the controls showed no hæmadsorption. It was also possible to show hæmadsorption to sheep red blood cells with an influenza *C* strain in a continuous line of cells isolated from rabbit heart.

Fig. 1 shows hæmadsorption 'rosettes' seen with myxoviruses and the kerato-acanthoma cell line. Very little difference is seen between the response in the case of mumps virus and two of the influenza *A* strains (*PR*8, and *FM*1) tested (Figs. 1*a* and *b*). In the case of the *A*2 strain (Fig. 1*c*), however, the 'rosettes' often take on a much heavier appearance. The reason for this occurrence is not known, but it may be related to the amount of 'free' virus on the

TIMBER TECHNOLOGY

Effect of Temperature on the Strain Recovery in Wood

UNTIL recently, strains occurring beyond the limits of plastic creep in wood were, because of the similarity to creep in crystalline solids, identified with micellar creep in cellulose and considered as plastic strains[1] Investigations carried out by Ivanov[2], in which swollen wood was subjected to cyclic loading and unloading with compression stresses above the limit of plastic creep along the fibre direction, showed that after each cycle of loading and unloading, both elastic and residual strains are increasing Moreover, Ivanov found that strains occurring during compression beyond the limits of plastic creep, when wood is compressed along the direction of fibre length until the moment of showing symptoms of failure, were not connected with microscopic displacement of wood structure components On the other hand, strains occurring beyond the limits of plastic creep when wood is compressed across the fibre direction are, to some extent, related to the deformations of cell cross-section shapes[3].

On the basis of present available research results, strains occurring in wood can be divided into three categories elastic strains, elastic lagged strains, and plastic strains

Investigations on the stress relaxation in wood showed that stresses are undergoing relaxation with the passage of time regardless of whether they are higher or lower than those occurring at plastic creep limit[4,5].

Moisture content and temperature of wood are exerting a decisive effect on the relaxation of stresses in such a way that the increase of moisture content and temperature is accelerating stress relaxation[6].

We have now determined the effect of temperature on the strain recovery in wood after compression across the fibre direction The detailed results of these experiments are to be published in the near future Experiments which are the subject of this report were conducted on green, maximum swollen beech-wood (*Fagus sylvatica* L) Samples of dimensions 20 × 20 × 30 mm were subjected to compression with a thrust amounting to 120 per cent of ultimate strength (σ_D) For ultimate strength, the stresses corresponding to the limit of plastic creep were assumed

Experiments consisted of the following independent treatments (1) loading of wood at 20° C followed by heating it to 100° C after a lapse of 24 hr from the moment of unloading, (2) loading of wood at 20° C followed by heating it to 40, 60, 80 and 100° C immediately after unloading, (3) loading of wood at 20, 40, 60, 80 and 95° C and observing the strain recovery at the same temperatures

Because the wood was wet and in a state of maximum swelling, water used as a heating medium was raised to the predetermined temperature in order to eliminate the possible effect of wood moisture-content changes on the results of observations In the evaluation of test results, the correction resulting from anomalies of the coefficient of linear temperature strains in moist wood was introduced

Maximum swollen wood, when heated in w
is subject to elongation in a tangential di
and contraction in radial and lo
directions[7-9]

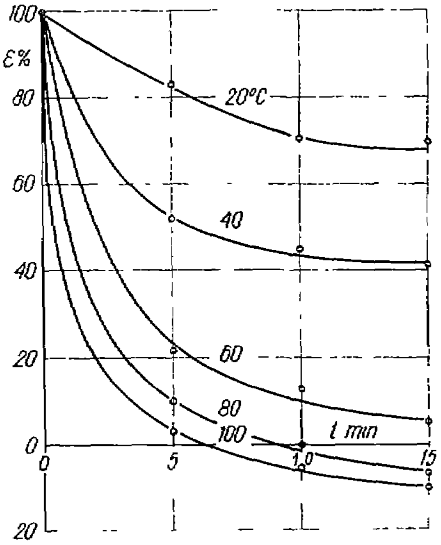

Fig 1 Effect of temperature on the strain recovery in beech-wood compressed across the fibre direction

Experiments in the first treatment showed that wood, like other substances with large molecules of chain structure[10], is also subject to the thermo-elastic strain recovery effect This phenomenon appeared also when the temperature of wood, previously steamed for 6 hr, was increased from 20° C to 100° C

The effect of temperature on the strain recovery, expressed in percentage of strain, and observed immediately after unloading, is illustrated in Fig 1 It is evident that the rate of strain recovery increases with the rise of temperature Also it may be noted that at 80° C and 100° C, wood sample dimensions in the direction of compression exceed initial dimensions in that direction.

From Fig 2 it may be seen that wood previously heated to various temperatures and loaded with the same compressing force (120 per cent σ_D) recovers its initial dimensions almost completely when heated during loading, and after unloading maintains these dimensions regardless of the magnitude of strain which occurs during loading

From the results obtained it is evident that when maximum swollen wood is heated it acquires a high degree of elasticity There is a probability that the total strain occurring at some moment after unloading the wood sample, and regarded commonly up to now as a plastic strain (durable, non-recoverable), is only apparently plastic, being the sum of
and real elastic strains The
elastic strain is intrinsically
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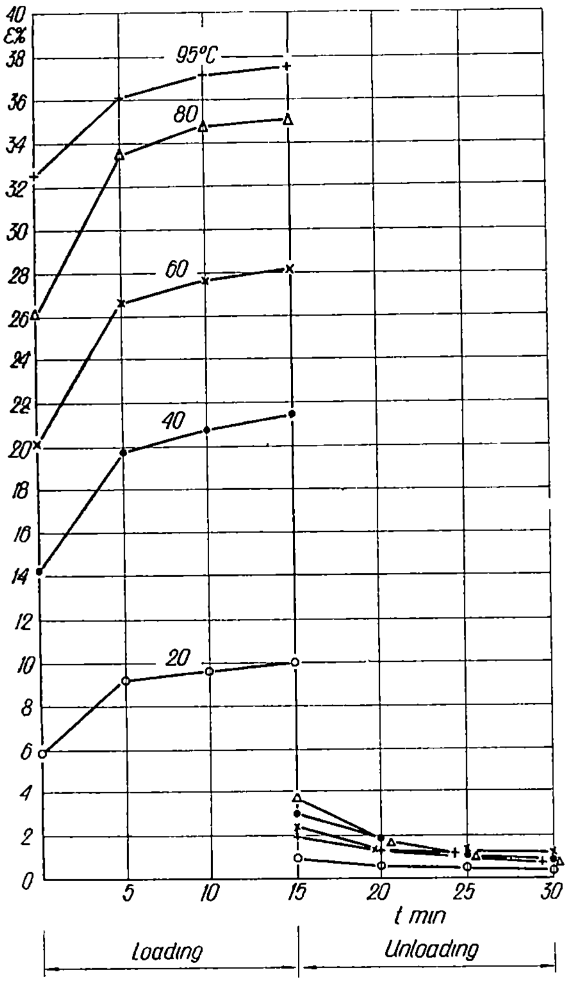
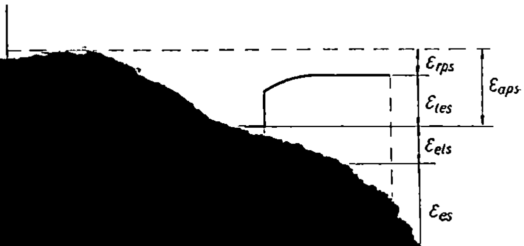


Fig 2 Effect of temperature on the strain and strain recovery in beech-wood loaded with equal compressive force of 120 per cent σ_D in the tangential direction

In view of these facts, the problem of dividing apparent plastic strains in wood into thermo-elastic and real plastic strains is of great importance

On the basis of these experiments we propose the partition of strain recovery in wood according to the scheme presented in Fig 3 Only those strains which remain after the first thermoplastic effect can be considered as the real plastic strains (ε_{rps}) It was experimentally corroborated that further increase of temperature, for example in time t_3 (Fig 3), results in very little, if any, increase of strain recovery. In order to determine the value of real plastic strain caused by compression of moist wood across the fibre direction, it is sufficient, it seems, to separate the thermoplastic strain (ε_{tes}) from apparent plastic strain (ε_{aps}) by accelerating strain recovery by raising the temperature of the wood



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MISCELLANEOUS

Philosophical Transactions of the Royal Society

In connexion with Dr Wightman's article in *Nature* of Oct 7, p 23, on the relationship between the Royal Society and the *Philosophical Transactions* in the seventeenth and early eighteenth centuries and his emphasis on the unofficial character of the *Transactions*, it may be interesting to note that the abridgement of these up to the year 1700, prepared by John Lowthorp, appears to have been officially sponsored by the Society. The fly-leaf of the third edition, printed in 1722 after the publication in the preceding year of a further abridgement of the *Transactions* for the years 1701–20 by Benjamin Motte, carried the following advertisement :

"May 5, 1703 At a meeting of the Royal Society. Sir John Hoskyns, V P. in the chair, Mr. Lowthrop Presented a Proposal for printing an Abridgement of the Philosophical Transactions This Design was Approved by the Society and He was Desir'd to proceed therein "

This is followed by the subscription

"May 12 1705
Impimatur
Is Newton, R S Pr "

(Lowthorp's name is so misspelt and the 'r' omitted from the word 'imprimatur' in my copy)

In contrast, the abridgement by Motte carries only a commendatory notice, dated July 26, 1720, signed "Edm Halley, Secr Reg Soc "

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EDUCATION FOR LEADERSHIP

IN his presidential review to the Rockefeller Foundation for 1960*, Mr G J. Harrar, who succeeded Mr Deak Rusk as president on July 1, 1961, pays particular attention to the deep interest which the Foundation has taken from the first in the welfare of people in the less-developed countries. Originally this was manifested through the fundamental studies and programmes in public health which its International Health Division undertook in many parts of the world Now it is equally manifest in the agricultural programme, which, beginning in Mexico, covers co-operative projects in Colombia, Chile, India and the Philippines, and the Foundation's researches on arthropod-borne virus diseases has at various times involved co-operative field stations in six different countries Supplementing these programmes and partly in support of them is the increased emphasis on training individuals from many countries for leadership in national and international development, and training is now a major sector of the Foundation's whole programme and is strongly oriented toward the less-developed countries Indeed, taking into account the increasing proportion of the Foundation's grants which seek to strengthen educational and research institutions overseas, education for leadership, particularly in the less-developed countries, could fairly be described as the dominant theme in the President's review; and it is presented with all the lucidity, cogency and balance which have marked the reviews of his distinguished predecessors

Mr Harrar notes first that the Foundation's fellowships are awarded to strengthen institutional faculties by enriching the experience of investigators and teachers, as well as for training younger individuals who plan careers in teaching and research Again, its travel awards have enabled many responsible leaders in all aspects of national planning to observe principles and practices in use elsewhere, and a considerable number of American scholars and scientists have worked for extended periods with colleagues in overseas institutions Further, individuals who have been associated with Foundation operating programmes or who have received training awards have been given assistance on their return to their parent institution to make them professionally more effective and to strengthen their departments and institutions Many grants, too, are made each year to institutions, departments and projects in the United States and abroad which seem of promise, the objective being to increase knowledge in its application for the benefit of mankind

In many countries the most urgent needs are for a steady rise in standards of living and for the broadening of scholastic, intellectual and creative opportunities; but in some, early priority must be given to improvements in sanitation, public health and medical care, and to increased food production as the basis of an adequate diet Until these requirements are satisfied, Mr Harrar points out, real progress in housing, transport, communications and other technologies is impossible scholarly activities and the creative arts are unlikely to flourish among sick or under-nourished people The need for more qualified persons to accept major responsibilities in government, education, science, technology, and economic and social development is manifest everywhere. Innate ability is not lacking, but opportunities for its full development are often grossly inadequate, and accordingly the most important single element in the progress of the less-developed or emerging States is leadership. Both the quality of training and the number trained are important, and these Mr Harrar fully recognizes are primarily a national responsibility, philanthropic and other foreign agencies can only help at critical points, particularly at the more advanced levels. Although most future leaders and investigators must necessarily receive their basic training at national institutions, and can only be sent overseas for training on a selective basis, it is for this reason that bodies like the Rockefeller Foundation are especially concerned with training for leadership

The urgent and growing demands being made on scientific and technological man-power and on professional man-power make it the more important that such resources should be used with skill and perception Here Mr. Harrar suggests that private foundations operating overseas have a unique opportunity to assist in creating patterns by which the limited numbers of highly trained individuals in less-developed countries can contribute most effectively to research and to the training of younger people If these leaders are properly situated, supported and encouraged, they can create islands of excellence which, with increasing numbers, may grow and coalesce into a firm base for genuine progress In view of the many opportunities for investment in excellence in the United States, the Rockefeller Foundation is increasingly selecting those which may ultimately pay substantial dividends both there and abroad, a significant proportion of the Foundation's grants to educational institutions in the United States are now in recognition of their part in training people from the less-advanced countries, and to assist the extension of various phases of institutional programmes in the United States into critical areas abroad

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* The President's Review from the Rockefeller Foundation Annual Report 1960 Pp 96 (Rockefeller Foundation, 111 West 50th Street, New York, 1961)

In this belief that the greatest challenge to private foundations is their work for the benefit of mankind, Mr Harrar discusses further the importance of educational developments in the less-developed countries and the contribution of the Foundation's scheme of fellowships and training grants for study abroad These have been used to assist particularly promising or enterprising individuals, with the object of encouraging the development of growing points of excellence The Foundation's interest in programmes for the control of specific diseases has almost entirely given place to this type of development There is, however, one exception, namely, the virtually world-wide problem of malnutrition, and for the past twenty years the Foundation has been attacking this problem at what might be regarded as its source—inadequately developed agricultural practices Attention has also been given to the adaptation of available food supply to biological needs, and three grants during the current year—one to Harvard University for a co-operative project with the Medical School in Cali, Colombia, one to the National Institute of Nutrition in Mexico, and one to the Institute of Nutrition of Central America and Panama in Guatemala—are cited as contributions in the Foundation's continuing programme for improving theory and practice in nutrition

The problem of food supply and nutrition is, however, closely linked with that of population and its growth, and here for thirty years the Foundation has sought opportunities to advance knowledge of all aspects of the reproductive process, while latterly the psychological implications of the reproductive process and sociological study of the pattern of population growth have received attention This latter aspect has received particular attention from the Population Council, which has done a great deal to increase world knowledge of the population problem, and last year arranged with the Johns Hopkins School of Public Health that the School should add to its staff experts in various phases of the population question Some of their time will be spent at Johns Hopkins studying matters of basic theory and practical application, but an appropriate portion of their time will be available to the Population Council for field-work in co-operation with government health services responsible for planning more orderly changes in the size of population.

The Rockefeller Foundation has participated in initiating this new arrangement, which should increase the number of competent investigators of the difficult problem of how to change public attitude and practice in this field For the past five years, too, the Foundation has been exploring possible ways of increasing the supply of well-trained diplomats for the execution of foreign policy and the effective representation of the interests of the emerging countries' interests abroad The critical importance of this factor has been repeatedly demonstrated by the events of the past year, but in this field assistance in training well-qualified leaders is a far more complex problem and directly involves issues of national independence, prestige and pride

One method adopted by the Foundation originated from the request of the representative of a South Asian country for help in strengthening the library of his Ministry of Foreign Affairs, which was quite inadequate for present-day needs. Some twenty-five countries have now sought such assistance, which has been provided for twenty-one after discussion with the ambassador or a senior official of each country, while representatives of more than twenty countries took the initiative in requesting further aid and guidance in training their young diplomats In consequence, the Carnegie Endowment for International Peace formulated a programme for creating a corps of diplomats thoroughly conversant with the practices and procedures of modern international relations Under this programme, to which, since April 1959, the Rockefeller Foundation has contributed 525,000 dollars, sixteen Fellows nominated by the governments of fifteen newly independent countries have been sent to the School of International Affairs at Columbia University or the Geneva Graduate Institute of International Studies, a part time seminar for younger diplomatic officers in embassies in Washington has been conducted by members of the Johns Hopkins University School of Advanced International Studies, and a training programme arranged in New York for young representatives of missions to the United Nations

A training and study programme at a more advanced level, involving a year of intensive study, has also been arranged at Harvard University and, taken together, the two programmes, to which the Foundation has contributed 668,200 dollars, represent a carefully designed attempt to meet the urgent needs of countries which almost overnight must develop a structure of government that requires competent and qualified foreign service personnel No one would suggest that such measures alone will solve the whole problem Stability and security must await the slow process of educating whole nations in the difficult art of responsible government, but the Rockefeller Foundation is clearly making a valuable contribution to a vital element in the provision of leaders of understanding and imagination

There are other fields also in which the Rockefeller Foundation is seeking to meet these needs of the emerging and under-developed nations. Mr Harrar refers to its efforts in dealing with the language problems in Peru and Bolivia, and intellectual communication between Latin America and the United States and among the Latin American countries themselves, but one of the most interesting sections of his review is that in which he describes the growing interest of the Foundation in the agricultural development of Africa, and particularly its efforts to enable the existing agricultural institutions of Africa to provide well-rounded organic programmes of agricultural research During 1960 the Foundation appropriated funds for this purpose to four institutions of university-level and one college of technology, as well as making awards to several experimental stations in East Africa for research on cereal crops and for studies of livestock physiology. Thus, 41,300 dollars went to the University of Khartoum faculties of agriculture and veterinary science, and 75,000 dollars to the University College of Rhodesia and Nyasaland for intensive studies of micro-organisms and their capacity for fixing nitrogen and on the range and adaptability of native species of grasses and their capacity to provide optimum grazing conditions for cattle. Lovanium University, in the Congo, received 26,300 dollars for the purchase of X-ray equipment for the study of the mineral content of tropical soils and the influence of weathering on them. The Department of Agriculture at the University College of Ibadan, Nigeria, received 40,000 dollars to accelerate studies of production economics and of the marketing, preservation and storage of produce and for studies of grass management systems for Ndama cattle, while 42,750 dollars went to Kumasi College of Technology for teaching and research equipment for agricultural science. Grants to the East African Agricultural and Forestry Research Organization, the Kenya Department of Agriculture and the East African Veterinary Research Organization brought the Foundation's total contribution in this field in 1960 to 405,350 dollars.

This is only a fraction of the Foundation's appropriation of 6,713,314 dollars for programmes in agricultural sciences in 1960 out of a total appropriation of nearly 33 million dollars, and of this some 2·6 million dollars were for the operating programmes in this field. It compares with appropriations of 10·25 million dollars in medical and natural sciences, nearly 4·5 million dollars for the humanities and nearly 4 million dollars for the social sciences, but it is interesting to note something of the same pattern in the allocation of grants by the Nuffield Foundation described in its latest annual report. Grants allocated for the Commonwealth Overseas amounted to some £375,000, including £250,000 for the proposed medical school at the University College of Rhodesia and Nyasaland, and its fellowship and scholarship schemes of £226,000 from grants which totalled £2·26 millions during the year, of which nearly £90,000 was for scientific research and just over £300,000 for medical research, the total amount for the Commonwealth Overseas being £600,000. While the Nuffield Foundation is clearly not directing the same effort to agricultural science as the Rockefeller Foundation, both Foundations are manifestly placing their emphasis on the support and fostering of excellence.

There are, indeed, passages in Mr. Harrar's review to the Rockefeller Foundation which seem to overlook the importance of an educated democracy and the dangers which can attend the use of the media of mass communication by the demagogue in the more backward countries. By and large, however, the efforts of the Rockefeller Foundation to help the emergent nations to develop the type of leadership and trained men and women they need are soundly and imaginatively conceived, and well designed to achieve their purpose if only they are given reasonable time.

SURVEY OF ANTIBIOTIC LITERATURE

Antibiotica

Herkunft—Arten—Eigenschaften. Von Prof. T. Korzybski und Prof. W. Kurylowicz. Pp. xix+1105. (Jena: Gustav Fischer Verlag, 1961.) 130 D.M.

THIS book, first published in Warsaw in 1955, now appears in German after being brought up to date. Its object is to summarize in a systematic way the enormous amount of antibiotic literature which has appeared since the discovery of penicillin. The authors believe that it will help in the very difficult problem of quickly identifying an antibiotic met with in antibiotic research if it has already been described in the literature.

In the introduction, the authors point out that it is not possible yet to classify antibiotics from a chemical point of view as so many of the antibiotics described are of unknown chemical structure. Hence, they chose to classify antibiotics according to their sources. There are seven chapters dealing, in turn, with antibiotics from bacteria, Actinomycetes, Fungi Imperfecti, Basidiomycetes and Ascomycetes, lichens and algae, higher plants, and animal sources. The first three chapters are further subdivided, Chapters 1 and 3 on the basis of a sub-classification of the source, while in Chapter 2, which comprises more than half the book, the principal basis of subdivision is the type of antibiotic activity displayed.

Despite the fact that it is not possible to classify all antibiotics from a chemical point of view, there are a few clearly recognizable chemical groups and these are treated together by the authors. Thus Section C of Chapter 1, which deals with antibiotics produced by bacilli, contains a useful general discussion of polypeptide antibiotics, and the polypeptide antibiotics produced by Actinomycetes are dealt with in a section of Chapter 2. This chapter also includes a section dealing with the Macrolide group of antibiotics (Erythromycin, Magnamycin, etc.) and the antibiotics active against moulds are divided into the polyene antibiotics and others, and a brief general discussion of the polyene antibiotics is given.

From the narrow point of view of the identification of a particular antibiotic, a basic classification on, for example, extractability into various types of solvent might have been preferable to that adopted. But the authors have a rather broader purpose; they appear to have made satisfactory use of order wherever it exists, and they have probably produced as good and workable a classification as is possible at the present time.

The articles on individual antibiotics aim at giving the name and description of the source; a summary of culture media, methods of cultivation, isolation and purification; the physical and chemical properties; the antibiotic spectrum, the toxicity found in animal experiments, and the ultimate importance in medical research such as clinical application, if any. Naturally, many descriptions are not complete, either due to the absence of information in the literature or because statements in the literature are not sufficiently precise.

The preface is dated June 1960, and the latest reference appears to be dated September 1959. Allowing for the great amount of work that must have gone into the preparation of the book for the press, this appears to represent as small a time-lag as could be hoped for, so that the book may be considered satisfactorily up to date. Extensive tests have failed to reveal any omissions so that the book's coverage of its field appears to be complete

Besides the pages of contents, which list all antibiotics described by name, there are an author index, an index of antibiotic names and an index of sources With these aids, no reader should experience any difficulty in finding what he wants

I have read a number of individual articles dealing with antibotics and in every case the article was found to present a very satisfactory and accurate summary of the information available in the literature

The bibliographies are very good and wherever possible references are included to review articles and monographs which contain comprehensive bibliographies

Careful perusal of the book from all points of view, therefore, suggests that it is destined to become an important work in the field of antibiotic research The price will, unfortunately, probably prevent many individual research workers from buying it, but it is a book which should be included in any library which makes a serious attempt to cover antibiotic interests In such libraries it will almost certainly prove to be in constant demand B K KELLY

TRAITÉ DE ZOOLOGIE

Traité de Zoologie

Anatomie, Systématique, Biologie Publié sous la Direction de Pierre-P. Grassé Tome 5, Fascicule 1 Annélides, Myzostomides, Sipunculiens, Echiuriens, Priapuliens, Endoproctes, Phoronidiens Pp viii + 1116 + 5 planches Fascicule 2 Bryozoaires, Brachiopodes, Chétognathes, Pogonophores, Mollusques (Géneralités, Aplacophores, Polyplacophores, Monoplacphores, Bivalves) Pp 1168 + 5 planches (Paris Masson et Cie, 1960) Broché 180 NF , cartonné 190 NF each

THE present welcome volume, in two fascicules, deals with a number of separate and not all closely related groups of the lower invertebrates as indicated in the titles Both fascicules contain almost precisely the same number of pages Each has a short list of errata, a detailed and excellent index and a full, expanded table of contents, which appears at the end They are both very well illustrated, each with 5 coloured plates and Fasc 1 with 904 and Fasc 2 with 954 text figures While many of the illustrations are borrowed but redrawn, a remarkably high proportion are original and they reach a very high standard, adding much to the usefulness and attractiveness of the volume

Apart from the editor, Prof Pierre-P Grassé, ten authors contribute to Fasc 1, two of whom, one from Brussels and one from Algiers, come from outside France, and all are experts in their own fields The majority of the text figures are line blocks and 18 have one or in some cases two colours introduced, and this is a commendable practice since it aids easier and quicker interpretation , Fig 857 in the present copy has the colour out of register, a point that is noted because the others are so exact Twenty-one figures are in half-tone The text of the chapter on the Phoronidea finished on p 1053 and is followed by a number of addenda which amplify or bring up to date points in the preceding sections and then by the index and table of contents Curiously, these pages are numbered MLV-MCXVI and the figures I-XXIX, but in Fasc 2 the pagination and numbering of text figures carry on with p 1054 and Fig 876 as if the pages numbered in Roman numerals did not exist In some places, for example, p 34 keferSteinii, p 120 dumerilii, etc , the double 'i' is employed instead of the now generally accepted single 'i' and on p. 994 the former name is misspelled Kleferstein Two forms, *Histriobdella* and *Branchiobdella*, the names of which suggest leeches, with which, *inter alia*, they have been placed and the systematic position of which has been subject to discussion, are here regarded as highly modified and as a parasitic polychæte and a parasitic oligochæte respectively Another anomalous form, *Acanthobdella*, some of the characters of which suggest Oligochæte affinities, has now been placed in the Acanthobdelliformes, a new order of the Hirudinea An anecdote well known in Great Britain will now be lost sight of as it has been shown, p 846, that *Golfingia macIntoshii* is a synonym of *G vulgare*.

Ten authors are also concerned with Fasc 2 and of these three come from outside France, two from Copenhagen and one from Leningrad In this fascicule there are only 4 text figures with colour introduced, but there are 63 half-tone blocks and of these 8 occupy the whole page and are of wash drawings, each showing about a dozen species The rest of the figures are in line block The first class to be treated in the longest chapter is the Bryozoa, falling into the two sub-classes proposed by G J Allman in 1856, the Phylactolaemata, all freshwater, and the Gymnolaemata, marine The latter were divided into three orders Cyclostomata, Cheilostomata and Ctenostomata by G Busk in 1852 and this grouping was accepted for more than seventy years In 1926 F Borg showed that the Cyclostomata (his Stenolaemata) were more widely separated from the other two than these were from one another, and E Marcus in 1938 placed them as sub-orders of the Eurystomata This classification, with its corollaries to include fossil forms, has been adopted in the present work It is rather strange to find Allman's monograph on fresh-water Polyzoa appearing under the bibliography of the Gymnolaemata

About half-way through the volume is a most interesting chapter on the Pogonophora In 1914 M Caullery described an enigmatic tubicolous worm-like creature with numerous tentacles, hence Pogonophora (πωγων, a beard), collected by the *Siboga* Expedition The material was not very well preserved and so a completely satisfactory account could not be given and indeed it was suggested that this animal called by its discoverer *Siboglinum weberi*, might only be the anterior part of an animal since no trace of an alimentary canal was found It was considered that the creature might be allied to the Enteropneusta and so M Caullery contributed a chapter on it in volume XI of the *Traité*, where indeed it belongs, in 1948 Since then, and particularly by Prof A V Ivanoff of Leningrad, a great deal of additional information has been furnished and now 11 genera with 43 species have been described from better-preserved material The editor has fortunately got Prof Ivanoff to contribute the chapter on the Pogonophora It is the first account and survey available to the general zoologist It provides a very complete account of the anatomy, histology and embryonic development, and in addition to the ordinary text figures mostly original, there are three coloured plates These depict the animals in three dimensions It is clear that they are deuterostomes with a single cœlomic sac in the protosome, which sends canals into the tentacles, a pair of cœlomic sacs in the mesosome and another pair in the metasome. It is concluded that they are related to the Stomatochorda and particularly to the enteropneusts. This connexion with the early Chordata together with the absence of an alimentary canal render them an important and intriguing class.

The next class calling for comment is the Monoplacophora, a name given by N. Odhner in 1940 to a group of Palæozoic Mollusca until then grouped with the Patellacea. In 1957, however, H. Lemche described a living form, *Neopilina galatheae*, referable to the group which had been collected five years previously by the *Galathea* Expedition from west of Costa Rica at a depth of 3,570 metres. Afterwards, H. Lemche and a colleague, K. G. Wingstrand of Copenhagen, gave a full account of it in the *Galathea Reports*. The editor of the volume, Pierre-P. Grassé, got these two authors to provide an account, which he translated, illustrated by a number of new figures, so that again we have the first general account of a most remarkable animal from the original describers. The well-developed cœlomic cavities are described as consisting of three pairs—the pericardial pair, the body pair which lie dorsally and are extensive, and the gonads. The apparent metamerism of a number of organs (muscles, nerves, gills, blood vessels, nephridia and gonads) is well marked and considered by the authors to be comparable with that in Annelida and Arthropoda. These points raise interesting problems concerning the relationships and derivation of the primitive molluscs. While the chapter was in the press another mollusc, for the present referred to the same genus *N. ewingi*, was reported (Clarke, A. H., and Menzies, R. J., *Science*, 1959), and although little information regarding it is available it seems to offer certain divergences from *N. galatheae* which will have to be taken into account before coming to definite conclusions on the problems above mentioned.

Here, then, is another addition to this widely used text-book that is indispensable to every zoological laboratory. It reaches the high standard in both text and illustration set by its predecessors and is indeed a noteworthy and commendable volume.

C. H. O'Donoghue

POLYMER CHEMISTRY

An Introduction to the Organic Chemistry of High Polymers

By Prof. Carl S. Marvel. Pp. viii + 82. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 36*s*. net.

THIS book consists of notes originally assembled for use in connexion with the Humble Lectures in Science, delivered by Prof. Marvel in 1956. It is intended for those just starting research in polymer chemistry.

Most of the introductory texts at present available in polymer chemistry attempt to be reasonably comprehensive in their choice of topics: this one is strictly limited to the organic chemistry of high polymers. Neither the physical chemistry of polymers and their solutions nor physicochemical methods of studying polymers are considered. After a very brief description of methods for the characterization of polymers, the author proceeds to a systematic consideration of various types of condensation polymerization. Both free radical and ionic methods of addition polymerization are then discussed, followed by chapters on co-polymerization and di-ene polymerization. A short account of reactions of polymers concludes the book.

As is only to be expected in a book of this size, individual topics are not dealt with in much detail. There are a number of occasions, however, where the discussion is so brief as to lack clarity. Particular examples are the section on the mechanism of free radical addition polymerization and the introduction to the section on co-polymerization.

The most serious limitation of the book is that it is already five years out of date. It is a pity that so little attempt seems to have been made to include work done after 1956. Because of the rate at which polymer chemistry is advancing, this leads to some important omissions. For example, topics which are omitted or receive only the briefest mention include stereospecific polymerization, polyaldehydes, polycarbonates, polymer stability and degradation, radiation methods, and new methods of anionic polymerization. Quite apart from their importance, these developments in the forefront of the subject are those most likely to arouse the enthusiasm of a beginner, which is surely one purpose any book of this kind should serve.

There are few trivial misprints. More serious is the appearance of pentavalent carbon on pages 47 and 48, unhappily in a place where the unwary reader might be misled into thinking that Friedel–Crafts-type catalysts used in cationic polymerization are covalently bound to the polymer chain. On page 47 is the statement that in every case of co-catalysis in the initiation of cationic polymerization the real initiator is believed to be a proton. This neglects the important cases where there is good evidence that carbonium ions derived from the solvent are chain initiators.

The book is, in general, attractively printed and strongly bound. At a cost of fivepence a page, however, it must be one of the most expensive chemistry books available.

I. C. McNeill

QUANTITATIVE BIOLOGY

Blood and other Body Fluids

Analysis and compilation by Philip L. Altman. Edited by Dorothy S. Dittmer. (Biological Handbooks.) Pp. xvii + 540. (Washington, D.C.: Federation of American Societies for Experimental Biology, 1961.) 10 dollars.

NUMERICAL information is becoming of ever-increasing importance in all branches of biology. While physicists, chemists and engineers started long ago to compile handbooks of data, the collection of biological figures is of relatively recent date. The first major systematic effort in this direction were the *Tabulae Biologicae* which were initiated in the 1930's. Some ten years ago the United States National Academy of Sciences and the United States National Research Council began to sponsor a series of biological handbooks which has so far led to the publication of ten volumes. Some deal with blood, nutrition, metabolism, general biology, respiration and circulation, and five others with various aspects of toxicology and pharmacology (acute toxicity, antibiotics, insecticides, tranquillizers and fungicides). The present volume is based on these earlier handbooks and is the first to appear under the general direction of the Committee of Biological Handbooks of the Federation of American Societies for Experimental Biology The material is presented in the form of tables, graphs, diagrams, nomograms and line charts Three hundred and eighty investigators have taken part in the compilation and much trouble has been taken to obtain accuracy, to eliminate misprints and to exclude material of questionable validity Apart from blood, fields covered include urine, lymph, hæmolymph, cerebrospinal fluid, body water, various secretions of the digestive tract, reproductive secretions, milk, sweat, sebum and the fluids of the ear and eye Among the species, man occupies the first place, but the information extends to numerous other vertebrate and invertebrate organisms

While the data of physical and chemical reference books are usually constants which have been measured with a known accuracy, biological data are, as a rule, not so definable. they are not constants and their scatter is in many cases not sufficiently known The figures given in the book are therefore bound to be of varying degrees of reliability, but this difficulty has been effectively met by detailed references to the sources of information In fact the references given may well prove to be one of the most valuable features of a very useful mine of factual information

H A KREBS

A NEW STANDARD WORK ON COCOA

Cocoa

By D H Urquhart Second edition (Tropical Agriculture Series) Pp xxv + 293 + 129 plates (London Longmans, Green and Co , Ltd , 1961) 40*s* net

THIS book was originally published in 1955 and two additional impressions were issued in 1955 and 1956 The new (second) edition attempts to bring the subject-matter up to date It contains one entirely new chapter (on manuring) and several of the chapters of the first edition have been combined in the second The total number of pages has thus been increased from 230 to 293 The book is one of a series on tropical agriculture of which five volumes have already been published. The author of *Cocoa* was formerly director of agriculture of Ghana, West Africa, and adviser in cocoa-growing to Messrs Cadbury Bros , Ltd , Bournville, Birmingham, England

The book includes all the detailed information needed by practical cocoa farmers, as well as students and agricultural extension workers Its usefulness is greatly enhanced by the excellent illustrations and the tables of data. Much progress has been made in the cocoa industry since the first edition of this book appeared in 1955, particularly in the control of pests and diseases of the cocoa tree, and the detailed specification of the atmospheric and soil environment necessary for maximum production of cocoa Thus, in the chapters concerning soils and manuring, the results of researches carried out in recent years in Ghana by the late C F Charter and in Belgian Congo by M V Homes have been incorporated into the new edition The establishment, maintenance and costings of the cocoa plantation, the rehabilitation of the derelict and unremunerative plantation, and the preparation and grading of the crop for market, receive detailed consideration in no less than seven chapters and nine appendixes, spanning a total of 159 pages or 50 per cent of the total number of pages comprising the book, thus stressing the essentially practical outlook That Urquhart's book is providing a much-needed standard work on cocoa and one which is a worthy successor to the classical works on the subject written by C J J Van Hall and by J H Hart, which have long been out of print, is indicated by the fact that the first edition and its two impressions were exhausted after only five years had elapsed The need for a Spanish version has already become manifest, and the task of translation has been undertaken by the Inter-American Institute of Agricultural Sciences at Turrialba, Costa Rica Publication of the Spanish edition is expected shortly, it should add to the increasing demand for the book by making it available to cocoa growers in tropical America

LONDON LIBRARIES

The Libraries of London

Edited by Raymond Irwin and Ronald Staveley Second, revised edition Pp 332 (London The Library Association, 1961) 36*s*

THE first edition of this work, published in 1949, was based on lectures delivered during a course at the School of Librarianship and Archives, University College, London, and was intended to supplement but not replace R A Rye's *Students Guide to the Libraries of London* That work has long been out of print and even the first edition of *The Libraries of London* is now largely out of date Accordingly, the new edition attempts to present a much fuller picture of the library resources of London All the chapters have been carefully revised and most have been largely re-written, there is a new chapter on the University College Library, and separate chapters on the University of London Library, on the British Library of Political and Economic Science, while the other university libraries are described in a separate chapter Additional chapters deal with the music libraries, the ecclesiastical and theological libraries, the industrial libraries, technical and professional libraries and those of the learned societies, and of the London County Council, while that on the Public Record Office is omitted Apart from the London Library, no attempt is made to describe the commercial and subscription libraries, and likewise there is no reference to the libraries associated with the offices of the great national newspapers

The individual chapters are the work of different authors, all authorities in their field, but what is gained in authority is sometimes offset in part by lack of balance, and diversity of scale and treatment That on the Science Museum Library, by the late H T. Pledge, is frankly episodical, and besides its historical or descriptive lacunæ, it has scant reference to future developments The Patent Office Library is more adequately covered, but there are some surprising omissions in the chapter on Government Departmental libraries, while that on industrial libraries is scarcely of greater value than as a reminder of the existence of such libraries , it is content to list and describe those of the British Aluminium Company, the Cement and Concrete Association, and the British Paper and Board Industry Research Association Nevertheless, used with imagination, this book, produced in accordance with the high standards which are customary with the Library Association, forms a most useful and convenient guide to the bibliographical resources of London

R BRIGHTMAN

Sensation and Perception

A History of the Philosophy of Perception By D W Hamlyn (International Library of Philosophy and Scientific Method) Pp xi+210 (London Routledge and Kegan Paul, Ltd , New York The Humanities Press, 1961) 25*s* net

THERE is something very refreshing about this book It deals with a complex theme without reducing the reader to fatigue and his patience to zero Broadly, the author takes as his 'matter' the trio—sensation, perception, judgment—and subjects them to a careful historical analysis In a word, we are told how these concepts have evolved with time, not forgetting that ideas need verbal expression for them to come into being However, the absence of such terms is not proof positive that the 'germ' was not alive in the mind of philosophers, at any given epoch, or even perhaps at some previous period

We begin with the classical figures, followed by a lively discussion of Hellenistic thought (Incidentally, the heading to Chapter 2 on page 31 looks very odd with this adjective spelt with one 'l') Next come chapters on the seventeenth century, rationalists, empiricists, Kant, Hegel, idealism, nineteenth-century sensationalism, the reaction, the twentieth century, conclusion There are an excellent bibliography and an adequate index

The connexion with *Gestalt* theory is well handled Perception, given that the whole is more than the sum of its parts, needs no learning "because it is innately determined" Perception, if passive, tends to sensation; if active, to judgment The former is unsatisfactory in various ways, especially to the scientist, who uses the second almost subconsciously. What must be avoided, as Wittgenstein remarked, is the introduction into this study of one's own experiences In the pre-perceptual sense at least, there is no private language

The style is pleasant and lucid fewer italics would have made it still more agreeable without any loss of emphasis F I. G RAWLINS

Analytical Chemistry of the Rare Earths

By Dr. R C Vickery (International Series of Monographs on Analytical Chemistry, Vol 3) Pp viii+137 (London and New York Pergamon Press, 1961.) 40*s* net

THE rare earths, as defined by the author of this invaluable little volume, comprise the lanthanon series to lutetium with the addition of yttrium and scandium

The analytical examination of mixtures of these elements has been rendered far less time-consuming in recent years by the application of the newer technique of X-ray fluorescence analysis Radioassay following neutron irradiation now permits the determination of millimicrogram quantities, and controlled ion-exchange experiments have enabled the chemist to separate the individual rare earths in a very pure form All these modern methods are described lucidly in this monograph, which also contains the most detailed description of the more classical, but still eulogized, absorption spectrum technique for the detection and estimation of these intractable elements Conventional emission spectrography is not neglected by the author, for despite the complexity of the spectra given by these elements, there are occasions where the arc, or preferably the spark technique, can give the most useful quantitative information A table is given of the main persistent lines of the rare-earths emission spectra, and various internal standard line-pairs are also presented in tabular form for specified concentration ranges

Polarographic methods as applied to the unique cases of samarium, europium and ytterbium are described briefly in the appendix

The earlier chapters deal with the more classical methods of gravimetric and volumetric analyses, and here the student will find a wealth of information on chemical separational procedures The analysis of ores, the separation of calcium and iron, the usages of selective organic reagents such as Beck's pyrocatechol, complications attending the Group III hydroxide precipitations, are all described with much attention to laboratory detail Although quantitative methods for the determination of the rare earths are now almost wholly instrumental, the uses of oxalate, iodate, bismuthate and complexan reagents are well worthy of recapitulation. In retrospect, we join with the author in expressing unqualified admiration of the skill, patience and ingenuity shown by the early chemical research workers who isolated, albeit in crude form, the first rare-earth elements

This monograph has much to commend it to the academic student, but for the rare-earth chemist it is most certainly an essential acquisition and will prove a valuable laboratory companion D T LEWIS

Paper Electrophoresis

A Review of Methods and Results By L P Ribeiro, E Mitidieri, and O R Affonso. Pp. xii+463 (Amsterdam Elsevier Publishing Company, London D Van Nostrand Company, Ltd , 1961) 72*s*

THIS book by Ribeiro, Mitidieri and Affonso represents a welcome and thorough classification of the already voluminous literature on ionography There is a wealth of references, 3,200, some of which have been published only in the more esoteric medical journals

The first part of the book includes chapters on the theory, methods and apparatus The theoretical chapter is written with great clarity and will be very useful to beginners in this field Even Ohm's law is included and there is a useful example on the results of Joule's effect "When high voltages are used there will naturally be an increase in current causing an elevation in temperature which may be so great as to ignite the paper "

In the subsequent part of the book on applications the emphasis is on proteins, but there are also chapters on hormones, vitamins, carbohydrates, nucleic acids, amino-acids and peptides, a sketchy chapter on inorganic substances, one on immunochemistry and one on other applications which deals with topics as diverse as amines, dyes and venoms

The large bibliography means that the treatment afforded individual references varies considerably throughout the book, ranging from minute practical detail to just the reference itself The book has few printing and translation errors and the diagrams are well produced Two points might, however, be mentioned on page 211 the structural formula given for the sugar unit after oxidation with periodic acid appears to be that for an α-diketone rather than for a di-aldehyde as suggested in the text, and for greater clarity the cyclohexane rings which appear in some of the formulæ throughout the book might, where appropriate, be amended to aromatic rings

The book will undoubtedly be a valuable asset to biochemists, chemists and clinicians particularly as there are more than three hundred references per ounce G V F SEAMAN

A MICROBIOLOGICAL EXAMINATION OF SOME CARBONACEOUS CHONDRITES

By Dr GEORGE CLAUS
New York University, Medical Center, New York
AND
Prof. BARTHOLOMEW NAGY
Department of Chemistry, Fordham University, New York

MICROSCOPIC-sized particles, resembling fossil algae, were found to be present in relatively large quantities in the Orgueil and Ivuna carbonaceous meteorites No such particles were found in two ordinary stony meteorites, namely, in Holbrook and in Bruderheim

There are nineteen known carbonaceous chondrites, all of which were observed to fall Orgueil fell in southern France in 1864, Ivuna in Central Africa in 1938, Mighei in Russia in 1889, and Murray in the United States in 1950 Carbonaceous chondrites contain water and a few per cent of organic matter

Wiik[1] has shown that carbonaceous chondrites may contain as much as 20 per cent water On the basis of his hydrogen isotope work, Boato[2] concluded that a large portion of this water in Orgueil and Ivuna is of extraterrestrial origin Carbonaceous chondrites have thin fusion crusts Mueller[3] and others found that after the development of the indigenous, hydrous-mineral components, the interior of these meteorites was not subjected to high temperatures The interiors of some of the carbonaceous meteorites apparently have not been heated to temperatures higher than approximately 200° C

Berzelius[4] and Wohler[5] extracted organic matter from carbonaceous chondrites. Berthelot[6] obtained saturated hydrocarbons from a sample of Orgueil In more recent years, Mueller extracted the Cold Bokkeveld meteorite and obtained organic matter which had high oxygen, nitrogen and sulphur content Calvin[7] suggested that a compound resembling cytosine might be present in Orgueil, and Briggs[8] presented analyses which may indicate the presence of purines Nagy, Meinschein and Hennessy[9] analysed hydrocarbons from Orgueil with a mass spectrometer and observed certain spectral features which resembled those of biogenic hydrocarbon mixtures

Urey and Craig[10] concluded that the carbonaceous meteorites are typical members of the high iron group chondrites These investigators reported that serpentine-type minerals and magnetite are present in carbonaceous chondrites Kvasha[11] suggested that chlorite, a hydrous layer-lattice silicate, is a mineral component in Staroye Boriskino.

Five carbonaceous chondrite samples were examined during this work. One Orgueil sample (*A*), Ivuna and Mighei, were obtained from the American Museum of Natural History in New York, and another sample of Orgueil (*B*) was received from the U S National Museum in Washington, D C The sample of the Murray meteorite was obtained from the Institute of Meteoritics, University of New Mexico, Albuquerque, New Mexico One of the two non-carbonaceous chondrites, Bruderheim, was received from the Department of Geology, University of Alberta, Edmonton, Alberta, and the other, Holbrook, was received from the American Museum of Natural History

Grains of the meteorite samples were crumbled in water or in glycerol on glass slides. A microscopic examination of the two Orgueil samples and of Ivuna revealed the presence of particles which were dissimilar in morphology to any known mineral form The 'organized elements' resembled, but were not identical to, the morphology of certain species of algae which live in water (The aquatic algae are capable of producing hydrocarbons[12] comparable with those previously found in Orgueil[9]) One form of the organized elements was morphologically dissimilar to any known terrestrial organism The organized elements did not show birefringence in polarized light, but they did show fluorescence (yellow) in ultraviolet light (Fig 1). A few specimens of microbiological contaminants (common, airborne bacteria, algae, etc) were present in the carbonaceous as well as in the non-carbonaceous meteorites Organized elements were abundant in the Orgueil and Ivuna carbonaceous chondrites but were absent in Holbrook and in Bruderheim

Somewhat similar but poorly defined particles were found in Murray and Mighei. Their lack of structure precluded identification with the organized elements from Orgueil and Ivuna (see Table 1)

Five types of organized elements have thus far been observed

(1) Small, circular forms (Fig. 1), surrounded by double walls which showed thickening and occasional sculpturing with protuberances Their diameter varied between 4 and 10μ and they appeared yellowish-green in transmitted light. The interior of the organized elements appeared to be either homogeneous or it was granulated

(2) The second type (Figs 2 and 3) resembled the first one; however, the surfaces of these organized elements were covered with spines, with other appendages or with a furrow The appendages usually appeared to be tubular in shape, and occasionally they penetrated through the walls. The diameter of the appendages varied between 0 5 and 2μ, and that

Table 1 Organized Elements and Contaminations in Five Meteorites

(The numerical values represent the number of particles in 1 mgm of sample)

Type of particles	Orgueil (*A*)	Orgueil (*B*)	Ivuna	Holbrook	Bruderheim
Organized elements	1650	1700	1680	—	—
Apparent contaminants					
(*a*) Bacteria	27	34	31	22	48
(*b*) Algae					
Achnanthes microcephala	1	—	—	—	2
Navicula sp	—	1	—	—	—
Nitzschia palea	—	—	1	—	1
Trachelomonas volvocina	—	—	—	—	1
(*c*) Others					
Conidiospore	—	—	—	—	1
Sponge spicule	2	—	—	—	—
Starch granule	1	—	—	—	—
Cellulose fibre	4	6	6	22	50
Total contaminants	35	41	38	44	103

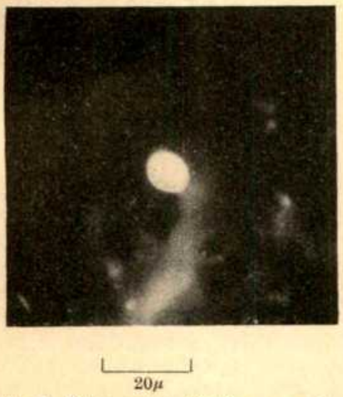

Fig. 1. Fluorescence effect in an organized element, type 1, in ultra-violet light

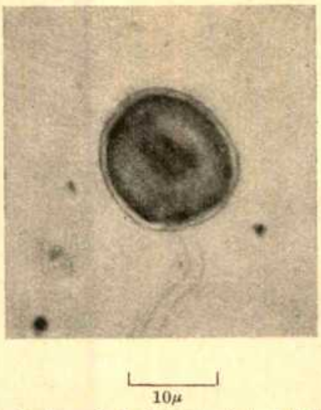

Fig. 2. Organized element of type 2 in the Orgueil meteorite

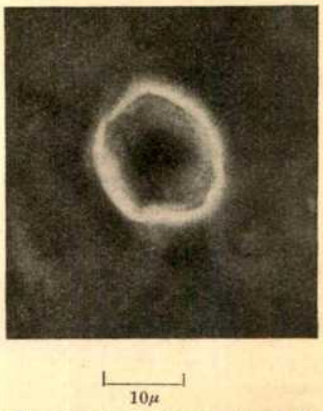

Fig. 3. Organized element of type 2 as seen under the phase-contrast microscope. Note internal structure of the organized element

of the organized elements was between 8 and 30μ. Solid appendages had club-like structures on their distal ends. Spines were arranged in pairs around the appendages. The furrow started at the single appendage on the apex of the organized elements.

(3) The third type of the organized elements had shapes like shields. They had no appendages. Their walls showed marked thickening and sculpturing; their average diameter was approximately 15μ.

(4) Cylindrical forms were seen which had thick walls and finely sculptured wall surfaces. Their length was approximately 20μ, their width was 10–12μ.

(5) An apparently hexagonal form was also observed (Fig. 4). This organized element may contain, however, 10–12 surfaces. Three of the surfaces were considerably thicker than the others and they served as the bases of the three, thick, tubular protrusions. The organized element seemed to be surrounded by a structureless halo, which was interrupted only by some matter that was protruding from two of the appendages. There were three vacuole-like and irregularly shaped bodies in the interior of the organized element.

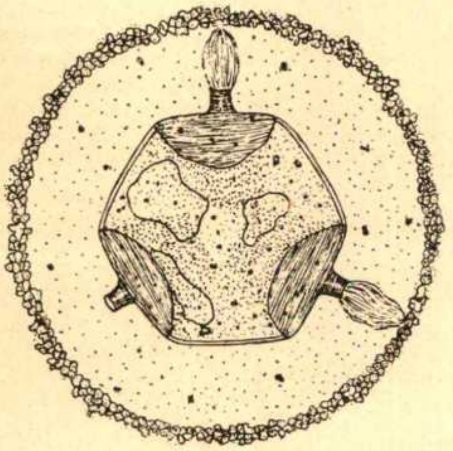

Fig. 4. Sketch of organized element of type 5. (Drawing is used because the original forms were not suitable for taking photographs)

The Orgueil meteorite samples contained each of the five organized element types. Only the first three types were present in Ivuna. Types 1 and 2 were by far the most abundant in both Orgueil and Ivuna.

There were other morphological features which should be mentioned. Several of the organized elements appeared to undergo 'cell-divisions'. Constrictions occurred in the middle portions of the elongated bodies in one form of this phenomenon, proceeding toward the interior and suggesting the formation of two 'daughter' bodies (Fig. 5). Several organized elements showed distortion and rupture of the walls. Often the interior appeared to be spilling out through the fractured walls.

Attempts were made to identify the chemical components of the organized elements with biological staining techniques; chlorzink-iodine, toluidine blue, methylene blue, Janus green *B*, Feulgen, Gridley, acid-carmine, periodic acid–Schiff, hæmatoxylin eosine and ninhydrin gave colour reactions. The reliability and the significance of the colour reactions in the organized elements are not known. It is interesting to note, however, that diffuse nuclear-staining material was found in the organized elements (Fig. 6).

From these morphological, optical and staining results we have interpreted the organized elements as possible remnants of organisms.

Although the origin of these organized elements cannot be answered at this time, indications of their

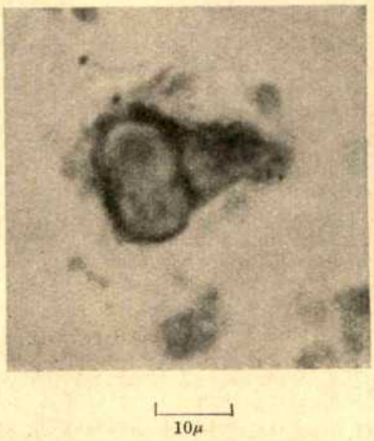

Fig. 5. Organized element of type 2 resembling 'cell division'

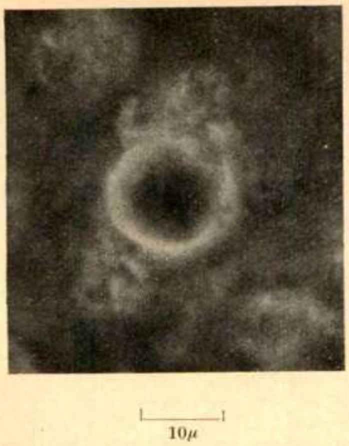

Fig. 6. Organized element of type 1 after Feulgen staining; note indications of diffuse nuclear material

origin may be summarized in the following observations:

(1) The high concentrations of the organized elements in Orgueil and in Ivuna could have developed only in an environment where moisture was continuously present for a prolonged period of time; yet the meteorite samples were kept in dry museum storage and would have disintegrated if subjected to an aqueous medium. Furthermore, they were outdoors for only a few hours after they had fallen. There are only traces, if any, of capillary water retained in the meteorites.

(2) The organized elements, types 1–4, resemble those species of Dinoflagellates or Chrysomonads which live only in water (in the sea or in lakes, and do not occur in the soils).

(3) Types 1–4 appear to be similar to but not identical with known terrestrial species. The fifth type of the organized elements is entirely dissimilar in its morphology to known terrestrial form.

(4) Terrestrial organisms resembling types 1–4 of the organized elements develop cysts under unfavourable conditions (such as a drying or dry environment). Only two or three of the organized elements in Orgueil and in Ivuna showed structures resembling cysts.

(5) The fact that the same types of organized elements were present in both Orgueil samples which fell in the temperate climate of southern France, and in Ivuna, which fell seventy-four years after Orgueil in an arid, tropical region of Central Africa, renders unlikely contamination with morphologically identical, locally derived, micro-organisms.

The morphological similarities are of interest in the light of unpublished results of one of the authors[13] which indicate a chemical similarity between Orgueil and Ivuna.

We are of the opinion that these observations suggest that the organized elements may be microfossils indigenous to the meteorite.

We would like to express our appreciation to Prof. Douglas J. Hennessy, of Fordham University, and to Dr. Warren G. Meinschein, of the Esso Research and Engineering Co., for their constructive criticisms. Dr. Meinschein originally proposed also a palinological examination of meteorites. We would also like to thank those persons with whom these results have been discussed: Prof. Harold C. Urey, of the University of California; Dr. Brian Mason, of the American Museum of Natural History; Dr. Jack Green, of North American Aviation, Inc.; Dr. C. Mervin Palmer, U.S. Public Health Service; Profs. Currier E. McEwan, Maxwell Schubert, Norman Cooper and Alan J. Johnson, of New York University Medical Center; Prof. Henrik P. deBoom, University of Pretoria, South Africa; Dr. Bela J. Cholnoky, Council for Scientific and Industrial Research, Water Research, Pretoria; and Prof. Alexander Wolsky, Fordham University. Profs. Urey, Wolsky, Hennessy, Schubert and Johnson and Dr. Meinschein read the manuscript and offered valuable advice regarding this presentation. The meteorite samples were provided by Drs. Brian Mason, E. P. Henderson, Lincoln LaPaz and Prof. R. E. Folinsbee.

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BRADYKININ

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BRADYKININ is a polypeptide discovered in 1949 by Rocha e Silva, Beraldo and Rosenfeld[1], who coined the name 'bradykinin' (slow-moving) because, when acting on the guinea pig intestine, it caused a delayed and slow contraction. They showed that it is formed by the action of certain enzymes, such as those in some snake venoms and trypsin, on plasma globulins. 'Kallikrein' is the name given to another group of enzymes which occur in the body and which act on the plasma globulins to form bradykinin-like activity. Much of the earlier work on these enzymes was that of Frey, Kraut and Werle, and was reviewed by them in 1950 [2]. A vasodepressor substance was shown to be present in urine[3], in pancreas[4], in blood[5] and in saliva[6], although it was not realized at that time that this vasodepressor substance was an enzyme, acting by the formation of a vasodilator polypeptide. The active principle in all these fluids was called 'kallikrein'. However, the kallikrein obtained from these various sources is not the same enzyme. Blood kallikrein, which is different from that in glands, behaves like the proteolytic enzyme plasmin[7] and is inhibited by proteolytic inhibitors[8]. The glandular kallikrein does not behave like a proteolytic

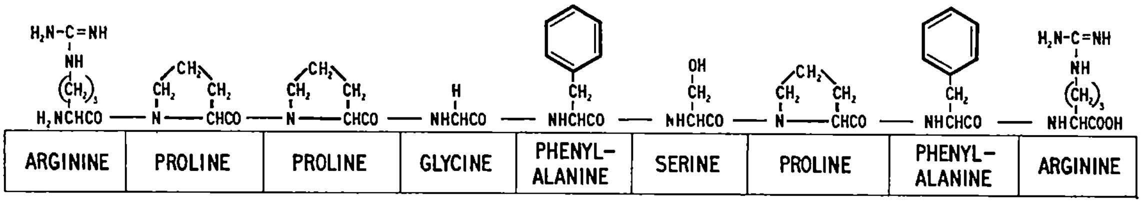

Fig 1 Structure of bradykinin

enzyme[9]—it does not hydrolyse substrates, like casein, nor is it inhibited by proteolytic inhibitors However, the kallikrein of the salivary gland hydrolyses certain synthetic esters, but not the corresponding amides[10] This gives support to the theory of Rocha e Silva and Hamburg[11,12] that bradykinin is formed by an esteratic action

Trypsin and plasmin are both proteolytic and esteratic enzymes—it may be that some of the kallikreins, particularly glandular kallikrein, are not proteolytic enzymes at all, but esterases It is possible that the two classes of enzymes—the slow-kinin-forming enzyme and the quick-kinin-forming enzyme described by Lewis[9] are the proteolytic and esteratic enzymes respectively Whatever the truth may be, it is certain that there are several different kinds of enzymes which act on the plasma globulins to form bradykinin, or at least peptides, which are closely related to bradykinin

It is not certain whether the peptide formed by these different enzymes is the same in each case and therefore the name 'plasma kinin' has been suggested to cover all the peptides formed from plasma proteins in this way For example, the name plasma kinin may be applied to the principles which are formed in blood under various conditions, such as when plasma is diluted[13] or when plasma comes into contact with a glass surface[14] Presumably these physical actions result in activation of one of the plasma-kinin-forming enzymes which are normally present in blood as inactive precursors Although the exact structure of all the peptides formed by these various enzymes is not known, we do know the amino-acid sequence of one plasma kinin—bradykinin After attempts by Andrade, Diniz and Rocha e Silva[15,16], who obtained bradykinin of high activity, it was finally isolated[17], and synthesized[18,19] From an incubated mixture of crystalline trypsin and pseudoglobulin Elliott, Horton and I isolated bradykinin using mainly the technique of elution chromatography on carboxy-methylcellulose The peptide was eluted from the columns using a salt gradient at constant *p*H The volatile buffer ammonium acetate was used as eluant so that the samples could be evaporated, thus avoiding contamination with salt

The pure peptide obtained in this way contains five amino-acids in the following molar proportions serine 1, glycine 1, phenylalanine 2, arginine 2 and proline 3 giving nine amino-acid residues or a nonapeptide

These are present in bradykinin in the sequence shown in Fig 1 (ref 20) The natural and synthetic products were found to be identical[21-23]

The main pharmacological actions of bradykinin are smooth muscle stimulation, vasodilatation, increase in capillary permeability, accumulation and migration of leucocytes and production of pain

Most smooth muscle preparations are stimulated by bradykinin even in minute amounts The rat uterus is contracted by 0 1 ngm /ml (10^{-10}) and the guinea pig ileum by 1 0 ngm /ml (10^{-9}), while the rat duodenum is relaxed by bradykinin 0 1 ngm /ml (10^{-10}) Some smooth muscle preparations like the rabbit jejunum and rat colon, which show spontaneous activity, are first relaxed and then contracted by bradykinin Bradykinin causes bronchoconstriction in the guinea pig[24], although it has no effect on the bronchioles in the dog[25] In non-asthmatic patients large doses of bradykinin, given as an aerosol, occasionally cause constriction of the bronchioles, asthmatics are more sensitive[26], but it seems doubtful that bradykinin is concerned specifically in asthma

Bradykinin is one of the most active vasodilator substances known In the cat hind limb bradykinin is as active as acetylcholine[27]. On the flow of blood in the human forearm bradykinin was found to be a more active vasodilator than histamine, acetylcholine, isoprenaline and 5-hydroxytryptamine[28] In some of these experiments as little as 0 1 μgm bradykinin injected into the brachial artery caused a strong vasodilatation in the whole forearm

It has been known for some years that crude mixtures containing bradykinin cause an increase in capillary permeability[29] and pain[14] With the availability of the pure peptide it was shown that pure bradykinin possesses both of these actions on local application[27]

In addition, bradykinin causes accumulation and migration of leucocytes[30] This effect, however, is produced only by relatively large doses of bradykinin (1–100 μgm /ml) and in such doses other peptides affect leucocytes in the same way

Bradykinin thus causes vasodilatation, increased capillary permeability, production of pain and accumulation and migration of leucocytes These are the cardinal signs of the early stages of the inflammatory response It therefore seems likely that bradykinin plays the part of a mediator in this reaction. Menkin[31,32] originated the view that specific polypeptides may be responsible for the inflammatory response It is possible that bradykinin is one of a group of peptides which account for this reaction as postulated by Menkin There is no direct evidence yet that bradykinin is formed during the inflammatory reaction, however, it is possible, as suggested by Hilton and Lewis[33], that bradykinin or perhaps the enzyme which forms bradykinin may correspond to the *H*-colloid of T H Lewis[34] and of Krogh[35], which together with *H*-substance (presumably histamine) accounts for many of the reactions to local tissue injury No specific antagonist of bradykinin is yet available in order to test such a hypothesis by inhibiting or preventing inflammation It has been claimed that phenylbutazone is an antagonist of bradykinin[36] As phenylbutazone is known to be a potent anti-inflammatory agent this would provide good evidence that bradykinin plays a major part in inflammation However, the action of phenylbutazone is not specifically to antagonize bradykinin, and many other drugs are partially antagonized by the large doses of phenylbutazone required (Lewis, G. P , unpublished results). Another anti-inflammatory agent—aspirin—specifically antagonizes the bronchoconstriction caused by bradykinin in guinea pigs[34] However, here again this cannot be taken as evidence of the role of bradykinin in inflammation. as aspirin does not antagonize those actions of bradykinin which would be involved in inflammatory reactions (Lewis, G. P., unpublished results).

Although there is no direct evidence of the part bradykinin may play in the reactions to local injury, there is evidence of the part it takes as a physiological mediator of functional vasodilatation, as shown by the experiments of Hilton and Lewis[33,37–39] on perfused salivary glands In these experiment it was found that during activation of the glands an enzyme, probably salivary kallikrein, escaped from the secretory cells into the tissue space and that this enzyme formed bradykinin, or at least a plasma kinin, when it came into contact with the plasma globulins When the gland cells are activated, the enzyme thus escapes both into the duct to appear in the secretion and into the interstitial space of the gland around the blood vessels to form the vasodilator polypeptide As kallikrein appears in many glandular secretions, it follows that all these glands have the plasma-kinin-forming mechanism which might therefore be the mechanism in such glands for producing functional vasodilatation In this way—by producing an increased blood flow in the gland—bradykinin supplies the gland with the fluid required during secretion. It might not only be in glands themselves that bradykinin has such a role; in tissues which contain an abundance of glands, intestine and skin, for example, it may also account for periglandular vasodilatation. For example, in the intestine it is possible that the activation of intestinal glands leads to bradykinin formation which would bring about the vasodilatation required during the assimilation of foodstuffs

In skin, Fox and Hilton[40] have found that human sweat contains a plasma-kinin-forming enzyme and by analogy with the salivary glands have concluded that bradykinin formation accounts for the vasodilatation in the glands during secretory activity In the case of sweat glands bradykinin plays an additional part. As the sweat glands are in such close proximity to each other in the skin it seems likely that when the glands are activated, during body heating, the bradykinin formation which occurs accounts for the vasodilatation in the whole skin This is the conclusion Fox and Hilton reached from their experiments on increased forearm blood-flow during body heating In thus providing extra fluid in the increased flow of blood to form further sweat to be secreted and evaporated, bradykinin plays a part in the regulation of body heat

The theory that bradykinin is the mediator of functional vasodilatation in glands and glandular tissue has been challenged on two points

First, Terroux, Sekelj and Burgen[41] claimed to have dissociated glandular activity and vasodilatation in the salivary glands As a measure of glandular activity they used oxygen uptake However, whether oxygen uptake reflects the glandular activity quantitatively is doubtful, as these authors themselves suggest that part of the metabolism in the secreting gland occurs through anaerobic glycolysis Their experiments show that small doses of atropine reduced the increased uptake of oxygen in parallel with secretion when the gland was activated by stimulation of the chorda tympani nerve, but did not affect vasodilatation in the gland This problem has been examined extensively by Hilton and Lewis[38], and we have shown that after atropine, even in doses larger than those generally used by Terroux, Sekelj and Burgen, nerve stimulation still produced an output of plasma-kinin-forming enzyme As the enzyme comes from the secretory cells, it is evident that although overt secretion is prevented, some of the biochemical processes associated with glandular activity are still stimulated. Therefore. the lack of parallelism between oxygen uptake and vasodilatation in the partially atropinized gland is not a relevant argument against the view that bradykinin is responsible for functional vasodilatation

Secondly, Schachter[42] observed that guinea pig saliva did not act on guinea pig serum to form bradykinin and concluded that functional vasodilatation was not the result of bradykinin formation However, Schachter also observed that the guinea pig serum used in these experiments did not form bradykinin with the saliva from cat, rat and man, but that guinea pig saliva did form a plasma kinin when incubated with the serum of other species It seems, therefore, that it is not that guinea pig saliva does not contain the plasma-kinin-forming enzyme but that under the conditions of this particular test the serum did not react with salivary kallikrein from any species. Plasma and serum are known to contain a complex mixture of enzymes, inhibitors and activators (see ref 43) and it may well be that in order to produce bradykinin from guinea pig serum by the action of salivary kallikrein *in vitro*, special treatment of the serum proteins is necessary

Functional vasodilatation is a feature of glands which act intermittently, like the salivary glands, and it requires a mechanism which produces a large increase in blood flow of rapid onset and short duration It might well be that in glands which are continuously active over relatively long periods of time, such as mammary and endocrine glands, no such functional vasodilatation occurs This would explain the absence of plasma-kinin-forming enzyme from the secretions of such glands as ovary, thyroid and prostate[44]

The part played by bradykinin in functional vasodilatation in glands and glandular tissues and its possible role in inflammation depend on two factors which limit the area in which the reaction goes on, that is, factors which localize the reactions First, it seems likely that the formation, the action and the destruction of bradykinin go on in the interstitial fluid In a gland, for example, this would mean that when the gland is activated the enzyme escapes from the secretory cells into the interstitial fluid; there it acts on the globulins and forms bradykinin The peptide dilates the blood vessels and is eliminated via the lymph. Hilton and Lewis[37] provided evidence that this actually occurs in the salivary glands and that under normal conditions it is not necessary for the peptide or the enzyme which forms it to enter the blood stream Thus, this physiological reaction would be physically limited to the interstitial space of a gland Secondly, there is an efficient mechanism in the body for destroying excess bradykinin In blood, and probably in interstitial fluid, there is an enzyme which rapidly destroys bradykinin and thus controls the action of bradykinin Without this mechanism bradykinin formation in the blood stream would result in generalized peripheral vasodilatation and vascular collapse This is, in fact, what might happen in certain cases of shock The reactions of certain proteins, such as antigen and antibody in the blood stream, may activate the plasma-kinin-forming system Bradykinin or a plasma kinin would then be formed in the blood stream and if it were associated with a reduction in the bradykinin-destroying activity of blood, it would result in vascular collapse and shock

However, there is as yet no evidence to indicate that bradykinin plays any part in generalized reactions All the evidence that we have suggests that bradykinin acts in local reactions, first, as the physiological mediator of functional vasodilatation and, secondly, as a mediator of the inflammatory response In such local phenomena bradykinin might well be classified as a local tissue hormone

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FOOD PROCESSING IN RELATION TO HEALTH

THE final session of Section I (Physiology and Biochemistry) of the British Association meeting held at Norwich on September 6 was devoted to "Food Processing in Relation to Health", but the four papers read did not—and indeed could not in the short time available—cover the complex nature of the problems implied by the title Nor were the full implications of the subject raised in discussion, an omission which may indicate that the audience were aware that many of the questions they might have asked cannot, in the present state of knowledge, be answered No contribution was included in the programme on the nutritive effects of food processing, a subject on which all too little information exists, but one on which it would have been useful, and perhaps salutary, to have had a summary Nevertheless, some other aspects of food processing entered into the other papers, and if all are viewed together an otherwise fairly complete picture emerges Miss D F. Hollingsworth (London) described trends over eighty years in food consumption Prof John Yudkin (London) discussed dietary fat, and Dr Magnus Pyke (Menstrie, Clackmannanshire) dietary protein in relation to health Finally, Dr J R Nicholls (London) reviewed the need for food preservation by means of additives and the precautions taken to ensure safety

As a background to any consideration of trends in food consumption, it is necessary to study trends in food supplies In the United Kingdom such statistics are readily available, for the years immediately before 1939 and for each year since the outbreak of war, in reports of the former Ministry of Food and the present Ministry of Agriculture, Fisheries and Food on food consumption-levels, now published annually in the *Board of Trade Journal* Earlier estimates, which are not fully comparable with present ones, but which serve to indicate trends, were made by a committee set up by the British Association in 1880 to estimate the apportionment of the national income between different foods and services, with particular reference to foods, for the years 1909–13 by a committee of the Royal Society as a basis for studying the adequacy of national food supplies in the First World War, and for the years 1924–28, independently, by Sir Alfred Flux and Sir Albert Feavearyear Examination of all these data shows that in eighty years the character of British food supplies has changed remarkably. In 1880, 55 per cent of the energy value of food supply was derived from grain and potatoes compared with 34 per cent immediately before the War, 43 per cent in the 'forties and 31 per cent in 1959 The marked changes in the other direction have been for proportions of the energy value derived from animal products, which increased from 21 per cent in 1880 to 27 per cent in 1959, from fats and oils, which increased from 5 per cent in 1880 to 17 per cent in 1959, and from sugar, which increased from 13 per cent in 1880 to 17 per cent in 1959 Over a longer time, increasing sugar consumption is perhaps the most remarkable of all, consumption in Britain having increased five-fold in the past century and twenty-five times over two hundred years Comparison of these trends with corresponding ones for the United States suggests that the proportions from fats and oils and sugar are unlikely to increase further, but that, if the standard of living continues to rise and manual work to decrease, the proportion from grain and potatoes may fall further with a corresponding increase in that from animal products Such a general trend towards more food of animal and less of vegetable origin (with the possible exceptions of sugar, fruits and green and other non-starchy vegetables) is to be found in all affluent societies, and its desired nature has been recognized since the writing of the Proverb, "Better is a dinner of herbs where love is, than a stalled ox and hatred therewith"

The nutritional pattern of the national food supply in Britain, after war-time and post-war changes, became fairly stable by 1956 For all nutrients evaluated, the national diet in 1959 was of greater nutritional value than before the War Its energy value was 5 per cent above the pre-war estimate and had scarcely changed since rationing ended in 1954 The most marked changes, compared with pre-war estimates, were for calcium and thiamine, which stood at 66 and 38 per cent, respectively, above the pre-war levels in 1947, and have remained almost unchanged since About one-third of the increase in calcium came from increased milk supplies and the rest from the addition of *creta præparata* to flour The restoration of thiamine to flour was largely responsible for the maintenance of the supply of this vitamin, and the restoration of iron and nicotinic acid to flour was the main cause of the increases in these nutrients Animal protein and vitamin A also showed substantial increases Increased consumption of dairy products was the main reason for the rise in animal protein The rise in vitamin A came in almost equal amounts from milk and its products, liver, vegetables containing carotene, and margarine fortified with vitamin A, which more than offset reduced butter consumption

Within this general trend towards a diet containing more foods of animal and less of vegetable origin there has been another, a striking trend towards the increased consumption during the past few years of the so-called 'convenience' foods, in which the manufacturer has relieved the housewife of much of the labour of preparation

Supplies of canned meat and canned vegetables are nearly three times those of the pre-war years and well above those available in the early 'fifties Supplies of canned fruit and home-produced canned soups were in 1959 more than twice as great as in 1953, while the supply of canned fish was three times as great Consumption of unsweetened evaporated milk (which acts as a substitute for cream) is steadily increasing, but that of sweetened whole and skim milk is declining

In the past five years the production of dried soups has doubled Since 1954 there has been intense competition in the breakfast-cereal market which has included the introduction of sugared cereals Biscuit production has doubled in the past ten years Custard powder is becoming less popular, but sales of table jellies, ice-cream and soft drinks are increasing 'Instant coffee' has rapidly gained ground, and 'instant tea has appeared Another development has been in frozen foods In the past ten years consumption of frozen vegetables (almost entirely peas) has increased about eight-fold, and since 1953 consumption of frozen fish, not all of which is purchased in the frozen state, has quadrupled A recent innovation is the sale of frozen partly prepared main dishes, such as chicken pie, and another even more recent is the production of dehydrated ready meals The growth of the self-service shop and supermarket has probably led to a greater revolution in the presentation of meat products to the public than of other groceries or provisions

According to the National Food Survey, nearly one-fifth of domestic food expenditure in Britain in 1959 was devoted to cooked and canned meats, other meat products, cooked and canned fish, quick-frozen legumes, canned vegetables, canned fruit, fruit juices, factory-produced cakes, pastries, biscuits and puddings, quick-cooking cereals, canned and dehydrated soups, and recent trends suggest that such expenditure will rise

In considering dietary fat in relation to health, Prof Yudkin said that under war-time and post-war rationing there were claims that fat consumption was too low and that this was responsible for dry skins, dull hair, a feeling of lassitude and an increase in the incidence of infection The claim to-day is that we eat too much fat and this results in a variety of illnesses, the most important being obesity and coronary heart disease. His view is that there was no evidence that we suffered from inadequate consumption of fat during rationing and scarcely any evidence that we are suffering from excessive consumption to-day The quantity of fat needed for human health is unknown

Obesity, an important medical problem, is due to eating more food than is needed Prof Yudkin made two points on this first, that our increasingly sedentary lives have decreased food needs, but many people are not eating less, and, secondly, that before the introduction of arable farming our prehistoric ancestors must have lived mainly on protein and fat, the outstanding dietary change occurring at the beginning of civilization being an increase in carbohydrate consumption In the past two centuries an additional change has been the remarkable increase in the consumption of sugar with a reduction in other forms of carbohydrate For these reasons he recommended that the best way to prevent or cure obesity is to eat much less carbohydrate and not to worry about fat intake

Turning next to consideration of coronary heart disease, Prof Yudkin said that there seems to be no doubt that coronary thrombosis has become more common in the past twenty years in the developed countries such as Great Britain, and that it is far less common in countries and communities where the diet contains little fat In spite of increasing research into the subject, the main evidence relating fat consumption and coronary thrombosis is still epidemiological and no direct evidence linking the two has been produced On the whole, populations with low consumption of fat have little coronary thrombosis But these are also populations with low standards of living, whose diets differ in ways other than in their fat content, and whose lives differ in ways other than diet. There are only one or two investigations in which the comparisons are of groups with similar diets but differing modes of life, or with different diets but similar modes of life, these investigations do not support the theory that dietary fat is the single factor causing coronary disease The investigations which compare the increased incidence of the disease with the changing consumption of fat equally do not show a close relationship The present evidence fits best the suggestion that coronary thrombosis is caused by a number of factors, these include heredity, lack of physical activity, smoking, mental stress and overweight Prof Yudkin concluded that there may well be other factors, and that one of these may be an excess or deficiency of one or more nutrients, such as fat in general or some kinds of fats in particular His main conclusion was, however, a plea for more research

Dr Pyke's theme was the simple one that a proper understanding of the chemistry of food and the physiological requirements of people makes it possible to arrange that food processing shall be entirely beneficial to consumers He illustrated his theme by a description of modern work on the assessment, in terms of amino-acids, of both the composition of proteins and human protein requirements He spoke of early work on the improvement in health brought about by increased consumption by children of liquid milk and, more recently, of dried skim milk This led to the modern approach in developing countries of studying the available protein foods, assessing their amino-acid content, mixing and producing a palatable product designed to meet the amino-acid needs of the children for whom it is intended He gave as a concrete example the production of 'Incaparina' by Dr Scrimshaw and his colleagues in Guatemala from maize, sorghum, cotton-seed, yeast, calcium carbonate, leaf meal and vitamin A This can be made into a palatable gruel acceptable by children suffering from protein malnutrition Its effectiveness was demonstrated by pictures of an affected child before and after treatment

Dr Nicholls spoke of man's need since prehistoric times to preserve and store foods so as to find the means of avoiding both glut and famine By tradition, salt, sugar, vinegar and other materials have been used to preserve foods In present urban life, other additions are necessary to prevent damage during transport and storage, and to produce for the housewife more attractive, more convenient foods of better keeping quality. Such additives must be of real practical value and must not impair significantly nutritive properties nor involve a health hazard. Dr Nicholls described the ways of testing and controlling the use of additives not normally accepted as food ingredients, including the work of the Advisory Committee on Poisonous Substances used in Agriculture and Food Storage, the Food Standards Committee, various scientific bodies and the chemical and food manufacturers' laboratories

D F HOLLINGSWORTH

SPEECH

A SYMPOSIUM on speech was held on September 5 during the British Association meeting in Norwich under the joint auspices of Sections I and J (Physiology and Psychology) The chair was taken by Prof O L Zangwill (Cambridge), who pointed out that this was a near-centenary occasion In 1868, the British Association met at Norwich and held a famous discussion on speech to which Broca and Hughlings Jackson were the main contributors In addition to commemorating this occasion, it was hoped that the symposium would serve to draw together recent lines of work on problems of speech and illustrate changing conceptions in the study of aphasia

Neurology of Speech

The discussion was opened by Sir Russell Brain (London), who began by paying tribute to Hughlings Jackson's remarkable contribution to the neurology of language When Jackson addressed the British Association at Norwich at the early age of thirty-three he had already evolved the main ideas which were later to transform our thinking about aphasia. In particular, he saw clearly the need for fuller understanding of the physiology of speech and in this respect physiology had lagged behind anatomy. Whereas the work of Broca and his successors threw much light on the areas of the brain involved in language, it had failed to clarify its nature as a physiological activity In Sir Russell's view, progress in the study of aphasia depends above all on our gaining more adequate insight into the physiological basis of normal speech.

Sir Russell proceeded to outline three lines of approach which might serve in some measure to bridge the gap between the anatomy and psychology of language First, important clues might be derived from genetic study In particular, we need to know how the infant's perception of his own voice monitors his speech production. In the adult, it is known that delayed speech 'playback' markedly dislocates the output of speech[1], and it is not extravagant to suppose that certain types of speech disorder owe their origin to derangement of aural 'feedback'. There is consequently a need for fuller analysis of the basic mechanisms involved in the acquisition and regulation of speech

Secondly, Sir Russell laid stress on phonetic studies and their implications for the physiology of speech Modern technical methods have made possible very precise analysis of the physical properties of speech sounds and have thrown a good deal of light on the characteristics of sound patterns on which intelligibility depends Yet we still know little of the central mechanisms which govern the recognition of speech sounds and which enable constant meanings to be attached to what we hear Context, it would seem, is an important consideration Thus, what particular consonant is heard has been shown to depend in many instances on the vowel by which it is followed[2] In the case of word-meaning, context is of even greater importance and raises in acute form the whole problem of serial order in central nervous activity[3]. In view of these considerations, Sir Russell was concerned to stress the need for concepts which do adequate justice to the flexibility of language. In particular, he proposed that the concept of the 'schema', originally put forward by Head and Holmes[4] in regard to the appreciation of posture, might find fruitful application to the physiological organization of speech[5].

Thirdly, Sir Russell paid tribute to the growth of communications theory. He approved of the use of statistical method in the analysis of speech, and of the view that the brain is to be regarded as an instrument for the estimation of probabilities. Indeed, what he had called the 'schema' might perhaps be defined as the physiological mechanism which enables the individual to gamble on the probability that a particular sound pattern is to be invested with a particular meaning. He added that probability theory is now being successfully applied to the analysis of vocabulary and its reduction in aphasia.

Techniques in the Study of Aphasia

Prof. R. C. Oldfield (Oxford) was mainly concerned with the development of techniques for the analysis of specific derangements of speech. He pointed out that whereas the clinical examination of dysphasic patients has changed little since the time of Hughlings Jackson, there has been some advance in methods designed to study particular facets of the disorder. For example, Head's tests[6] were modelled on quantitative physiological procedures and were intended to assess particular derangements of symbolic function. Unfortunately, Head's theoretical views had not found general acceptance and his tests are nowadays seldom used *in toto*. Weisenburg and McBride[7] developed some more standardized tests of intellectual function, but in view of the variability of performance in aphasia it is doubtful whether their use has led to significant advance. In general, Prof. Oldfield saw little purpose in studying aphasic cases in all their bewildering variety; better, he counselled, stick to purely linguistic issues and concentrate research effort on a narrow front.

Among new methods of particular promise, Prof. Oldfield singled out the work of Penfield and his associates[8] on the electrical stimulation of the exposed human brain cortex. As is well known, stimulation within the so-called 'speech areas' reproduces many of the clinical symptoms of aphasia, among them speech arrest, misnaming, distortions and ellisions, and wrong use of words (paraphasia). It is noteworthy, moreover, that the use of this technique has failed to sustain the classical distinction between 'sensory' and 'motor' speech zones set up on the basis of clinical study. Prof. Oldfield also directed attention to the recent use of intracarotid injection of sodium amytal[9] as a means of establishing hemisphere dominance, that is, the hemisphere in which speech is located. As with cortical stimulation, transient and reversible dysphasia may be produced. Such methods, Prof. Oldfield suggested, have obvious possibilities for the experimental analysis of disordered speech.

An account of some psychological work on nominal defects and related problems was then given. As Prof. Oldfield pointed out, naming an object involves more than the mere association between symbol and referent, and disorders of naming cannot be viewed in terms of the failure of specific associative links. It is necessary, for example, to consider redundancies within the total system within which we search in seeking a particular name. More generally, Prof. Oldfield urged that statistical study of the products of disordered speech is well worth undertaking, and its arduousness is nowadays much reduced by the availability of tape recorders and electronic computers. Such study, Prof. Oldfield suggested, might well model itself on the methods employed by Yule[10] and Zipf[11] in the statistical study of normal vocabulary. Preliminary examples of the application of quantitative methods to aphasia were described[12].

In conclusion, Prof. Oldfield once again laid emphasis on the need to select limited problems and to develop techniques appropriate to their study. Although clinical issues are often dramatic, it is not always the most interesting problems that lend themselves to scientific attack.

Why we have Two Ears

The third speaker was Prof. Colin Cherry (London), who dealt with problems in the general field of speech perception. He began by directing attention to axial symmetry in bodily structure and its significance for biological adaptation. In particular, the possession of two ears is important not only for directional listening but also for effecting maximal separation of sound sources and hence optimal discrimination of speech in a noisy environment. Prof. Cherry was therefore particularly concerned to determine the features which enable the brain to infer 'what goes with what' and thereby to create unified and meaningful auditory percepts (*Gestalten*).

An important light is thrown on speech perception by consideration of the facts of binaural fusion. In the case of tones of high frequency, the half wavelength of sound is less than the inter-aural separation, and here fusion appears to depend on an 'averaging process' which extracts the fluctuating envelopes of the sounds and rejects the high-frequency steady tones[13]. This may be expected greatly to extend the range of frequencies from which stable *Gestalten* can be built. Prof. Cherry further laid stress on the role of head rotation in producing time-differences at the two ears and thereby facilitating the discrimination of speech. As he rightly pointed out, the sense of hearing appears to be closely related to the sense of balance, and posture and head adjustments are clearly of great importance in exploring the acoustic world.

Prof. Cherry then touched briefly on the relations between hearing and speech. As he has shown, an individual can listen to and repeat a spoken message virtually concurrently ('shadowing') and it is noteworthy that even stammerers can often perform this task without appreciable handicap[14]. This might suggest not only that speech and hearing form a single integrated activity but also that impediments of speech may be due to faulty aural monitoring rather than to executive disability. Indeed, such findings do much to reinforce Sir Russell Brain's plea that greater attention should be devoted to the 'feedback' mechanism in the regulation of normal speech.

The problem of binaural speech perception was next considered, with special reference to dichotic listening. Some years ago, it was shown that if two different messages are fed simultaneously through headphones, one to each ear, the subject can 'shadow' either message at will but is rendered almost totally unaware of what is reaching the 'neglected' ear[15]. In real life, however, we seldom listen to competing sources of information in so simple a manner. More commonly, the listener is assailed by a barrage of voices which have different mutual time-relations at the two ears and various other small differences. In separating them, it is necessary to assume a kind of inductive inference based in part on past experience. Can this be studied?

As a first step, Prof. Cherry described an experiment still in progress in which the subject wears headphones and listens to a message recorded on a special tape recorder. This enables various time intervals to be introduced between right and left headphones and thus causes the fused sound image to move from side to side in the listener's head. A second voice which may likewise be caused to move is then introduced. With the first voice set at a given position, the second is moved to right or left of it randomly by various amounts, and at each setting the subject guesses whether the shifted voice is to the right or left of the fixed voice. Although the statistics of the guesses show some measure of uncertainty, the task can in general be performed. On the other hand, if sounds provided by random noise generators are substituted for the voices, it becomes almost impossible to accomplish; indeed the listener hears two noises as a single fused sound[16]. The next step in the experiment is to proceed either by removing clues from the voices (for example, by removing larynx pitches) or by adding clues to the random noise sources (for example, by introducing a sharp resonance); in each case the measure of uncertainty shows the extent to which the clues in question are utilized. Although the results are not yet complete, Prof. Cherry held out good hopes that the technique would prove powerful in enabling us to specify "what there is about human voices that enables them to be separated so efficiently".

It will be seen, then, that Prof. Cherry was concerned to isolate the factors involved in the segregation of complex auditory experiences. This analysis, he submitted, must be set out in the mathematics of statistical inference. It must be supposed that the brain is able to carry out a kind of 'correlational analysis' which enables it to identify certain invariant clues under an infinite variety of uncertain transformations. These clues are to be regarded as statistical invariants, and correlations (or hierarchies of correlations) are required to assess them. So far, he had been concerned only with first-order, short-term correlation functions[17], which allow a start to be made in describing the logic of certain perceptual operations. These computations, he suggested, which involve operations of short-term storage, coincidence detection and averaging of sensory data, may well be 'natural' to the central nervous system. The ultimate test of such an approach is, however, physiological, and Prof. Cherry concluded by asking physiologists whether, when considering the brain as a computer, it had been looked at from this point of view.

In his closing remarks, Prof. O. L. Zangwill contrasted the approach to speech evidenced by the contributors to this symposium with that of their predecessors ninety-three years ago.

First, he said, it is plain that much less weight is attached nowadays to consideration of structure. Unlike Broca, Sir Russell Brain had brought along no colourful charts to demonstrate the cerebral seat of speech and evidently did not regard anatomical issues as of prime importance. Although the problem of cerebral localization of course remains, it now seems doubtful whether clinico-pathological study alone can throw important light on the mechanism of speech. Indeed, neurologists appear to be turning increasingly to neurophysiology, experimental psychology and communications theory to help in the elucidation of clinical phenomena. In this field at least, pathology has proved itself a disappointing guide to our understanding of normal function.

Secondly, Prof. Zangwill directed attention to some striking changes in our general conception of the speech processes. In Broca's day, it was customary to break down speech into a multiplicity of elements, sensory and motor, and to suppose that each of these possessed determinate cerebral localization. Even Hughlings Jackson had not been wholly free from this tendency to over-simplify. To-day, it would seem, our approach is decidedly anti-analytical; no one had spoken of sensations, images or related products of introspective abstraction. Indeed, the emphasis had been almost wholly on higher-order organization and on the brain mechanisms conceived to underlie it. This had been particularly well brought out by Prof. Cherry in his valuable studies of binaural integration and the perception of speech.

Lastly, Prof. Zangwill commented on the astonishing extent to which statistical thinking now dominates our approach to problems of language. Statistics were unknown to Hughlings Jackson, but it would appear that his successors can ill afford to disregard them. No longer can speech be envisaged in terms of reflexes and habits of an essentially particulate nature, and no longer can disorders of speech be viewed in terms of the loss of particular reactions or classes of reaction. As Prof. Oldfield so well showed, analysis of the products of disordered speech cannot be satisfactorily undertaken without recourse to considerations of probability theory. Although information theory may give difficulty to the non-mathematical, clearly it must be accepted if our knowledge of speech is to progress.

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OBITUARIES

Prof. H. V. A. Briscoe

HENRY VINCENT AIRD BRISCOE died in London after a short illness on September 24, 1961, his seventy-third birthday. He was professor of inorganic chemistry in the University of London at the Imperial College of Science and Technology during 1932–54 and thereafter professor emeritus and Fellow of the Imperial College. Briscoe was educated first at the City of London School and then the Royal College of Science, which at the time was on the point of being incorporated within the framework of the Imperial College. He graduated with honours in chemistry and then first assisted Sir Edward Thorpe in a revision of his *Dictionary*. Although he was still a junior member of the teaching staff of the Imperial College, during the First World War he was engaged in responsible and secret work (which similarly occupied much of his time during the Second World War) and later took charge of the Organic Chemistry Department at Sir John Cass College before being appointed in 1921 to the chair of inorganic and physical chemistry at Armstrong College (now King's College), Newcastle upon Tyne. He became head of the Chemistry Department in 1925 and later served as dean of the Faculty of Science in the University of Durham. Six years following his return to the Imperial College, on the retirement of Prof. J. C. Philip, he became director of the Laboratories of Inorganic and Physical Chemistry, a position he held until his retirement.

In addition to his academic positions, Briscoe had many outside interests: among other things he was the first director of the Northern Coke Research Committee's laboratory, president of the Research Association of British Paint, Colour and Varnish Manufacturers, visitor and manager of the Royal Institution of Great Britain and Ireland, vice-president of the Royal Institute of Chemistry, as well as being a member of Council and officer of the Chemical Society, and chairman of a local section of the Society of Chemical Industry. After the Second World War he was also greatly concerned with the improvement of the supply of laboratory technicians in university work and was chairman of the Imperial College Technician Training Committee, of the similar University of London Committee and of the National Joint Committee on the Recruitment and Training of Science Laboratory Technicians.

Briscoe's scientific publications cover a wide range of interests. Although his first publication in 1912 with P. W. Robertson was on the migration of the *para*-halogen atom in phenols, he afterwards moved into the inorganic and physical area. Among his earlier works were precise atomic weight determinations of vanadium, tin, thallium, boron and bromine. He considered himself a disciple of T. W. Richards and was similarly devoted to rigorous purification and the development of new methods, such as those for handling volatile hydrolysable halides. He was also aware of the possibility of the variation in isotopic ratios of materials of different geological origin, and for his studies on boron developed a flotation method for the measurement of comparative densities of boron trichloride which gave values to within about $\pm 2 \times 10^{-6}$ unit. This density method, which was typical of Briscoe's experimental skills and ingenuity, was also applied to examine heavy water and he was among the first in Great Britain to be concerned with the use of deuterium and later oxygen-18 as tracers in exchange reactions. Briscoe also made contributions to the chemistry of rhenium oxides, halides and sulphides, and of selenium.

Throughout his career, Briscoe was much concerned with more applied aspects of chemistry and made many contributions to the knowledge of coke, paints and drying oils, on which he was an authority, purity of water, insect pests in foodstuffs, etc. During the 1930's he became very interested in the silicosis problem and hazardous industrial dusts and devised methods for collection and analysis of dust samples. For this work he was awarded the Consolidated Goldfields of South Africa Medal by the Institution of Mining and Metallurgy. Inspired by a lecture given by Langmuir at the College, and anticipating current interest by many years, he also worked with Sebba on the reduction of evaporation from water surfaces through the use of monolayers of cetyl alcohol.

Briscoe will be remembered by his colleagues and students for his approachability and kindly manner. He is survived by his wife and a son and daughter—both members of the medical profession.

G. WILKINSON

Prof. Carl Hermann

PROF. CARL HERMANN, director of the Crystallographic Institute at the University of Marburg, died unexpectedly in his sleep on September 12 at the age of sixty-three. He was a pupil of Max Born and a fellow research student with Werner Heisenberg, at Göttingen. Later he became a lecturer at Stuttgart, where he and Paul Ewald initiated the *Strukturberichte* which (with its successor *Structure Reports*) gives a detailed abstract of every known crystal structure determination, and which is a valued work of reference in every modern scientific library. He also developed, with Prof. C. Mauguin, a systematic nomenclature (now known as the Hermann–Mauguin notation) for the 230 space groups, from which the symmetry can be derived at a glance, and he travelled widely in the early 1930's, gathering material for the two volumes of the *Internationale Tabellen zur Bestimmung von Kristallstrukturen.*

Hermann and his wife Eva were guests in my home at the time when the National Socialist party doubled its strength in the General Election of June 1932, which later brought Hitler to power, and I can well remember their dismay, for they realized even then what would follow. Later, Carl Hermann refused to conform to the political conditions imposed on academic staff and he took a position as physicist at the I.G. Farbenwerke at Ludwigshafen, where he continued crystallographic research of high quality on the nature of chemical bonds and on symmetry in n-dimensional space. He and his wife were imprisoned throughout much of the Second World War for their increasing activities in hiding and helping innumerable Jews to escape from the terrors of Nazi persecution. Only his eminence as a scientist and the intervention of his friends saved him from the carrying out of a death sentence, but these years left their mark on him.

In 1947 he was appointed to the new chair of crystallography in the University of Marburg and he threw himself with great energy into the teaching of the subject and into the building-up of an active research school But he also gave much of his time to the work of the Society of Friends, of which he and his wife were members, and to efforts to promote international understanding His presence at conferences was the more valued because of his great gift for languages, if a new language interested him, he learnt it

The statement put out by the Rector and Senate of the University of Marburg says of him. "Carl Hermann was a man of absolute integrity, and of kindness to others which had no limit For many years he was a member of the Disciplinary Court of the University and it was always he who tried to understand and to excuse the defendant . "

He is not likely to be forgotten, for he has left many friends KATHLEEN LONSDALE

Prof. E. Taylor Jones

THE death on September 29 of Prof Edward Taylor Jones at the age of eighty-eight will have been noted with deep regret by the many who benefited from his unique qualities both as a teacher and research supervisor His own education followed a pattern that was familiar at the turn of the century. His studies at University College, Bangor, and the University of Berlin were followed by a time as professor of physics at Bangor and a long period as professor of natural philosophy in the University of Glasgow, a period prolonged by the advent of war in 1939 His interests throughout his life extended over a wide field of physics and he seemed, in fact, to be equally interested in physics and in electrical engineering Thus, his earlier researches were in the classical field of electromagnetism with special reference to the mechanism of oscillatory devices such as the singing arc, the induction coil, the Tesla coil, and the magneto

Research into the behaviour of the induction coil may well have led to his very early attack on a variety of problems in electron diffraction. He was greatly excited by the elegant de Broglie approach to wave mechanics and soon followed the lead given by G. P Thomson at the University of Aberdeen in his famous work on electron diffraction This was a notable switch in interest and could have resulted in a greater volume of original work but for the limitations of means and research students of that time Nevertheless, on average he supervised the work of some five research students throughout his active career I myself realized, as one of his research students, that Taylor Jones made many fruitful suggestions while never stultifying the initiative of members of his team He preferred to play the part of adviser and in this he gave freely of his store of knowledge

He will doubtless be remembered by most as an inspired teacher of the very large ordinary classes These seemed to fascinate him He took great pains to make his courses lively and even at times hilarious, obviously believing that above all interest must be generated His extremely expert clowning in some of his demonstrations made it inconceivable that an understanding of the fundamentals should be beyond any student In spite of this unique approach to his ordinary classes there was considerable discipline in his handling of classes of more than two hundred students With the advanced classes he was content to follow more orthodox patterns of instruction He must have roused within many a life-long interest in physics by his vivid proof that science could be fun

Prof Taylor Jones had many publications to his credit and the list of these shows his versatility and widely ranging interests Physics made startling advances throughout his life and he was excited by many of them He did not confine his research interests closely and he never looked with favour on team-work In this respect he belonged with the individualists of his earlier period Those who knew him as a friend realized how fully he enjoyed his life spent in physical science. S. C. CURRAN

Dr. Madeline Kerr

IT was with deep regret that the colleagues and friends of Dr Madeline Kerr learned of her death on August 22, a few months after she had resigned from her post of senior lecturer in social science in the University of Liverpool because of ill-health.

Dr Kerr was primarily a social psychologist, but the richness and variety of her interests in the cultural process took her work into the sociological and anthropological fields Dr. Kerr graduated at Manchester and, after working in the Psychological Service of the Liverpool Education Committee, returned to take up the Knight fellowship in psychological medicine At Manchester she was influenced by Prof T H Pear and retained an interest in the interaction of social status and personality traits From Manchester she went to London, where she took her doctorate and worked with Prof. Lancelot Hogben She then joined Prof Susan Stebbing to teach psychology in the Department of Philosophy at Bedford College She was a great admirer of Susan Stebbing and this was reflected in the logical austerity of her seminar teaching

In 1947 Dr. Kerr was given leave of absence to act as social psychologist to the West Indian Social Survey, where she organized a programme of psychological testing. In the West Indies, Dr Kerr found an environment in which her interests in the graphic and plastic arts and in music combined with her scientific interests to make her immediately acceptable to the subjects of her study, and she made lasting friendships with members of all classes in West Indian society from simple peasants to members of the Government Some of the findings of her work were published in *Personality and Conflict in Jamaica* (1952)

On her return to England it was natural that she should join Prof T S Simey, who had also worked in the West Indies, in the Department of Social Science of the University of Liverpool Here she continued her intensive investigation on families, and in 1958 published *The People of Ship Street*, which described the existence of a matriarchal society in a working-class area of Liverpool.

In Liverpool she devoted most of her energies and her great enthusiasm to her students, for whom nothing was too much trouble and with whom she made close and enduring friendships She will be greatly missed.

D CHAPMAN

NEWS and VIEWS

Royal Society Medal Awards

THE following awards of medals have been made by the President and the Council of the Royal Society. *Copley Medal* to Sir Hans Krebs, Whitley professor of biochemistry in the University of Oxford, for his distinguished contributions to biochemistry, in particular his work on the ornithine, tricarboxylic acid and glyoxylate cycles; *Davy Medal* to Prof. D. H. R. Barton, professor of organic chemistry in the Imperial College of Science and Technology, University of London, for his distinguished researches in organic chemistry, particularly on the structure and stereochemistry of natural products of the terpene and steroid series, and the analysis of the conformation of cyclic structures; *Sylvester Medal* to Prof. P. Hall, Sadleirian professor of pure mathematics in the University of Cambridge, for his distinguished researches in algebra; *Hughes Medal* to Prof. A. H. Cottrell, Goldsmiths' professor of metallurgy in the University of Cambridge, for his distinguished work on the physical properties of metals, particularly in relation to mechanical deformation and to the effects of irradiation.

David Sarnoff Award in Electronics :
Dr. C. H. Townes

DR. CHARLES H. TOWNES, a leading American physicist and provost of the Massachusetts Institute of Technology, has been presented with the David Sarnoff Award in Electronics of the American Institute of Electrical Engineers. The award cited Dr. Townes for research in physics leading to major advances in communication technology. He is well known for his work on the theory and application of masers, on which he holds the fundamental patent. Dr. Townes was appointed provost of the Massachusetts Institute of Technology earlier this year. He was a member of the technical staff of Bell Telephone Laboratories during 1939–47, then joined the faculty of Columbia University as associate professor of physics. He was appointed professor in 1950, served as executive director of the Columbia Radiation Laboratory during 1950–52 and as a chairman of the Physics Department during 1952–55. Dr. Townes's awards include the Morris Liebman Memorial Prize of the U.S. Institute of Radio Engineers (1958), the Stuart Ballantine Medal of the Franklin Institute (1959), the Comstock Award of the U.S. National Academy of Science (1959) and the Rumford Premium of the American Academy of Arts and Sciences (1961).

National Physical Laboratory :
Dr. J. V. Dunworth, C.B.E.

DR. J. V. DUNWORTH has been appointed deputy director of the National Physical Laboratory (Department of Scientific and Industrial Research) in succession to Dr. G. Macfarlane, who early next year will take up his recent appointment of director of the Royal Radar Establishment, Malvern (*Nature*, 192, 18; 1961). Dr. Dunworth is at present deputy director of the Atomic Energy Establishment, Winfrith. He is forty-four years of age and was born in Manchester and educated at Manchester Grammar School and the University of Cambridge. Shortly before the outbreak of war in 1939 he was invited to become a member of Sir John Cockcroft's team investigating problems in the use of radar. He continued in this work until 1944, working on 150-cm and 10-cm radar development, mainly at the Radar Research Establishments at Christchurch and Malvern. Late in 1944 he went to Canada with Sir John Cockcroft to work with the National Research Council on atomic energy development. Soon after the end of the War he returned to Cambridge to take up his fellowship at Trinity College and was appointed a demonstrator in physics in the Cavendish Laboratory. Later, in 1946, he rejoined Sir John Cockcroft at the Atomic Energy Research Establishment, Harwell.

The Botanical Survey of India : Prof. H. Santapau

FOR a long time the Botanical Survey of India remained in hibernation and the superintendent of the Sibpur Botanical Garden was also the officer-in-charge of the Botanical Survey with its headquarters in the Indian Museum at Calcutta. About eight years ago the Survey was re-organized and now has a new headquarters at Calcutta together with a number of regional offices in different parts of the country. Dr. J. C. Sen Gupta, who was chief botanist for the past several years, retired last summer and was succeeded a few months ago by Father H. Santapau, for many years professor of botany at St. Xavier's College, Bombay. Father Santapau was born in Spain in 1903. He went to India in 1928 and has ever since been intimately associated with educational institutions in India. Although his original education was in Barcelona in Spain, he later studied in the Royal College of Sciences and Imperial College of Science and Technology, London, from which he obtained the diplomas of A.R.C.S. and D.I.C. and the B.Sc. honours and Ph.D. degrees of the University of London. In 1947 he was elected Fellow of the Linnean Society of London and in 1953 a Fellow of the National Institute of Sciences of India. Father Santapau is the author of a large number of research papers and books dealing with the results of his explorations in parts of the Western Ghats (especially Mahabaleshwar) and the Dangs Forest in Saurashtra. A few years ago he wrote a comprehensive *Flora of Khandala* (a second edition of which has now been published) and in 1958 the *Flora of Purandhar*. Among his other publications special mention may be made of one on the *Acanthaceae of Bombay* and another dealing with a *History of Taxonomic Researches in India on the Angiosperms*.

Twenty-five Years of Television

DURING the first week of November, the British Broadcasting Corporation celebrated the inauguration of the world's first high-definition television service —on November 2, 1936 (*Nature*, 138, 394, 793; 1936). At the beginning two systems of transmission, the Baird and the Marconi-EMI, were used in alternate weeks to judge under practical conditions which was the better. After three months of this experimental service, and following the recommendation of H.M. Postmaster-General's Television Advisory Committee, the transmissions were confined to the Marconi-EMI system on a standard basis of interlaced scanning at

405 lines per picture, and a picture frequency of 25 per sec It was in this fashion that what is generally conceded to be the best public television service in the world opened well in advance of the United States, where the first public television transmissions did not start until April 1939

It is a tribute to the excellent pioneer work of the engineers and scientists who formulated the technical standards adopted by the Government in 1937 to note that these have remained unchanged in Britain for twenty-five years Associated with the celebrations of this silver jubilee was a dinner given by the British Broadcasting Corporation at Grocers' Hall, on November 7, presided over by Sir Arthur fforde, chairman of the B B C The Prime Minister proposed the toast of the service and paid tribute to the pioneer work of all those concerned not only with the technical achievement but also with the foundation of the new techniques of programme provision and presentation, for both informative and entertainment purposes Many of those responsible for these developments were present on this occasion, and they and their successors must have been encouraged to work towards the further developments which will undoubtedly occur in the next quarter of a century

Milk Powder

Even the richest cows' milk contains some 86 per cent of water, and it has long been realized that if milk could be dried satisfactorily at a moderate cost to produce a product with practically the same nutritive value as the raw milk from which it came, the resulting powder would have many important uses It is not surprising to find, therefore, that the manufacture and use of dried milk are greatly increasing in many parts of the world, and that even in Britain the amount now made is three times as much as it was before the Second World War The Government's Milk and Milk Products Technical Advisory Committee, realizing that many potential users of milk powder might not know of the merits and value of the modern product, or about the different types that are available, sponsored the preparation of a booklet by its Milk Products Sub-Committee (Ministry of Agriculture, Fisheries and Food, Department of Agriculture and Fisheries for Scotland, Department of Health for Scotland *Milk Powder* Pp v+18 (London H M S O , 1961) 1*s* 3*d*)

The booklet succeeds in giving a most valuable account of the subject, and is divided into two parts, the first of which deals with the manufacture of milk powders, their composition, bacteriology, bulk reconstitution to form liquid milk and their nutritive value The first part contains also a section on the physical and chemical properties of milk powders in which important properties such as particle size and shape, bulk density, flow properties and solubility and dispersibility in water are discussed The second part gives an account of the commercial uses of dried milks in baking and in the manufacture of margarine, chocolate, manufactured meat products, soups and ice-cream It deals also with the use of milk powder in canteens and institutions, and with its value in feeding-stuffs for certain classes of farm livestock such as very young calves, chicks and turkey poults The booklet ends with a list of eighteen references from which further details can be obtained This small but most informative booklet will long remain a standard work of reference on a subject which is bound to increase in importance as food science and food technology develop with increasing application of these subjects to world food problems

Royal Ontario Museum : New Mineral and Gem Gallery

A new mineral and gem gallery at the Royal Ontario Museum, University of Toronto, has been financed by a 75,000 dollar grant from the International Nickel Company of Canada, Ltd The exhibit of minerals on show in the Gallery will include material from the Leonard Collection of gemstones, the Andre Dorfman Collection of minerals and the MacIntyre–Porcupine Collection of gold ore and nuggets The mineralogy collections now at the Royal Ontario Museum were begun at Victoria College, Cobourg, at the end of the nineteenth century, at the Provincial Museum, Toronto, and with the purchase by the University of Toronto of one of the collections assembled by the late Dr W F. Ferrier In 1912, when the Royal Ontario Museum was established, these collections were brought together.

The Cotton, Silk and Man-made Fibres Research Association

Speaking at the annual general meeting of the Cotton, Silk and Man-made Fibres Research Association on October 12, the director, Dr D W Hill, said that the work of the Association was now organized in fourteen departments, of which five were newly constituted, and the research programme was controlled by a new Research Committee served by nine sub-committees, including two new ones All the 110 members of staff declared redundant on the merging of the two research associations had found satisfactory positions elsewhere The report of the Council, presented at the annual meeting, in describing research and development during the year refers to the application of the principle of the Shirley 'automatic size box' to the feed-box of a mangle and to progress in overcoming difficulties associated with a self-brushing device for laying the fibres in warps during sizing It also refers to work on heat transmission through assemblies of aluminized fabrics and cotton blankets with the view of providing information on which can be based the design of clothing for protection under conditions more exacting than those met in ordinary life Special attention has been paid to the development of a cold-pad process for bleaching cotton–acetate unions without hydrolysing or delustring the acetate, and considerable progress has been made in work on the flow of liquids in contact with or through textiles Work of package dyeing has directed attention to methods of achieving complete wetting-out of the package

Men and their Jobs

For his inaugural lecture delivered at Birkbeck College, University of London, on October 16, Prof Alec Rodger took as his title "Occupational Versatility and Planned Procrastination". Prof Rodger indicated that existing surveys to discover the requirements of man-power are usually inaccurate, and that, despite what is being done to prevent occupational waste, there is much human wastage for which remedial work has to be done. At present, 10,000 cases pass through the industrial rehabilitation units of the Ministry of Labour each year Prof Rodger suggested that facilities which are at present intended mainly for the disabled or chronically employed should be extended to other sections of the community. With ten more occupational psychologists in the Ministry of Labour, considerable help could be given in providing better guidance for both adults and young people To avoid real human waste, however, the training people received for jobs should be given the widest possible base, and specialization should be postponed as long as possible, until the individual and his advisers are reasonably sure he is in the right occupation or profession

Nature Reserves

The forty-fourth annual report of the Society for the Promotion of Nature Reserves for the year ending March 31 is largely made up of accounts of the activities at the fourteen reserves which come under the jurisdiction of the Society (Pp 44+2 plates. London: Society for the Promotion of Nature Reserves, British Museum (Natural History), 1961. 10*s*.). At Woodwalton Fen, Huntingdonshire, a study of the breeding biology of the large copper butterfly was continued and the eggs and larvæ on more than a thousand food-plants are being counted at regular intervals. Observations will be carried out on the preference shown by the butterflies when egg-laying for docks growing in the open fen and those growing in the dykeside. Suggestions for introducing the butterfly on an experimental basis to Blackmoor Copse Nature Reserve, Wiltshire, have been considered, but it has now been decided to postpone a decision until further observations have been made at Woodwalton Fen. A spider new to the British list, *Asagena phalerata,* was recorded at Linwood Warren Nature Reserve, while at Calf of Man, Isle of Man, a red-breasted flycatcher (the second Manx record) was captured in June, and Manx shearwaters were taken on land during the summer. The Manx shearwater has not been seen on the island for nearly two hundred years. At Belfairs Great Wood, Essex, the reed mace grass veneer (*Crambus paludellus* Hubner) has now occurred two years running in a garden abutting on the Reserve, and is probably breeding on reed mace in one of the small ponds

Electrotechnology in Australia

In the annual report of the Division of Electrotechnology of the Australian Commonwealth Scientific and Industrial Research Organization for the year ended June 30, 1960, reference is made to the three-weeks visit paid by Prof. H Frohlich, professor of theoretical physics in the University of Liverpool, to Australia at the invitation of the Division The object of the visit was to discuss and advise on the various aspects of the research work of the Dielectrics Section of the Division, which is concerned with the study of the alkali halides, aliphatic long-chain compounds—particularly the growth of large single crystals and the production of pure compounds, the hydrates of calcium sulphate; and the theories of dielectric loss and of superconductivity The Division is responsible for the maintenance of the Commonwealth standards of measurement of electrical and magnetic quantities, and its activities are dealt with separately in the annual report under the headings of direct current, power frequency, audio- and radio-frequency, and magnetics With the completion of the calculable capacitor, considerable progress has been made with necessary additional equipment and techniques for the absolute determination of the ohm. The screening efficiency of screened rooms with especial emphasis on the microwave region of the spectrum has been investigated, and work on the probability problems associated with various random processes has been carried out experimentally by means of the probability distribution analyser designed and constructed in the Division. The report concludes with a list of the publications by members of the staff, and their names and positions as at June 30, 1960

Solid Propellant Test Rig

Messrs P. A. Hilton and Co, engineers, of Radlett, Hertfordshire, announce that they have completed the development of a solid propellant rocket motor test rig for use in universities and technical colleges. The rig consists of a stainless steel rocket motor suspended on a swinging parallel motion linkage which pushes against a cantilever spring beam. Movement under load is transmitted to a pen recorder indicating thrust, on paper moved at constant speed by a synchronous electric motor The area of the diagram produced is related by the calibration to the total impulse Chamber pressure is transmitted by flexible pipe to a bourdon tube which in turn operates a recorder pen running parallel with the thrust pen. The fuel used is a form of cordite supplied in cartridge form giving a specific impulse of about 180 sec and requiring no more elaborate storage facilities than are accorded to shotgun ammunition. The motor is equipped with a blow-out diaphragm to diminish explosion risks The rig permits measurement of thrust, total impulse, specific impulse and burning time, and it is possible to study the effect of chamber pressure and nozzle design on these quantities by substituting nozzles of different diameters and profiles

The rig is of simple design, of which the recorder is the weakest link, since it is not certain that the relation between diagram area and total impulse is strictly linear and since the mechanism for applying tension to the paper is rather crude. The rig, however, should prove valuable for teaching and demonstrating in engineering departments wherever an interest in rocket motors and combustion exists and it has been constructed on a scale such that departments with quite limited facilities can use it The rig is a welcome addition to the very limited range of standard teaching apparatus available in the sphere of gas dynamics and combustion Further details can be obtained from the manufacturers.

Summer School in Analytical Chemistry

The fifth triennial summer school in analytical chemistry, organized by the Royal Institute of Chemistry with the participation of the Society for Analytical Chemistry, will be held in the Manchester College of Science and Technology during September 9–15, 1962. The School will consist of four separate, but concurrent, courses· *Course* 1, "Physical Methods of Organic Chemistry", under the direction of Dr. D W Mathieson, reader in pharmaceutical chemistry, School of Pharmacy, London, *Course* 2, "Recent Developments in Inorganic Analysis", under the direction of Mr W T Elwell, chief analyst, Imperial Chemical Industries, Ltd, Metals Division, Witton, Birmingham, *Course* 3, "The Determination of Toxic Substances in the Air and in Effluents", under the direction of Mr H. E Stagg, chief analyst, Imperial Chemical Industries, Ltd., Dyestuffs Division, Blackley, Manchester, *Course* 4, "Newer Instrumental Techniques", under the direction of Dr. V. S. Griffiths, reader in spectroscopy, Battersea College of Technology, London. Further information can be obtained from the Education Officer, Royal Institute of Chemistry, 30 Russell Square, London, W.C.1.

Theoretical Chemistry Summer School at Oxford

Once again Prof. C. A. Coulson and the Delegacy for Extra-Mural Studies of the University of Oxford are organizing a summer school in theoretical chemistry. The school is to be housed in St. Antony's College, with lectures in the Mathematical Institute. Further information may be obtained from the Secretary, Delegacy for Extra-Mural Studies, Rewley House, Wellington Square, Oxford. The dates of the School are September 8–22, 1962, accommodation is strictly limited and the closing date for applications is April 30, 1962.

British Institution of Radio Engineers: Awards

The following awards have been made by the British Institution of Radio Engineers for outstanding papers published in the Institution's *Journal* during 1960: *Clerk Maxwell Premium*, to Dr. G. T. Wright (University of Birmingham) for his paper on "A Proposed Space-Charge-Limited Dielectric Triode"; *Heinrich Hertz Premium*, to Mr. R. A. Waldron (Baddow Research Laboratories, Marconi's Wireless Telegraph Co.) for his paper on "Features of Cylindrical Waveguides containing Gyromagnetic Media"; *A. F. Bulgin Premium*, to Dr. A. Kraus (Rohde and Schwarz, Munich) for his paper on "Reflection Coefficient Curves of Compensated Discontinuities on Coaxial Lines and the Determination of the Optimum Dimensions"; *Arthur Gay Premium*, to Mr. E. Davies (Marconi's Wireless Telegraph Co.) for his paper on "The Application of Printed Wiring to Development of Small Batch Production with Particular Reference to Television Equipment"; *Leslie McMichael Premium*, to Mr. C. H. Dix and Mr. W. E. Willshaw (Hirst Research Centre of the General Electric Co.) for their joint paper on "Microwave Valves: a Survey of Evolution, Principles of Operation and Basic Characteristics"; *Lord Rutherford Award*, to Mr. E. C. Fellows (Associated Electrical Industries Research Laboratories, Aldermaston) for his paper on "Energy Stabilization of a 4-MeV Electrostatic Accelerator using Controlled Corona Discharge"; *Charles Babbage Award*, to Mr. E. M. Bradley (International Computers and Tabulators) for his paper on "A Computer Storage Matrix using Ferromagnetic Thin Films".

The Night Sky in December

New moon occurs on Dec. 7d. 23h. 52m. U.T. and full moon on Dec. 22d. 00h. 42m. The following conjunctions with the Moon take place: Dec. 11d. 00h., Saturn 2° S.; Dec. 11d. 14h., Jupiter 2° S.; Dec. 20d. 11h., Aldebaran 0·7° S.; Dec. 26d. 21h., Regulus 0·5° S. In addition to these conjunctions with the Moon, Venus is in conjunction with Antares on Dec. 11d. 20h., Venus being 5° N. Mercury and Mars are too close to the Sun for observation. Venus is a morning star, but is also too close to the Sun for easy observation. Jupiter and Saturn are evening stars, setting about three hours after the Sun, but conditions for observation are not favourable. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Dec. 1d. 1h. 19·3m., σ Leo (*D*); Dec. 1d. 2h. 13·5m., σ Leo (*R*); Dec. 12d. 17h. 56·7m., 45 Cap (*D*); Dec. 18d. 19h. 46·6m., + 11° 445 (*D*); Dec. 20d. 4h. 25·0m., γ Tau (*D*); Dec. 23d. 23h. 30·4m., 81 Gem. (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The Geminid meteors are active during December 9–14, maximum activity being on December 13. The radiant is at R.A. 7h. 28m., Dec. + 32°, and conditions for observation are very favourable. The Ursid meteors are active on December 20–22, but conditions for observation are unfavourable. The winter solstice occurs on December 22d. 03h.

Announcements

Mr. R. Blyth has been appointed editor of *The Pharmaceutical Journal*. He has been responsible for the *Journal* since June this year. He joined the *Journal* as assistant editor in 1957 from the same post on *The Chemist and Druggist*.

The Royal Agricultural Society of England offers annually a medal together with an award of one hundred guineas for research work of outstanding merit carried out in the United Kingdom which has proved, or is likely to prove, of benefit to agriculture. Recommendations from heads of university departments or research organizations for the next award must be submitted to the Secretary, Royal Agricultural Society of England, 35 Belgrave Square, London, S.W.1, not later than January 31, 1962.

A symposium on "The Role of Science in the Development of Natural Resources with particular reference to Iran, Pakistan and Turkey" is to be held in Lahore during January 8–13. The symposium is sponsored by the Scientific Council of the Central Treaty Organization.

The sixty-eighth annual meeting of the American Mathematical Society will be held in Cincinnati, Ohio, during January 22–25, in conjunction with the annual meeting of the Mathematical Association of America. Further information can be obtained from the associate secretary, J. W. T. Youngs, American Mathematical Society, 190 Hope Street, Providence 6, Rhode Island.

The Department of Municipal Engineering of the Manchester College of Science and Technology is organizing a short course of lectures on "Problems of Radioactivity at Water and Sewage Works", which will be held in the College during January 11–12. Further information can be obtained from the Registrar, Manchester College of Science and Technology, Manchester 1.

The International Atomic Energy Agency is organizing a conference on "New Techniques in Biology and Medicine" at Mexico City during November 21–December 1. Special attention will be directed to the use of isotopes in these fields. Further information can be obtained from the International Atomic Energy Agency, Vienna.

The Chemical Research Committee of the Indian Council of Scientific and Industrial Research is organizing a symposium on "Carbohydrates, Cellulose and Cellulose Industries" at Ahmedabad Textile Industry's Research Association, Ahmedabad 9, during January 29–30, 1962. Further information can be obtained from the Director, Ahmedabad Textile Industry's Research Association.

The name of one of the authors of the communication entitled "Mouse Lymphoma Cells with Different Radiosensitivities" in *Nature* of November 11, p. 572, was omitted; the communication was by Dr. P. Alexander and Z. B. Mikulski.

THE UNIVERSITY OF OXFORD EXPEDITION TO PERU, 1961

THE University of Oxford Expedition to Peru, 1961, has recently returned to the United Kingdom after completing approximately three months field work in the Central Andean Region south of Huancayo. The investigated area covered 600 sq miles between latitudes 12° 25′ and 12° 50′ S , and longitude 75° 15′ and 75° 30′ W , at altitudes from 13,000 to 18,000 ft O D Six scientists completed an integrated study which concentrated on the geological and botanical features of the country

No previous work had been done on the geology of the area although the region was adjacent to country mapped by Dr J V Harrison of the Department of Geology and Mineralogy, Oxford R C Herrera constructed an initial geological map on the scale 1 50,000 This work was augmented by detailed stratigraphical sections of the Mesozoic and Tertiary, and by systematic collection of fossils, especially from the Cretaceous limestones A small number of volcanic and acid intrusive specimens were collected for Prof B Giletti of Brown University, in conjunction with a programme of age determination of Andean rocks The results of the investigation will be used by Dr J J Wilson, of Lima, in his work on the Mesozoic history of the Andean Geosyncline

The botanical work, carried out by J R Lloyd and J K Marshall, was divided into five parts First, a collection was made of the flowering plants, ferns and bryophytes of the whole area Concomitantly, a map and general survey of the plant formations in the region were completed Thirdly, the quadrat method was used for a more intensive examination of samples from the formations of all districts. Completeness and precision were given to this phase by the detailed study of all the communities of a selected 10 sq mile plot Material comparable with the recently discovered fern-allay, *Stylites*, was found to be widespread in the *Distichia* bogs, and an autecological investigation was made of this and other genera Finally, analysis of the changes of day and night temperature of four different flowering plant genera was carried out. The botanists were accompanied for a short time by Dr O Tovard, a Peruvian taxonomist, who aided in collecting and general survey

S R S Walker carried out work on the various soils, the majority of which were skeletal lithosols with a marked absence of horizons His work was closely linked with the ecological programme of the botanists, and the samples will be analysed at the Department of Botany, Cambridge.

A 1 50,000 map of the north-eastern part of the area was compiled by the surveyor, M R Bewsher, this supplemented the smaller-scale published maps, Stadia methods were used to map at 1 5,000 a limited area of particular interest to the botanists In addition, Bewsher recorded continuous meteorological observations at six stations

M J Troughton undertook a geographical description of the study area, and in particular of the Ayhuicha Valley centred on the village of San José de Acobambilla This is an isolated and particularly self-sufficient peasant community, and he was fortunate in being able to carry out the investigation during the 'faena' or collective community work period. At this time the men are recalled to the village from work among the herds on the puna grazing areas, thus presenting a good opportunity of observing the various activities of such a population.

Throughout the Expedition, great use was made of animal transport—mainly pack mules, which, in spite of their temperament, were ideally suited to the rough mountainous terrain. The mules, the extreme hospitality of the local Indians, and a working knowledge of Spanish were found to be great aids to exploration in the Andes of Peru. R. C. HERRERA

ELECTRON MICROSCOPY IN THE UNITED STATES

THE nineteenth annual meeting of the Electron Microscope Society of America was held at the Pittsburgh Hilton Hotel, Pittsburgh, Pennsylvania, during August 23-26 The Local Arrangements Committee, headed by Dr Robert V Rice, is to be especially commended for the arrangement and success of this meeting, which brought together more than 706 active workers in electron microscopy from 24 American States, Canada, Australia, Great Britain, Belgium, Germany and Japan It was the largest meeting in the history of the Society There were ten general sessions of contributed papers, five in the biological fields, five in non-biological areas , in addition, a special session on techniques and three symposia were features of the programme Nineteen scientific exhibits were presented and twenty-one commercial exhibitors participated in the meeting. Some 180 authors contributed papers on the technique and applications of the electron microscope to work in biology, chemistry, medicine, cancer, metallurgy, electron diffraction and related fields

A limited number of programme abstracts are still available from Dr A R Taylor, Parke, Davis and Co , Detroit, Michigan One hundred and three new members of the Society were elected, making the total membership 1,040.

In the symposium on "The Contribution of Electron Microscopy to Polymer Morphology", organized by Dr R G Scott (Pioneering Research Division, DuPont Experimental Station, Wilmington 98, Delaware), seven experts in this field discussed X-ray diffraction applied to polymers, spherulites, electron diffraction applied to single crystals, morphology of polymers, fibre microscopy and electron diffraction applied to synthetic fibres Arrangements for this symposium were planned over a period of two years and summarized the new phases of technology and applications of electron microscopy in the field of polymer science.

Dr Rubin Borasky (Electron Microscope Laboratory, Graduate College, University of Illinois, Urbana, Illinois) arranged and directed the symposium on "Ultrastructure of Protein Fibers—Collagen, Muscle, and Keratin" The papers on physical, chemical and mechanical properties of protein fibres in muscle and keratin summarized the recent contributions to the science of fibre structure, especially collagen.

The symposium on "The Fine Structure of Viruses" arranged by Dr. A. F. Howatson (Medical Biophysics Department, University of Toronto, Toronto 5, Ontario) was especially noteworthy in that the presentation of papers on recent advances in fine structure, animal virus structure, electron microscopy of nucleic acids and proteins, structures of bacteriophages and their variations, and work on the fine structure of foot-and-mouth disease virus brought together in a very logical sequence the information on the recent improvements in the techniques of replication, shadow-casting, thin sectioning and "negative contrast" staining and their application and interrelationships in obtaining new information on the structure of viruses both *in situ* and isolated.

Fifth International Congress for Electron Microscopy

Arrangements for the Fifth International Congress for Electron Microscopy to be held in Philadelphia during August 29–September 5, 1962, were announced at the meeting.

The Electron Microscope Society of America will be the host for the International Federation of Electron Microscope Societies at this Congress to be held, coincidently, on the twentieth anniversary of the Electron Microscope Society of America. Efforts are being made to have the Congress include the most significant developments since the last Congress in Berlin in 1958. The general theme will emphasize new and unique contributions of electron microscopy toward the advancement of science. In addition to contributed papers, the programme is being organized to include special papers by invited speakers which will set the theme of each session. In the interest of the earliest possible dissemination of the new material to be presented at the Congress, the Committee has completed arrangements for each active member at the Congress to receive the bound copy of the scientific papers at the time of registration. This will contain an abbreviated paper, including some micrographs or illustrations, for each scientific contribution presented at the Congress.

Further information can be obtained from the Secretary, Fifth International Congress for Electron Microscopy, 7701 Burholme Avenue, Philadelphia 11, Pennsylvania.

A. R. Taylor

THERMIONIC CONVERSION OF HEAT TO ELECTRICITY

A SYMPOSIUM on the thermionic generation of electricity was held at Bankside House, London, on May 11. It was organized by the Central Electricity Generating Board and attended by workers in the field of thermionic generation from a number of public and private concerns in Britain.

An opening address by Dr. K. H. Spring, of the Research and Development Department of the Central Electricity Generating Board, was followed by a paper from Mr. R. V. Harrowell, also of the Board, and Dr. J. Myatt (Atomic Energy Research Establishment, Harwell) on the efficiency and power of a diode generator. This paper examined the simplified expression for efficiency

$$\eta = \frac{\varphi_c - \varphi_A}{\varphi_c + P_L/I_A}$$

where φ_c and φ_A are cathode and anode work functions, P_L is the power loss and I_A is the anode current. When η is plotted against φ_c, the resulting humped curve is asymptotic to zero efficiency as φ_c is increased indefinitely, and for $\varphi_c = \varphi_A$, $\eta = 0$. It was pointed out that emphasis is seldom laid on the fact that this efficiency curve is valid only for a matched load at each value of φ_c: it is then true that $\eta = 0$ at $\varphi_c = \varphi_A$. But it is quite possible, under non-matched conditions, to have a high efficiency even when $\varphi_c = \varphi_A$, and this, or an intermediate state, may be desirable in certain circumstances. More important is the fact that, under matched conditions, maximum efficiency may sometimes be sacrificed for an increase in power, in this case φ_c will be less than its value at maximum efficiency.

A low anode work-function is important for the realization of high efficiency. The effective work-function of a metal anode in cæsium vapour depends on the cæsium coverage of the surface. A value below both the figure for clean metal and the bulk cæsium work-function can be achieved with a correct balance between the anode temperature and the saturated cæsium temperature: a value of 1·1 V has been quoted.

A study of the work-functions of anodes opposite oxide cathodes has been made by Dr. B. J. Hopkins (University of Southampton). The work-function of a clean nickel anode (~ 4·7 V) fell to less than 2·5 V. when the cathode was in the most active condition. Dr. Hopkins considers that barium oxide evaporates from the cathode in the molecular form and is deposited on the anode. Three methods of measurement were used: two depend on the determination of the contact potential difference from retarding field curves, the other uses the contact potential difference obtained by the Kelvin method. If the contact potential difference between the cathode and a known reference surface is also measured, then the anode work-function can be found by eliminating the cathode work-function from the two expressions, alternatively, the cathode work-function can be measured from a Richardson plot, although this would give a less trustworthy result.

Mr. D. C. Gore (Central Electricity Generating Board) read a paper entitled "The Thermionic Diode in Large-Scale Power Production from Fossil Fuels". This dealt with the diode as a thermodynamic topping device in a conventional boiler, using the upper part of the available temperature-range, which is not usable in a steam cycle. The mechanical arrangement would be for the diodes, in cylindrical form, to envelop the boiler tubes, the outer, cylindrical cathodes would be heated by the furnace gases. If a diode efficiency of 20 per cent could be attained and the efficiency of the conventional plant were 35 per cent, the total plant efficiency would be raised to 45 per cent by the introduction of diode 'toppers' passing three-quarters of the available heat. Any saving in fuel costs would probably be considerably offset by the relatively short life of the diodes, but Mr. Gore believes that if the very difficult problems of materials can be solved there are reasonable prospects for reducing generating costs. Some of the problems of materials and techniques were then reviewed by Mr. E. Cohen (Central Electricity Generating Board).

Mr. D. A. Watt (Atomic Energy Research Establishment, Harwell) and Mr. J. A. McWilliams (Boucher and Co., Kidderminster) described two engineering design studies of experimental in-pile thermionic generators: one has a planar geometry and uses ceramic–metal seals, the other, with a cylindrical geometry, has been simplified by omitting the ceramic–metal seals and using a continuous stainless steel envelope, which also forms the anode load. In the planar device the cathode is a coating of natural or depleted uranium carbide–zirconium carbide (25 · 75) on a tantalum 'top-hat', the inside of which contains uranium carbide–zirconium carbide enriched with uranium-235 as the fuel. To overcome the serious compatibility problem of fuel material in contact with tantalum at 2,000° C., the cylindrical version uses a rod of uranium carbide–zirconium carbide made of depleted or natural material at the end in contact with the tantalum cathode support and enriched material as the fuel at the free end. An output of about 300 W. was expected from an experimental model of the cylindrical diode being made.

The use of ion injection for space charge neutralization was discussed in a paper by Mr. G. G. Isaacs (G.E.C. Hirst Research Centre). In this type of device, ions are generated by an auxiliary discharge and, therefore, inert gases may be used instead of the chemically active alkali metal vapours. The experiments reported indicated a high degree of neutralization of space charge. A net output of about 200 mW. was obtained at an efficiency of about 1 per cent, however, no attempt had been made to optimize the design. As well as freedom of choice of filling gas, a further advantage of this kind of device is its potentiality for generating alternating current.

Mr. J. A. Thorp (A.E.I., Rugby) spoke on "Auxiliary Ion Sources in Cæsium Diodes". One method of neutralization of space charge is to produce positive ions from cæsium atoms in contact with a hot surface the work-function of which exceeds the ionization potential of cæsium. Mr. Thorp dealt with an investigation using matrix-type cathodes of low average work-function but with some high work-function spots at which ionization occurs. It is possible, however, for some ions to be trapped in a potential well at the mixed work-function surface and thereby be rendered ineffective. In the work described, a hot tungsten filament between anode and cathode has been used to ionize the cæsium. When the filament was biased one or two volts positive relative to the cathode, the anode current was several times greater than when the ionizer was unbiased. This indicated that the potential well at the filament was removed and positive ions were released.

In an analysis of the processes of ionization and de-ionization in a diode, Dr. L. G. Sanders (Atomic Energy Research Establishment, Harwell) considered the comparative effects of ionization by electron collisions and photons, and the so-called resonance ionization (also used by Mr. Thorp). It appears that the first two types of ionization are small compared with resonance ionization at the cathode, provided, in the case of electron collisions, that the electrons are at cathode temperature. However, if the electrons are at a temperature two or three times greater than the cathode temperature of about 2,000° C., electron collision ionization can make an appreciable contribution. The electron temperature may well be of this order due to barrier and Thomson heating. A simple approach, based on the current balance at the electrodes, has shown that the highest vapour pressure necessary is that which just neutralizes the negative space charge. If the pressure has to be higher than this amount in order to lower the cathode work-function by partial cæsium coverage, then the electrode spacing should be decreased to limit the plasma resistance.

In a review of progress in the United States, Dr. B. C. Lindley (C. A. Parsons and Co., Ltd.) reported that the trend there is towards the use of refractory metal cathodes and high cæsium pressures: the former enables high cathode temperatures to be used, while the high vapour pressure (~ 10 mm. mercury) ensures, even at 2,000° C., a reduction in work-function due to the presence of a monolayer of cæsium, electrode spacings of the order of the electron mean free path minimize heat conduction through the plasma. Most of the American groups visited were working on the close-spaced vacuum converter as well as the cæsium version.

"The Crossed Field Thermionic Converter" was the title of the final paper, which was read by Dr. G. D. Sims (University College, London). In this device, space charge is overcome by crossed electric and magnetic fields. Dr. Sims described experiments that have established beam interaction with the accelerator electrode in the initial apparatus used. He believes there is little doubt that the accelerator current, which persists even with extremely high magnetic fields, arises as a result of instability in the electron cloud, this gives rise to either amplification of noise or oscillation—resulting in radio-frequency fields which cause electrons to lose sufficient energy to be drawn to the accelerator. This represents wasted power. Further experiments are being planned with the view of the suppression of accelerator current.

R. V. HARROWELL

RESEARCH IN THE LEATHER INDUSTRY

THE seventh biennial conference of the International Union of Leather Chemists Societies was held in Washington, D.C., during August 20–25.

The International Union was founded in Europe after the Second World War as a confederation of national member societies concerned with the leather industry, and research and technology related to it. From an early stage in its development, it had as one of its members the American Leather Chemists' Association, and in the twelve years since its foundation it has come to include among its twenty-five member societies a steadily increasing number from Asia, Africa and South America, the expanding leather industries of which are based on locally available supplies of hides, skins and tanning materials. The decision to hold this year's biennial conference outside Europe for the first time, and to combine it with the annual meetings of the American Leather Chemists' Association, is evidence of the growing collaboration between American and European leather chemists and their colleagues in other continents. Thanks to its efficient organization by the host society an enjoyable, lively and instructive conference resulted

As a craft industry, organized in relatively small units, the leather industry has in the past paid too little attention to the more fundamental types of research, and is to-day still handicapped in competition with the producers of synthetic and substitute materials by the allocation of insufficient funds to research and development Not only the scale of research effort needs to be increased, however, but also the communication of the results in a form in which they are assimilable and can be applied in tanneries These points were emphasized by M Riviere (Strasbourg) in the John Arthur Wilson Memorial Lecture with which the conference began, and also by speakers in the ensuing discussions, public and private It was evident that there was a deeply felt need for closer collaboration between research workers and the industry, and that while national and international conferences such as the present one help to disseminate scientific information among the research workers, they do not necessarily ensure that this information is put to the best practical use in the industry itself, to achieve this, a programme of education is needed to acquaint the tanner with scientific discoveries which he can use, but also to acquaint the scientist with the needs and difficulties of the industry

Of the sessions which were devoted to scientific papers, the outstanding ones were those devoted to work on the chemistry and structure of collagen. The president of the Union, W Grassmann (Max-Planck Institut, Munich), reviewed present knowledge of this subject as a preliminary to a discussion of the results recently obtained by his group The light and dark bands revealed by electron microscope photographs of collagen fibrils have different amino-acid compositions By the investigation of peptides isolated from heat-denatured collagen by degradation with trypsin, it has been shown that some regions of the peptide chains contain more of the polar amino-acids than occur elsewhere, while in other regions the amino-acids are mainly glycine, proline and hydroxyproline, with some alanine and serine In the non-polar regions, the sequence is usually glycine–proline–*X*, where *X* is either glycine, serine or alanine In the polar regions, which give the dark bands, the sequence is less regular, and glycine does not occur at every third position Carefully controlled denaturation of tropocollagen (soluble collagen obtained by leaching young skins in mildly acidic conditions) results in the production from collagen (mol. wt 360,000) of α-collagen of mol wt 110,000 and β-collagen of mol wt 215,000 in equal amounts, and the denaturation is thought to cause multiple breaks in the rod-like tropocollagen, following the uncoiling of the collagen helices. According to F O Schmitt *et al*, the long spacing segments are built up of parallel bundles of tropocollagen molecules, whereas in the fibrils of native collagen, neighbouring molecules are displaced relative to one another by one-quarter of their length, and this view has been confirmed by staining techniques followed by photometric measurements along the lengths of the segments The collagen molecules are bound together laterally by covalent cross-links, which are the cause of the limited swelling and the insolubility of collagen in water As was shown in a paper by H Hörmann (Max-Planck Institut), the covalent bonds consist of hexoses the two hydroxyl groups of which form ester links between different peptide chains in collagen Grassmann indicated that tannage consists generally of an increase of cross-linking of molecular chains within dark band regions of fibrils, although some tanning materials also form compact deposits surrounding the fibrils, and without any true tanning action

K Kuhn (Heidelberg) described experiments on the end-to-end linkage of tropocollagen molecules involving their treatment with trypsin and pepsin to remove the peptide appendages which are thought to protrude from the ends of these molecules The results were held to indicate that the end-to-end polymerization of tropocollagen molecules was mainly due to electrostatic forces, but this view was questioned by Schmitt in the discussion following the paper The production of gelatin fractions from skin collagens, and their re-constitution as fibres, was described in a paper by A Veis and J Anesey (Northwestern University, Chicago) with J Cohen (Armour Leather Co, Chicago), while A G Ward, N T Crosby, D G Higgs, R Reed and G Stainsby (University of Leeds) described experiments the aim of which was to break the insolubilizing cross-links of mature bovine collagen after alkaline pretreatment The rod-like particles of collagen may be dissolved to yield products similar in many respects to those from young skin Some beautiful electron microscope photographs were shown by L P Witnauer and L D Kahn (Eastern Regional Laboratory, U S. Department of Agriculture, Philadelphia), collagen fibrils precipitated by inorganic ions from solution had various solubility characteristics, as well as a variety of forms

Of the other twenty-five papers presented at the conference, the majority were concerned with leather technology rather than with questions of general scientific interest, and reference can be made to only a few of them H G Turley (Rohm and Haas Co, Philadelphia) compared and contrasted American and European practice in chrome tanning, and indicated that the different methods used are determined by economic factors as much as by different objectives P J van Vlimmeren (T N O, Waalwijk, Holland) described work on masked chrome tannages, stressing the importance of the stage at which the masking agent (formate, phthalate, acetate or oxalate) is added to the tanning liquor Because of the different rates at which chrome complexes are formed, the most appropriate stage differs for various masking agents and, when chrome sulphate alone is used, it may be best to add the material to pickled pelt as a dry powder produced by the slow evaporation of chrome sulphate solution, this gives strongly masked anionic complexes which break down only slowly in cold solutions E Heidemann, P. Fuhren and H Kroll (Technische Hochschule, Darmstadt) discussed the tannages of synthetic polypeptides (used as model substances) with chrome They have produced them as gelatinous materials or as voluminous flakes in which all the carboxyl groups are probably accessible to chrome complexes, and their results suggest that the majority of the cross-linkages between carboxyl groups involve complexes containing a pair of chromium atoms

In a session devoted mainly to the physical testing of leather, E Baumann (Bally Shoe Factories, Schoenenwerd, Switzerland) stressed the need for simple and rapid test methods, which are essential in testing a biological material such as leather for factory control The abrasion testing of sole leather was discussed by R G. Mitton, M Millar and F R

Morgan (British Leather Manufacturers' Research Association), who showed that, despite the obvious complexity of the wearing process of shoe soles, there is a good correlation between the results obtained in abrasion tests and durability measured in wear trials of shoes. In favourable cases, the closeness of the correlation seems to be limited by the unavoidable errors associated with the wear trials (and amounting to a few per cent) rather than to any other cause.

R. G. MITTON

INTERNATIONAL UNION OF FOREST RESEARCH ORGANIZATIONS

THE thirteenth Congress of the International Union of Forest Research Organizations was held in Vienna during September 11–16 under the presidency of Mr. James Macdonald, director of research and education in the Forestry Commission, London. Vienna was the birth-place of the Union, and the small body of specialists, members of Austrian, German and Swiss forest research institutes, who first met (at Mariabrunn) in 1893, would surely have been gratified at the ramifying growth and vigour of their brain-child. More than 400 delegates, an all-time record, attended the Congress from about three dozen countries, with particularly strong contingents from Finland, Germany, Japan, Scandinavia, the United States and the U.S.S.R.

All working sessions were held at the Wilhelm Exner Haus, Hochschule fur Bodenkultur, and the opening and closing ceremonies at the more central Haus der Industrie. The Congress was formally opened by the Federal Minister of Agriculture and Forestry, who reviewed the great changes—territorial losses, damage, over-exploitation and neglect—wrought by two World Wars in the forest economy of Austria and emphasized the growing importance of the forests, both State and private, not only as suppliers of timber, internally and abroad, but also as stabilizers of mountain slopes and guarantors of the water supply. The Marshall Plan had given a great impetus to rehabilitation, and now an extensive programme of torrent- and avalanche-control, embracing both engineering works and reforestation, was under way, backed by a carefully conceived plan of research.

The main work of the Congress was, as usual, carried out in the sections, of which (inclusive of bibliography) there were ten, which constitute the living heart of the Union. The section leaders, who continue in office between Congresses, are responsible for keeping in touch with the research workers in their respective fields of action, for distributing information of interest to specialist working groups and other members of their section, for initiating action as necessary, and preparing for and conducting their sectional meetings at Congresses.

Section 11 (Forestry Influences), under Dr. H. Storey of Washington, dealt with three main topics: management of forested watersheds for water supply (where stress was laid on the need for new measurement techniques and instrumentation), the role of forests and engineering structures in the control of floods and erosion (emphasizing the supplementary functions of biological and engineering methods) and shelterbelts, and ended with appointing working groups to initiate and co-ordinate work in each of these fields. Section 21 (Site Factors), under Prof. de Philippis of Florence, had forest typology, the influence of forest stands on the soil, and biological factors of forest regeneration as its main topics, and set up a joint working group with Section 25 in order to study what are the best criteria for assessing the quality of sites and the relationships between environmental factors and the potential productivity of sites. In Section 22 (Forest Plants) under Dr. C. Syrach Larsen of Hørsholm, the chief topics discussed were seed production problems in forest trees and the growing of pines in the tropics and subtropics, a working group was formed on provenance research. This Section has hitherto concentrated on tree breeding, but is becoming increasingly interested in physiology. Dr. Leibundgut of Zurich presided over Section 23 (Silviculture), in which a great variety of subjects was discussed, notably silvicultural problems raised by the general unsaleability of small-dimensional timber—a topic of world-wide interest—for which a new working group was set up, silvicultural problems of tropical forests, soil amelioration and weed control in nurseries, and root formation and competition. This Section is also busy, in collaboration with the Bibliographical Section, on terminology in its field. Section 24 (Forest Protection), under Prof. A. Biraghi of Florence, dealt mainly with insect population dynamics—especially the influence of manuring of forest stands on the development of populations of noxious insects, about which there is some controversy, mycorrhiza research, international co-operation in forest disease research and in quarantine measures, and problems of smoke and industrial fumes damage to forest growth. It also created a fifth working group to study the important question of fire control.

Section 25 (Growth and Yield), under Prof. S. Firat of Istanbul, discussed mainly the use of climate site indices—concluding that these cannot by themselves afford an accurate guide to the quality class of individual forest sites and setting up a joint working group with Section 21 (see above), the use of electronic digital computers in forest research—recommending *inter alia* the compilation of a register of existing computer programmes of interest to foresters and an index of organizations and individuals that are actively using computers, increment determination in uneven-aged tropical forests (a matter of great present-day interest) and increment determination by borings—in both of which, it was agreed, investigations were not sufficiently advanced to permit anything but tentative conclusions. This Section has a permanent working group that advises the Union on all matters concerned with aerial photography, photo interpretation and photogrammetry, and maintains effective links with Commission 7 of the International Society of Photogrammetry, the report of this group was approved and its membership is being widened. The Section also recommended the formation by mathematical specialists of advisory groups for helping forest statisticians, both by suggesting improvements in current university curricula on statistical methods and by organizing the collection and distribution of case studies and other information of value for the statistical planning of forest research. Prof. J. Speer of Munich led Section 31 (Forest Economics), which formulated and recommended a special accounting system for forest commercial enterprises and a corpus of economic terms that could be used by all countries It also discussed the difficult topic of the measurement of forest work productivity with Section 32 (Operational Efficiency) the other main topics of which, under the chairmanship of Prof V Sundberg of Stockholm, were the effects of noise and vibration on the forest worker and the establishment of university courses in operational efficiency Section 41 (Mechanical Conversion), led by Prof J Campredon of Paris, discussed many aspects of this subject and decided to enlarge its field of study so as to embrace all forest products (this to be the new name of the Section), with three initial working groups for wood quality, behaviour of wood exposed to fire, and sawing and machining

Finally, on the initiative of Austria and Germany, the Union decided to create a new section, Section 11, on the History of Forestry, with Prof von Mantel (West Germany) as leader

The Bibliographical Section, which is actually a joint committee of the Food and Agriculture Organization and Union representatives, met as usual several days before the Congress, under the chairmanship of Prof E Saari of Helsinki It was concerned mainly with the prosecution of two long-term projects—further accurate translations of the classification now widely accepted in the world of forestry science (*The Oxford System of Decimal Classification for Forestry*, of which English, French, German and Spanish authorized editions have already appeared) and speeding up the production on cards of a basic forest terminology in the English language, as an essential preliminary to the multilingual terminology project, sponsored jointly by the Food and Agriculture Organization and the Union

Prof J Speer (West Germany) was elected to be president in succession to Mr Macdonald, whose term of office expires at the end of the year Dr V L Harper (United States) was elected vice-president Elections to the Permanent Committee (previous members in italics) were Prof N Anutschin (U S S R), *Prof. T Bunusevac* (Yugoslavia), Prof J. Campredon (France), Dr E Holmsgaard (Denmark), *Prof A Horky* (Austria), *Prof M. Kreutzinger* (Poland), Prof A de Phillipis (Italy), Dr D R Redmond (Canada), Dr H Shirley (United States), and Dr Saito (Japan), leaving two places to be filled by representatives from India and Latin America, not yet designated

The International Council met on the concluding day, and agreed to the revised statutes which take into account recent decisions of and developments in the Union, the major changes being to strengthen the position and powers of the enlarged Permanent Committee which is its executive body between Congresses A new class of member—associate member—has also been created and the organizing of the secretariat, following its recent relinquishment by the Food and Agriculture Organization, has been entrusted to the president himself

The Union accepted an invitation from Germany to hold the next Congress there After the Congress at Vienna, about 130 of the delegates took part in strenuous study-tours that lasted for 11 days of magnificent weather and covered the whole country One tour concentrated on silviculture and management, another on the many aspects of forest protection, and the third on mechanical developments in soil-cultivation and trenching, tree planting and tending, timber extraction and milling

F C Ford-Robertson

NUTRITION AND POULTRY

The Scottish Group of the Nutrition Society held its sixty-second meeting on September 30 in the College of Agriculture, University of Edinburgh It took the form of a symposium entitled "Nutrition and Poultry". As the chairman, Prof S J Watson (University of Edinburgh), pointed out in his opening remarks, since the subject was "Nutrition *and* Poultry" it was not confined to problems of poultry feeding but instead embraced broader aspects of nutrition in which work with poultry has contributed to knowledge

Dr W. P Blount (British Oil and Cake Mills, Ltd , London) opened the proceedings with a very thorough exposition of the use of birds as experimental animals He said that apart from some cage birds which have special uses, poultry were the most commonly chosen birds for laboratory work, and he emphasized the advantages of their cheapness, availability and ease of management Illustrating his talk with a series of informative slides, he described different forms of housing, from brooders for small groups of chicks to intensive houses for many thousands of adult birds He discussed optimal conditions of light, temperature and humidity, systems of feeding and ingredients of poultry diets. He concluded with a survey of the common diseases of poultry, and indicated that many of them could be controlled by good management and the judicious use of drugs such as antibiotics and coccidiostats

In the following paper Dr M E Coates (National Institute for Research in Dairying, Shinfield) dealt with problems of fundamental nutrition for which poultry have proved peculiarly suitable subjects, particularly in vitamin research The examples quoted ranged from Eijkman's classic observation of polyneuritis in hens fed on polished rice, which led to the discovery of vitamin B_1, to modern work on the isolation and function of vitamin B_{12}, a field in which work with poultry has played a major part The developing chick embryo was cited as a very suitable organism for investigating the biochemical role of nutrients, as evidenced by experiments on the metabolism of vitamin B_{12} and studies in tissue culture of the function of vitamin A She described how chicks can be relatively easily maintained in a germ-free state and are thus becoming increasingly important in investigations of the relation of the alimentary microflora to nutrition

Prof S Lepkovsky (University of California), who has used birds to study the nervous control of food intake, gave a fascinating dissertation on hunger, appetite and satiety He described the influence of the central nervous system through hunger- and satiety-centres in the hypothalamus He illustrated the psychological effects of these states amusingly and accurately in a series of lantern slides of "Hubert", an unfortunate character from a comic cartoon who was obliged to subsist on a low-calorie diet

It has become common practice to include in diets for livestock non-nutritional additives that increase growth-rate and level of production. In reviewing additives for poultry diets Dr. J H Taylor, (Cyanamid Products, Ltd , London) classified them into those that alter the microbial flora to the benefit of the host animal and those, largely having hormonal activity, that produce increases in growth or productivity by modification of metabolic processes The former class of compounds was of greater practical importance, and had the largest effect when the level of performance was poor Detectable quantities of some antibiotics given in the diet appeared in the serum If there was reason to suppose that the beneficial effect of the antibiotic was systemic it was desirable that blood-levels should be increased If, however, the response to an antibiotic was solely due to modification of the gut flora, such enhancement was unnecessary The hormonal food additives such as œstrogens and thyroid-active agents could be of use in increasing fat deposition and improving carcass quality, although there was some danger of residues in the tissues if œstrogens were given in the food

The final paper in the symposium was an entertaining account by Prof M F M Meiklejohn (University of Glasgow), speaking in his capacity as editor of *Scottish Birds*, of wild birds that have been used as food for man He began by reading from the *Book of Leviticus* the catalogue of birds that were prohibited as food by Mosaic law, and showed how nearly it corresponded to the list of birds customarily considered as unfit for consumption The choice of wild birds for human food depended on their palatability and the ease with which they could be captured In general, sea birds were less palatable than land birds, although there were instances of inhabitants of remote islands subsisting on some of the colonial nesting species Of the land birds, those that lived in flocks and hence were more easily caught in quantity were most frequently a source of human food Many of the traps and nets used to-day by ornithologists derived from the catching devices evolved long ago for the capture of wild birds for food Prof Meiklejohn posed a number of questions, which the audience attempted to answer, about the relation between the food of wild birds and their suitability for human consumption

The lively discussion that followed each of the papers fully justified the choice of subject for this symposium On this occasion at least, the more homely aspects of the domestic bird were overshadowed by its role in the field of nutritional science

M E COATES

THE EUROPEAN NUCLEAR ENERGY AGENCY

THE third report of the European Nuclear Energy Agency[*], presented to the Consultative Assembly of the Council of Europe by Prof S Balke on September 26, records substantial progress in the first joint undertakings Work on the Eurochemic site was inaugurated in July 1960 and the detailed pre-project for the plant was approved in June 1961 The Halden heavy-water reactor reached boiling in October 1960 with the first fuel charge of natural uranium, and installation of a second fuel charge of enriched uranium will permit a power of 20 MW to be reached before the end of the year joint operation of the reactor has been extended to the end of 1962 Construction of the *Dragon* high-temperature gas-cooled reactor began in April 1960 civil engineering work on the site is well advanced and supporting research and development are progressing in parallel

A group was set up in November 1960 to study the nuclear propulsion of ships, and a meeting of experts has been convened to determine the areas of nuclear research where particular need for international co-operation exists and the objectives and means of collaboration

The European–American Nuclear Data Committee, created in June 1959, reviews the measurement programmes of member countries, inventories the equipment available, and has compiled a list of requests for new measurements so as to facilitate collection of the necessary data It is also assisting countries which have only recently begun to work in this field, and the Agency, in July 1961, decided to establish a European system of supervision and emergency warning which involves the immediate notification of any abnormal increase in environmental radioactivity recorded in a member country A study-group on food irradiation was set up in January 1960 to examine the economic and technical possibilities of the application of food irradiation so as to harmonize national programmes and propose joint projects where appropriate International seminars for university teachers and technical executives were again organized in 1960 and 1961, and a specialized symposium on criticality control in nuclear industry at Karlsruhe was attended by one hundred specialists

The Agency has also undertaken the examination of regulatory, administrative and legislative provisions introduced by European countries to conform with the radiation protection norms adopted in 1959, and these norms are being revised, in liaison with Euratom and the International Atomic Energy Agency, to take account of the latest recommendations of the International Commission on Radiological Protection A Convention on Third Party Liability in the Field of Nuclear Energy, dated July 29, 1960, has been signed by sixteen members of the Organization for European Economic Co-operation The Agency also participates in the investigation of problems posed by the insurance of nuclear risks, and particularly the application of tax regulations governing technical reserves and insurance premiums to these risks A first set of regulations applicable to research or experimental reactors has been elaborated by the Control Bureau responsible, under the Agency's Steering Committee, for carrying out the Security Control Convention In addition, the Agency centralizes economic data bearing on the development of the nuclear industry, and the result of an investigation, published in 1960, predicted that nuclear power stations will provide less than 3 per cent of the capacity of the European electricity production in the coming five years and less than 10 per cent in fifteen years

The practical value of international co-operation in this field is fully established by the year's work, and the experience gained in co-ordinating scientific work shows that in this way the less advanced countries gain indispensable experience that they could not acquire by themselves, while the other countries also benefit. This effort should be continued on as wide a geographical basis as possible, for the value of the results is increased by the scope and variety of the means used and relative costs are reduced. The report emphasizes that this experience in nuclear research is valid in other scientific fields and that European scientific progress calls for a concentration of human, financial and industrial resources only attainable by international co-operation. Annexes to the report include a report on the possibilities of joint European action in nuclear propulsion of ships and L. Kowarski's report on new trends in atomic research and their international significance.

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[*] Organization for European Economic Co-operation—European Nuclear Energy Agency Third Report on the Activities of the Agency Pp 253 (Paris Organization for European Economic Co-operation–European Nuclear Energy Agency, 1961)

UNIVERSITY FINANCE IN BRITAIN: 1959–60

THE annual returns from universities and colleges in Great Britain* in receipt of Treasury grants for the academic year 1959–60, now covering twenty-one universities and three colleges, is prefaced by a somewhat longer general survey than last year, in which university expenditure on research and academic salaries is considered more particularly.

The University Grants Committee has already made a recommendation to the Chancellor of the Exchequer regarding the location of a fourth new university, in addition to those recommended in July 1960, to be located at Colchester, Canterbury and Coventry, but this location is not disclosed. The Committee considers that the establishment of any new universities beyond these four should wait on the report of the Committee on Higher Education, and meanwhile academic planning boards are being appointed for the Universities of Essex, Kent and Warwickshire. It is expected that the expansion proposed by existing Scottish universities will provide the additional places needed in Scotland by the early 'seventies. Apart from this, the University Grants Committee emphasizes the importance of the enthusiasm and interest of a sponsoring area in determining their decisions on the location of new universities, as well as the presence of industries and some local financial support, the supply of lodgings and the attractiveness of the location to academic staff.

Following Sir Arthur Rucker's review of the application by the universities of the procedures recommended by the Gater Committee, a Building Sub-Committee has been established under the chairmanship of Sir Keith Murray to advise the University Grants Committee on its procedures for assessing non-recurrent grants for building works. A sub-committee has also been set up to assess, and advise the Committee on, the proposals for development in Oriental, African, Slavonic and East European studies which come forward from the universities, and a committee has been appointed with Sir Edward Hale as chairman, to make a comparative study of the methods of teaching undergraduates now being used in the universities and colleges of Great Britain in the fields of arts and pure and applied science. The report was obviously written before the Chancellor of the Exchequer made his statement in July, and it remains to be seen whether and how far the provisional building programme which the Committee has agreed with each of the universities, in the light of the Chancellor's statement on January 25, 1961, for each of the four years 1962–65, will be affected. To meet the expansion to 170,000 students by the early 'seventies and make good the backlog of past years, a building programme of £175 million at 1960 costs is required. The Committee has no apprehensions on the quality of students expected in the next ten years; it stresses again the importance of halls of residence; it welcomes the attention which the universities are giving to their existing degree structure, including the postgraduate structure, and generally it is impressed and encouraged by the attitude of the universities to the projected expansion.

The report records a solution to the question of assistance for St. David's College, Lampeter, by association with the University College of South Wales and Monmouthshire, which will assume sponsorship for St. David's for a trial period of ten years, during which, from August 1, 1961, the University Grants Committee will consider applications for recurrent and non-recurrent grants.

The number of full-time students in British universities again increased, to 104,009, compared with 100,204 in 1958–59, and statistics collected in October 1960 show a university population of more than 106,000. There were 6,443 full-time and 2,400 part-time students from overseas within the British Commonwealth, and 4,208 full-time and 2,068 part-time students from foreign countries. Of full-time men students 36·8 per cent were in arts, 24·7 per cent in pure science, 19·7 per cent in technology, and 11·9 per cent in medicine; these figures compare with 36·8, 24·2, 19·5 and 12·5, respectively, in 1958–59. For full-time women students the corresponding figures for 1959–60 are 62·0, 21·1, 1·3 and 11·8, respectively, and for 1958–59, 62·6, 20·7, 1·0 and 11·9. Full-time advanced students of pure science numbered 4,512 (34·5 per cent), of technology, 2,442 (18·7 per cent), and of medicine, 1,052 (8·1 per cent); 3,585 students were taking postgraduate courses in teacher training.

Of the full-time students, 83,083 were reading for a first degree, 3,605 for a first diploma, and 16,649 were engaged in research or other advanced work; the corresponding figures for 1958–59 were 80,805, 3,928 and 14,839, respectively. Of the new full-time students, 25·2 per cent were in pure science, 16·0 per cent in technology and 9·0 per cent in medicine; in 1958–59 the corresponding figures were 24·1, 16·2 and 8·9 per cent, respectively. Of all full-time students, 76·0 per cent were men and 24·0 per cent women, a proportion practically unchanged from 1938–39, when the corresponding percentages were 76·7 and 23·3; 17·2 per cent were at the Universities of Oxford and Cambridge, 20·9 per cent at the University of London, 39·1 per cent at other universities and university colleges in England, 5·9 per cent in Wales and 16·9 per cent at Scottish universities. The proportion of full-time students residing in colleges or halls of residence was 27·0 per cent, compared with 26 6 per cent in 1958–59, 50·3 per cent were in lodgings and 22 7 per cent at home, but these proportions vary widely between men and women and between institutions

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* University Grants Committee. Returns from Universities and Colleges in receipt of Treasury Grant, Academic Year 1959–1960. Pp. 76. (Cmnd. 1489.) (London: H.M.S.O., 1961.) 4s. 6d. net.

The proportion of assisted students was 82 4 per cent, compared with 80 5 per cent in 1958–59 and 71 9 per cent in 1953–54, ranging from 93 6 per cent in Wales, 89 0 per cent in English universities, excluding those at Oxford, Cambridge and London, to 73 4 per cent for Scotland Full-time teaching and research staff increased to 11,474 (10,823 in 1958–59) and 25 6 per cent are now in pure science, 15 0 per cent in technology and 17 3 per cent in medicine

Of the recurrent income of £59,800,092 (£52,273,306 in 1958–59), £43,170,107 was from Parliamentary grants (72 2 per cent), £5,990,466 from fees (10 per cent), £1,731,106 (2 9 per cent) from endowments, £1,533,673 (2 6 per cent) from local authority grants, £451,328 from donations and subscriptions, and £4,730,590 from payments for research Non-recurrent Treasury grants amounted to £19,852,747, an increase of £3,298,264 on the previous year, and of this, £13,839,308 was spent on building work, £1,914,375 on professional fees, £2,966,333 on furniture and equipment and £1,132,731 on site and property purchases Of the recurrent expenditure of £58,505,072—an increase of £3,978,797 on that for the period 1958–59—6 8 per cent was spent on administration, 44 0 per cent on salaries and superannuation of teaching staff, 11 1 per cent on departmental wages, 13 9 per cent on departmental and laboratory maintenance, 3 6 per cent on repairs and maintenance of buildings and 9 7 per cent on rates, insurance, heat, light, etc Expenditure on libraries, at £2,350,294, remained at 4 0 per cent of the total

HEALTH SERVICES IN SMALL FACTORIES

A CONFERENCE on "Health Services in Small Factories" was convened jointly by the World Health Organization Regional Office for Europe and the International Labour Organization at Dun Laoghaire, Dublin, Ireland, during May 2–16 (*WHO Chronicle*, 15, No. 9 , 1961) The forty participants in the conference included medical officers, officials from Ministries of Labour, and representatives of employers' and workers' organizations from more than twenty countries

Between 80 and 90 per cent of factories in most European countries employ less than 100 workers Of the 220,000 factories in the United Kingdom, 150,000 employ 10 or fewer workers ; more than a million people work in factories employing 25 or less, the number of such factories having nearly doubled in the past twenty-five years. In Italy, 95 per cent of industrial plants have less than 100 workers, in Switzerland 90 per cent, in Belgium 96 per cent, in Denmark 99 per cent, and in Sweden 92 per cent

While the problem of providing health services in large factories in Europe is well on the way to solution even though different methods are used in different countries, the smaller factories in most countries of Europe do not have health services on or near their premises The risks to which their workers are exposed are certainly not less than in the larger factories and in many cases are greater, since the working environment in terms of atmosphere, ventilation and layout is considerably poorer

Instruction in special hazards and supervision and training in handling dangerous materials, such as toxic chemicals or radioactive isotopes, are less efficient as a rule than in large factories While periodic medical examinations are compulsory for workers in certain dangerous trades, there are usually no medical facilities within the factory beyond a first-aid box—the foreman is often in charge of this, but he is usually untrained in health matters

There was general agreement among the participants in the conference that the provision of health services to these small undertakings is a real and urgent need, but there was less agreement about how to provide them Among the difficulties are the diversity and dispersion of factories, which may be scattered over considerable distances. It is feasible for a doctor to provide health care for 3,000 workers in one factory, but not for thirty factories each with 100 workers The cost of an autonomous health service is prohibitive for the small employer, and health hazards are often not understood or appreciated by employers and employees

The conference discussed schemes now being tried out in various parts of Europe to provide health services for small factories Four main tendencies are discernible

In Czechoslovakia and in eastern Europe as a whole, these occupational health services are provided as part of a general programme of health protection covering both curative and preventive medicine and organized by the State

In France, the Ministry of Labour is responsible for implementing general regulations on hygiene and security Employers are obliged to set up health services which, for the majority of small factories, take the form of inter-factory services The responsibilities of the physicians in these services are, in principle, the same as those of all industrial medical officers, that is, they are entrusted with preventive medicine, treatment being provided only in emergencies and for accidents

In Italy, health services in small factories are mainly the concern of the National Institute for the Prevention of Accidents, which has some thirty branches scattered throughout the country

In the Netherlands, the United Kingdom and Scandinavia, inter-factory occupational health services are provided on a voluntary basis, generally by means of a non-profit-making body on which doctors. employers and workers are represented. Organizations of this type show a tendency to grow in size and popularity as new factories join the service, and to extend to other areas Their role is mainly preventive , but in some cases, they provide treatment also

In a separate statement, the employers' representatives attending the Conference declared that, while agreeing that medical services in small factories should essentially be preventive, they nevertheless felt that these services should be encouraged to provide other treatment as well as emergency treatment The advantages to be gained are a more efficient use of man-power, an increase in production (since workers would not lose so much time visiting hospitals for treatment) and improved morale They stressed their opinion that such services should be organized on a voluntary basis

The representatives of the workers' organizations welcomed the important developments now taking place in the field of health supervision for their members, though many of them considered that progress in this field was still too slow It was their opinion that, by accepting the goal of occupational health services for all workers, the International Labour Organisation has opened an important chapter in the history of occupational health

UNIVERSITY OF SUSSEX

THE first prospectus* of the University of Sussex describes the origins of the University since it was first promulgated in 1911, the way in which it has been constituted, proposed developments and the kind of administrative and teaching and research arrangements which are being made for students

Courses leading to degrees in the Faculty of Arts and Social Studies will be organized and pursued in a a different way from that followed in other British universities The Faculty will be divided not into departments concerned with particular academic disciplines but into schools of study where different specialisms will be studied in relation to each other

In 1962–63 there will be three such schools—the School of Social Studies, the School of English and American Studies, and the School of European Studies A School of Afro-Asian Studies will follow later. Each School will have a dean at its head In addition, there will be professors and lecturers in particular subjects The schools will not be super-departments, and the same subject may be studied in more than one school

Details of the science curriculum are still under consideration It is intended to provide degree courses which will prepare students not only for careers as professional scientists, in the academic world, in industry, and in Government scientific establishments, but also for careers as teachers, and in industrial, commercial and public administration There will be a three-year course in natural science, leading to a classified honours degree During this course students will study three subjects chosen from pure mathematics, applied mathematics, physics, chemistry, and biology In addition, the curriculum will require some study (which will be examined) of the history of science and of the impact of science on society

For a limited number of selected students, there will be a second honours course lasting a year, in which one science only will be studied, and a successful candidate will be awarded classified honours in that subject The student who has completed this further course will thus have a specialized knowledge of a single science, and some understanding of the social sciences

There will also be a course lasting three years in engineering science This will be a general course, it is not intended to provide specialized courses in civil, mechanical or electrical engineering

During the years 1962–63 and 1963–64, only the subjects mathematics (pure and applied), physics and chemistry will be offered It is hoped to begin courses in engineering in 1964 and in biology in 1965.

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* The University of Sussex Prospectus, 1961-62 Pp 26+2 plates (Brighton The University of Sussex, 1961)

METABOLISM OF THE HUMAN RED BLOOD CELL

SOME aspects of the metabolism of free fatty acids by the human red blood cell *in vivo* have been studied by Dr. D Mendelsohn of the Department of Pathology and Microbiology, University of the Witwatersrand, Johannesburg (*S. Afric J. Med Sci*, 20, Nos. 1 and 2, July 1961) Following the oral ingestion of glucose by a normal subject, an increase in erythrocyte-free fatty acid concentration occurs, reaching a maximum in 1–1½ hr In contrast, the diabetic patient shows a much smaller rise in red cell-free fatty acid during a glucose tolerance test, in many diabetic cases no increase could be detected at all This defect in the diabetic erythrocyte could be corrected by the intravenous administration of insulin during the performance of a carbohydrate tolerance test

Although the findings do not permit any definite conclusions to be drawn, it is suggested that the human red blood cell is able to synthesize lipid from carbohydrate *in vivo* This faculty seems to be impaired in the human diabetic erythrocyte.

Mendelsohn has also examined the interrelationships of carbohydrate and lipid metabolism in the human erythrocyte *in vitro*, all the findings observed when investigating the metabolism of lipids by the human red blood cell *in vivo* have been reproduced *in vitro* The results of the investigation suggest that under certain *in vitro* conditions, that is, in a phosphate buffer containing glucose and brilliant cresyl blue or methylene blue, the mature human red blood cell is able to synthesize fat from carbohydrate Any exchange of fat between red cell and serum which might have been expected to cause a rise in erythrocyte lipid-levels has been eliminated by incubating the cells in phosphate buffer instead of serum. The fact that normal red cells showed an increase in total esterified fatty acid together with free fatty acid indicates that the rise in cell-free fatty acid was not due to lipolysis of complex lipids with subsequent release of fatty acids

A much less noticeable rise in the level of free fatty acids was apparent in the erythrocyte of an insulin-deprived diabetic patient when compared with a normal patient Addition of insulin to the diabetic red cell suspension corrected this deficiency. It is suggested that under the *in vitro* conditions described, the mature human erythrocyte appears to be able to synthesize fat from carbohydrate whereas in the diabetic red cell this faculty is markedly impaired Mendelsohn has also examined some of the mechanisms which might be responsible for the abnormal behaviour of the diabetic erythrocyte

STUDENT MENTAL HEALTH

IF one asks an examiner of the old-fashioned, bluff, extraverted type why students fail in their examinations he will possibly reply "Because they are insufficiently prepared" No doubt he will be content to leave it at that, but if one continues the Socratic questioning and demands: "Why are they insufficiently prepared?" one will be told "Because they haven't worked enough" But this is not the complete answer, even supposing that one is told that their lack of work depends on a poor personality, laziness, or something of the sort.

These students who pose such problems are, as Dr Malleson pointed out at a conference on the mental health of students recently held in London*, those who have surmounted the difficult hurdles of the 'eleven plus', the Common Entrance, the General Certificate of Education examinations, and so on in the past. Why have they failed at the university?

The papers read at the conference suggest that there is no simple answer Although the student may have been successful at school, there are psychological difficulties associated with university life which may interfere with his work These range from home-sickness, if he lives in a hostel, or domestic unrest associated with parental quarrelling, if he lives at home to such grave psychological illnesses as schizophrenia, depression and the severe crippling neuroses.

Student failure may mean that a valuable young man gives up further education and takes up work inferior to his intellectual capacity It means, moreover, a great deal of expense to the nation It is estimated that a student costs about £700 a year to educate at a university If he gives up his academic career, as most students that fail do, after a year and a half, he has wasted at least a thousand pounds of public money When one considers the number of students the loss is potentially enormous

What is to be done about it? The answer lies, to some extent, in the work of the psychiatric advisers, who have the opportunity of examining students complaining of psychological symptoms. Many universities have such a unit and, as Dr Malleson emphasizes, if only four students a year are saved from giving up their academic careers in a year the adviser has earned his keep

Such an advisory unit is not primarily to treat nervous or mental illness, although one of its functions is early diagnosis, but to help the much larger number of students to overcome the usual adolescent difficulties Among those who complain of comparative trivialities the more sinister illnesses are found and even these, if discovered early, can sometimes be helped

The time has passed when students should be thrown ruthlessly into university life to sink or swim Such a policy may have sounded bold and manly, but many young men with otherwise a fine intellectual future have sunk, have worried over a triviality until they have broken down or taken their lives uselessly

Probably all those who are student advisers will agree that we do not know everything regarding the problems of academic life It is one of the advantages of an advisory unit that there is an enormous amount of material for research purposes, and, since the student is a picked man, derived from an intellectual *élite* Ultimately this work should throw a valuable light not only on student problems but also on mental health in general.

CLIFFORD ALLEN

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* World University Service Student Mental Health Report of a Conference held at Campbell Hall, London, 8th–9th April, 1961 Pp 44. (London World University Service, 59 Gloucester Place, 1961.) 2*s* 6*d*

TRAWL FISHING IN THE TROPICAL ATLANTIC

Catch-rates and Composition

IT is notoriously difficult to assess relative productivity between trawl fisheries in different parts of the world because of the inherent differences in fishing power of the vessels operating each fishery Though it may be possible[1] to apply factors to convert the catch-rates of vessels of similar type, but of different size, to a standard form, this is generally only practicable within a limited geographical area The investigations of the Colonial Office vessel, *Cape St Mary,* off West Africa in 1952–57 and off South America in 1957–58, working with the same officers and gear on both sides of the Atlantic, offer an opportunity to compare the standing crop, in terms of catch-rate, of the demersal fish of the two areas Mitchell and McConnell[2] have described their investigations off Georgetown on the Guiana coast, and an account of the work off West Africa is now in preparation[3]. Salzen[4] has described some early results from Ghana with the same vessel, though with a different crew

The *Cape St Mary* was a 100-ft (between perpendiculars) steel trawler of rather typical North Sea design, of 238 gross tons and with 385-h p Diesel engine; details of her construction and gear are given in ref. 2 The trawls, in both areas, were of the Peter Carey type, 72 ft on the head-rope, and were normally worked with 25-fathom bridles

In the silty water and on the muddy deposits of those parts of the tropical Atlantic coast-line where there is heavy run-off of surface water from the land, there are few, if any, reef corals, and the ground is suitable for trawling; the demersal fish fauna is superficially similar on both coasts, comprising a large number of species, relative to temperate faunas, principally of Sciaenidae, Drepanidae, Pristipomatidae, Ariidae and Cynoglossidae, off West Africa, and of Sciaenidae, Pomadasydae, Gerridae and Ariidae off British Guiana On harder, sandy deposits the fauna comprises principally Sparidae, Lethrinidae and Lutjanidae off West Africa, and Serranidae and Lutjanidae off British Guiana However, the percentage composition of the catch is rather different off West Africa and off that part of South America (Table 1) which was investigated

There appears to be a much greater dominance of Sciaenidae in the British Guiana catches than in those

Table 1 Percentage Composition of Commercial Landings at Four Tropical West African Ports, from Various Sources, and of the *Cape St Mary* Landings at Georgetown, British Guiana

	Freetown	Abidjan	Takoradi	Lagos	Georgetown
Sciaenidae	34	21	44	25	67
Polynemidae	15	14	13	14	<1
Drepanidae (or Ephippidae	14	3	3	2	<1
Ariidae	11	2	—	14	4
Trygonidae	2	5	18	8	<1

from the African equatorial regions (Table 1) of comparable latitudes In this respect the Guianian fisheries appear to resemble more closely those of Mauretania and Angola, to the north and south of the African equatorial zone, where a single species (*Sciaena aquila*) dominates the landings, the more so since a single species (*Micropogon furnieri*) constituted up to 43 per cent of the total landings in Guiana.

In the final analysis of the catch-rates of the *Cape St. Mary* off West Africa[3], it was convenient to divide the data according to the fauna comprising each catch a Sciaenid fauna on soft deposits down to the level of the thermocline at about 55–65 m , a Sparid fauna on sand and shell-sand down to 90–110 m , and on mud between the thermocline and 110 m ; and a deep fauna, mainly of small, useless species, from 110 to 450 m , in which elements of the semi-abyssal continental slope fauna occurred Results in ref 2 are expressed in relation to deposit and depth but can readily be re-arranged to conform to the West African results, since a similar division of the fauna by deposits was recognized in that work

Table 2 Catch-rates (kgm /hr) of the *Cape St Mary* on Four Sectors of the West African Continental Shelf between 13° W and 17° W and on the South American Continental Shelf between 7° N and 8° N

	Sulima	Freetown	Conakry	Gambia	Georgetown
Sciaenids, etc	183	195	295	178	187
Sparids, or Serranids, etc	21	53	53	130	59
Deep fauna	14	—	43	90	7 5

The results (Table 2) show that the catch-rates off West Africa and South America are very similar, with a single significant anomaly; this is the high catch-rate of snapper off Gambia This region lies at the northern extremity of the equatorial zone on the West African continental shelf, near the limit of the tropical Sciaenid fauna and is characterized by a rich fishery for large snappers (*Dentex*, *Lethrinus*, etc) which, furthermore, extends much deeper—at least to the continental edge—than does the tropical Sparid fauna farther to the south A similar situation occurs at the southern end of the equatorial region in West Africa, to the South of Cape Lopez

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Preliminary Bionomic Data from West African Fisheries

The determination of bionomic parameters in tropical fisheries is a matter of some difficulty, due particularly to the lack of seasonal or periodic changes in the environment, which renders useless many of the classical techniques in temperate and boreal seas[5] It has been shown[6] that demersal fish off Sierra Leone have, in general, no periodic cycle of feeding activity during the year, and it is known from general fisheries investigations there that many species are reproductively active throughout the year, juveniles of a few centimetres long being taken in the trawl debris in most months

However, recent investigations at Freetown[3] have shown that some species—fortunately including some of the more important commercial species—do, in fact, have a period of peak breeding activity which appears to have an annual periodicity, it has been possible to use the annual peak recruitment to the trawl catches of juvenile fish, and their subsequent growth, to determine rates of growth and mortality, using Petersen's technique

This evidence is derived from the inspection of the monthly length frequency distributions taken from trawling data—from commercial, exploratory and experimental trawling, the technique depends for its success on a number of factors that a single population can be sampled monthly, or more frequently, over a period of years, that the samples should be taken with a very small-meshed net so that the 50 per cent selection point of the net falls on fish of not more than six months old, that the juveniles should be present on the same grounds as the adults, without ecological separation and, finally, that there should be no significant difference in size between the sexes It appears that Sciaenidae and Drepanidae are the most likely families to fulfil all these requirements, and give the most satisfactory estimates of growth- and mortality-rates of the species investigated.

The results obtained for eight demersal species are set out in Table 3 in terms of length-for-age data

Table 3 Length-for-age Data, Lengths expressed as Total Length (Snout to Tip of Longest Caudal) in cm
Doubtful estimates in parenthesis

	I	II	III	IV	V	VI
Pseudotolithus elongatus	15 0	24 5	31 5	36 0	39 2	41 2
Pseudotolithus senegalensis	(15 0)	(28 0)	(42 0)	—	—	—
Drepane africana	16 0	23 0	25 5	—	—	—
Chaetodipterus lippei	15 5	24 0	—	—	—	—
Galeoides decadactylus	11 0	19 1	26 3	34 0	—	—
Pentanemus quinquarius	8 5	15 5	20 5	31 0	—	—
Pagrus ehrenbergi	(18 0)	(32 0)	—	—	—	—
Pristipoma jubelini	(15 0)	(22 0)	(30 0)	—	—	—

In a single species, *Pseudotolithus elongatus* (=*Corvina nigrita*), the data sufficed for a more detailed analysis than is included in Table 3 This is an abundant species in the estuarine and inshore catches, and the monthly length frequency distributions showed very satisfactory progression of peaks, month by month There was an annual peak of recruitment to the catches in June and July of small fish about 5 cm long which appeared to be the result of a spawning peak during the dry season at the turn of the year, confirmed by evidence from the gonadial cycle

From an investigation of the length/weight relationship of the species it is possible to derive an estimate of both relative and instantaneous growth in weight, and this is shown in Table 4

Table 4 *Pseudotolithus elongatus*, Growth in Length and Weight (h = relative, g = instantaneous growth in weight)

Year	Length (cm)	Weight (gm)	h	g
I	15 0	22 0	—	—
II	24 5	109 0	3 95	1 60
III	31 5	222 0	1 03	0 71
IV	36 0	337 0	0 52	0 42
V	39 2	403 0	0 19	0 17
VI	41 2	439 0	0 09	0 09

From dissections of the length frequency distributions of a large sample taken in November 1959, with a very fine-meshed net, it was possible to make an estimate of the relative numbers of individuals in

Table 5 *Pseudotolithus elongatus*, INSTANTANEOUS MORTALITY-RATE (i), ANNUAL MORTALITY-RATE (*a*), AND ANNUAL SURVIVAL-RATE (*S*), BIOMASS IN KGM OF THE SURVIVORS OF 1,000 FISH AT YEAR I (*P*)

Year	Log $n - \log n+1$	*i*	*a*	*S*	*P*
I	—	—	—	—	11 5
II	0 89	2 049	0 87	0 13	12 9
III	0 63	1 450	0 76	0 24	12 1
IV	0 63	1 450	0 76	0 24	2 2
V	0 43	0 990	0 62	0 38	1 0

each year-class and from these estimates, using Ricker's exponential tables[7] to make an estimate of mortality-rates These are shown in Table 5

From these estimations of mortality and from the length/weight relationship it is possible to estimate the relative biomass of the survivors year by year of a single-year class (Table 5) It is clear that in this species the relative weight contributed to the population by fish of more than 3 yr old is relatively very small, and it is clearly desirable that exploitation should begin before this time In fact, an estimate was made of the composition of the commercial landings at Freetown of this species and showed that 97·8 per cent of the individual fish landed were in their second or third year of life, and only 1·9 per cent were older fish.

In general, the tropical West African demersal fisheries appear to depend on only a relatively small number of year-classes of the constituent species, and this fact may be expected to have important consequences in any management policy—variations in the brood-strength of a species from year to year, for example, may be expected to have a far greater effect on the availability of a fish than is the case in a cold-water species, where the landings may depend on 10 or 20 yr -classes—and this may, in part, be an explanation of the puzzling fluctuations in abundance which are so characteristic of fisheries in this part of the world

The evidence on which these data are based will be published in full in the Colonial Office Fisheries Publications in due course

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A NEW APPLICATION OF THE FARADAY MAGNETO-OPTICAL EFFECT FOR DIAGNOSTIC MEASUREMENTS OF TRANSIENT MAGNETIC FIELDS

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EXTENSIVE use has been made of high-speed photography in plasma research but only to record plasma geometry, brightness, and the presence of instabilities and shock-waves We now describe a new technique for recording time variations in a longitudinal magnetic field which makes use of the Faraday magneto-optical effect, uses simple inexpensive apparatus and is easily incorporated into experiments already using streak camera observations

In an earlier paper[1] we described methods for superimposing streak (distance-time) and optical interference fringe photographs on the same emulsion By combining this and the new technique it should be possible to determine geometry, space-time distribution of electron number density in a high-density plasma (about 10^{17} cm $^{-3}$), and line-integrated variations of magnetic field along the line of sight with one streak camera

The magnetic field inside a plasma is usually determined from magnetic probe readings, but such readings are limited, since the physical presence of the probe introduces impurities and results in a perturbation of the plasma Even when the probe readings are only taken as valid up to the time when the plasma hits the probe, the problems of providing screening against radiation and protecting the recording oscilloscope from extraneous Earth currents is formidable The lifetime of plasma probes is also short

An interesting technique has been described in ref 2, it provides an independent method of measuring the longitudinal magnetic field inside a plasma without inserting probes and also gives the electron density But the method is only applicable to plasmas having a sufficiently low electron density to give a frequency below the propagating microwave frequeney and a magnetic field strength sufficiently large to give a measurable ratio of electron Larmor frequency to propagating frequency (w_L/w) In our experiments we work in the optical region with wave-lengths of 5×10^{-5} cm and magnetic fields of about 10^4 œrsted This means that we cannot use the Consoli–Dagai technique, since $(w_L/w) \simeq 10^{-5}$ However, this low ratio of (w_L/w) means that the electron density can be more accurately determined from the optical fringe pattern[3] and when the electron density distribution is known it is relatively easy to compute the associated magnetic field distribution inside the plasma[1] The magnetic field distribution determined in this way is, of course, a function of the magnetic field strength at the plasma boundary. which must be determined independently We determine the magnetic field at the boundary by making use of the Faraday magneto-optical effect in the walls of the plasma discharge tube

The experiment described here is our first experiment using the magneto-optical technique and is designed to measure the driving magnetic field inside a theta piece[4-6] The cylindrical discharge tube was made of extra dense flint glass, Chance number 70030, and has a Verdet's constant of about 0 09 where the Faraday effect is given by

$$\theta = rHL$$

Fig 1 Theta pinch at 1 mm ambient pressure

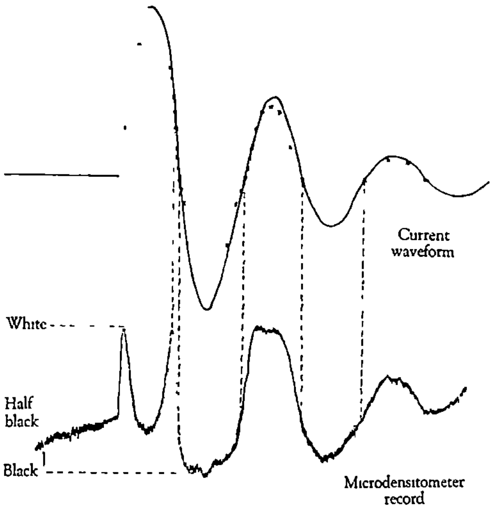

Fig 2 Theta pinch waveforms ×, from densitometer record

θ is the rotation of the plane of polarization in minutes, H is the strength of the magnetic field in œrsted directed along the optical axis; L is the path-length in cm , r is Verdet's constant and is given by the old classical theory[7] as

$$r = \frac{2\pi N e^3 w^2}{c^2 n(f - mw^2)^2}$$

where N is the number of electrons/unit volume, e and m are electron charge and mass, c is the speed of light, $2\pi(m/f)^{1/2}$ is the electron 'free period', w is the angular frequency of propagation, n is the refractive index in the absence of magnetic field

We have repeated the derivation of r allowing for the fact that H has a Fourier spectrum in the transient case and for an H component of angular frequency Ω we find that the frequency components of H modulate Verdet's constant so that w must be replaced everywhere by $(\Omega + w)$ Fortunately, in our experiment $(\Omega/w) \ll 1$ and Verdet's constant is not appreciably modulated

To measure the rotation of the plane of polarization we use a polarizer and an analyser to convert rotation into a change in intensity in the transmitted light Since the intensity is proportional to the square of the cosine of the angle between the plane of polarization of the light incident on the analyser and the plane of polarization for maximum transmission through the analyser, the analyser and polarizer are set with their planes of polarization at 45° to give maximum sensitivity and easy determination of magnetic field direction. The external driving field is derived from the rotation of light in the cylindrical wall The light is restricted to a sufficiently narrow band-width 5400–6400 Å by the polarizer At a suitable part of the camera sweep the light source is pulsed and a short time afterwards the theta pinch is initiated Light from the light source is also allowed to enter the camera without passing through the discharge tube to provide a light-intensity datum

Fig 1 shows a streak camera photograph of a theta pinch in deuterium, ambient pressure 1 mm of mercury fed by a 6 μF condenser charged to 9 kV Reading the photograph from top to bottom the first track is of light from the light source direct, the second track is of the polarized light passed through the discharge tube and shows clearly the modulation produced by the theta pinch magnetic field

Fig 2 shows the current in the theta piece monitored across a small series resistance together with a microdensitometer reading of the second track in Fig. 1 The vertical lines show the correlation between points of zero magnetic field and half-black light intensity points It will be noted that there is an extra half-black point corresponding to the large rotation produced by the high initial magnetic field To calibrate the apparatus the theta piece was evacuated and fed with current from a known condenser, thus providing a damped oscillation of the magnetic field

Fig 3 shows the associated streak photograph and Fig 4 the associated current and microdensitometer waveforms Once again there is an extra half-black point and two humps, which provide excellent self-calibration since they define a rotation of 45° in the discharge tube.

Using magnitudes of magnetic field defined by the damped current waveform and the first 45° point we find that the Verdet's constant for the discharge tube glass was 0 094 min /œrsted cm and using the calibration waveforms we have superimposed on the current waveform in Fig 2 the points obtained from the microdensitometer trace in Fig 2 It will be seen that the correlation is excellent and that the optical method fills in the initial rise that was too fast to be recorded by the current oscilloscope There was no need in this experiment to use the light-intensity datum trace in the interpretation of the microdensitometer traces shown in Figs 2 and 4, since we were fortunate in having a special high-intensity lamp developed by Dr. Gabriel of the Atomic Weapons

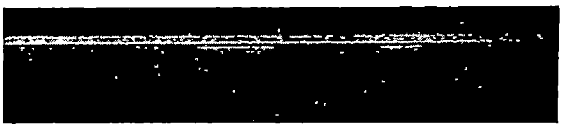

Fig 3 Calibration streak photograph

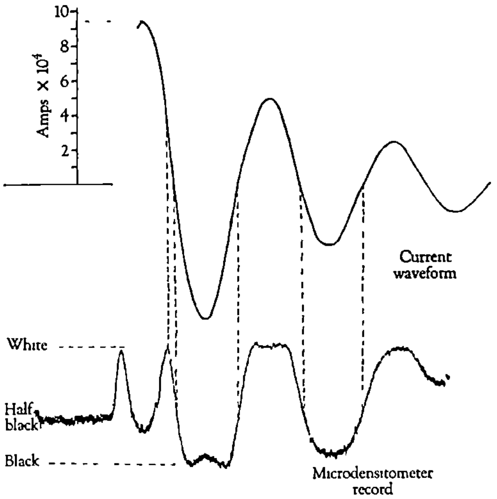

Fig 4 Calibration waveforms

Research Establishment[8] that gives a light pulse of about 10 µsec. duration which is remarkably constant However, we feel that the datum trace would have corrected for a lamp of inferior type. It is also fortuitous that the polarizer and analyser make the light a sufficiently narrow band for the purpose of the experiment and high-quality interference filters are not required

Fig 5 is a schematic diagram of our next experiment and is included to show how the technique may be extended to measure not only the driving magnetic field but also the trapped magnetic field in a theta pinch The diagram shows how the end windows of a pill-box type discharge tube can be used to measure the trapped field on the axis which is expected to have small divergence at the ends of the theta piece. The main feature of the optics is an iris diaphragm placed between a small supplementary lens and the exit lens feeding the light from the centre of the theta piece to the camera By controlling the diaphragm aperture the transmitted light through the centre will have a good 'signal-noise' for measurement of trapped field to be made even when the plasma fills the whole cross-section[1]

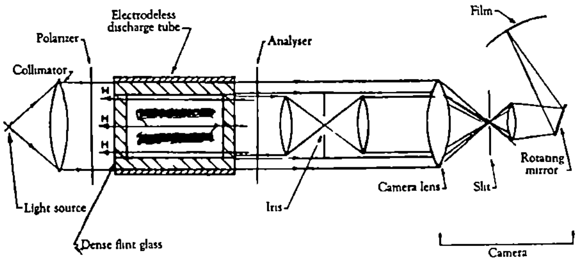

Fig 5 Proposed optical system

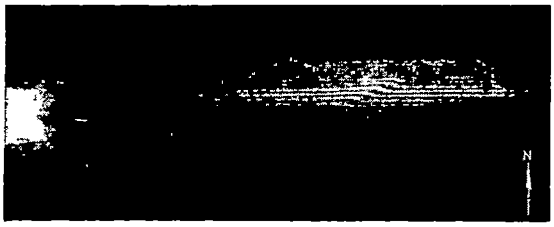

Fig 6 Streak-interferogram at 1 mm ambient pressure

Fig 6 is a streak camera record of a theta piece with interference fringes superimposed and is added for sake of completeness so that the reader can visualize a composite picture showing fringe shift and trapped and driving magnetic fields We wish to thank Beryl Richardson for her invaluable assistance in the experiment We are also indebted to Dr Gabriel for supplying the special light source and the Director of the Atomic Weapons Research Establishment for permission to publish this article

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SOME PHYSICO-CHEMICAL PROPERTIES OF PARACRYSTALLINE SPHERULITES OF BIOLOGICAL ORIGIN

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THE occurrence of lipoid in a mesophase in certain mammalian tissues has already been reported[1] Matter in this form possesses paracrystalline or liquid crystalline properties whereby the molecular compactness and regular orientation, characteristic of a true crystal, is preserved when, in a given thermal range, liquid mobility is demonstrable There are theoretical reasons for believing[2] that a mesophase of this kind is ideally suited for protoplasmic structure, in the case of certain lipoid complexes, it has now been shown[3] that a mesophase with characteristic liquid crystalline properties exists, naturally, in specialized cells of the ovary, adrenal and other tissues, though not in depot fat; pathologically, a similar mesophase may be present in plasma lipoprotein and in the soft parts of atheromatous deposits in arteries Under experimental conditions, similar lipoid complexes can be produced by pulsating the lipoprotein fraction against normal arterial intima at physiological pressures[4]

Known for years to physicists and physical chemists[5], the mesomorphic form of matter has not been extensively studied in biological material, nor in relation to disease The following report therefore describes further some of the properties of the spherulitic liquid crystals observed in various forms of lipoid mesophase in tissue

Material for study was obtained by making squashed preparations of the appropriate tissues in microscopic films, or, when bulkier yields were necessary, by homogenizing the tissue in water For general use, an abundant yield of paracrystalline substance was readily obtained by homogenizing segments of liver, taken by biopsy or at autopsy, from rabbits fed with 0 5–1 0 per cent cholesterol for three months or more From this homogenate, intracellular and cell-free lipoid were separated by centrifuging, and independently studied in aqueous or other suspensions More exact details of technique are described elsewhere[3], and paracrystalline substance has also been studied in lipæmic plasmas, resected atheromatous vessels in patients, corpora lutea in surgically excised ovaries, and other sources Examined in microscopic films under polarized light, fresh preparations of the tissues mentioned show abundant birefringent particles intra- and extracellularly including, in the soft lipoid components spherical droplets 0 5–2µ in diameter These anisotropic droplets (spherulites) have firm but not rigid, uniformly spherical outlines at 4–25° C , each droplet contains a polarization cross with arms parallel to the planes of polarization. When the film is rotated, the arms do not change direction or undergo extinction, when the analyser is rotated, the cross is extinguished at 180° intervals The sign of birefringence is positive and, with the polarizer only *in situ*, a faint interference pattern may still be seen These droplets are therefore liquid crystals of spherulitic structure, without surface attachments to the suspending liquid or, when intracellular, to the cytoplasm.

On a heated stage at 30°–37°, the spherulites tend to increase in size by limited confluence and are then deformed in outline when compressed; the polarization figure loses its symmetry but retains a roughly cruciate pattern and a positive sign of birefringence At 37°–42°, liquid flow is present, with birefringence, at higher temperatures, a true liquid, transparent, isotropic melt results From this, a mesophase with considerable liquid crystalline growth of spherulites and an orderly arrangement of polarization colours re-forms on cooling At 0° C or below, the spherulitic structure is destroyed, the degree of destruction being more or less proportional to the rate and intensity of cooling, placed for a few seconds in liquid nitrogen, the liquid crystals are instantaneously and permanently destroyed, with liberation of free cholesterol in amorphous or true crystalline form, true crystals of fatty acids and other indeterminate particles Individual spherulites show paracrystalline growth when kept for some hours in water or salt solutions, but not in glycerol They are destroyed by organic solvents and dehydration In white light, they are greenish and refractile, the larger spherulites may show a weak reaction with sudan dyes, when treated with benzpyrene[6], they show faint blue fluorescence in ultra-violet light

These properties of the spherulitic component of this lipoid mesophase indicate that the constituent molecules are radially arranged, unstable to changes in temperature, dehydration and organic solvents, but stable at 4°–37° and, in this state, penetrable by water and triglycerides. The spherulitic structure is, however, resistant to disruption by various other physical agencies, including ionic and non-ionic detergents, lipase, rat-liver esterase, trypsin and ultracentrifuging in salt solutions at 80,000g When subjected to ultrasonic vibrations (20 kc /s), no structural change can be detected in 30 sec but disintegration begins thereafter and is complete in 3–5 min. γ-Radiation up to 5 M rads from a cobalt-60 source produces no change nor does irradiation with 4-MeV. electrons from a linear accelerator.

It is known[7] that some of the lipoid in abnormal, hyperlipæmic plasma is resistant to the action of clearing factor, and this may also be related to the presence of a stable mesophase When heparin, or a comparable sulphated polysaccharide, is injected intravenously into a patient with essential hyperlipæmia (or a cholesterol-fed rabbit) with an abnormal, low-density β-lipoprotein plasma component, the classical clearing effect is often not obtained This anomaly is due to the abnormal β-lipoprotein which resists, or may even inhibit[8], the action of lipoprotein lipase, considered by some authorities to be the active clearing factor[9]. If the abnormal lipoprotein is then separated by centrifugal flotation from the uncleared plasma and examined at 22°–37° under polarized light, much of it is present in the form of a spherulitic mesophase, similar in properties to that already described in atheromatous and other tissues This abnormal lipoprotein complex can therefore exist within the plasma colloid as a mesophase, more stable than the chylomicron emulsion of normal lipæmia, and immune to one of the principal physiological clearing mechanisms This is the form of lipoprotein which, when pulsated against excised arterial segments, produces atheroma-like deposits in a similar lipoid mesophase[4] It is therefore possible that the presence in circulating blood of this form (S_f 12–20) of low-density lipoprotein is of direct causal importance in atherosclerosis.

Histochemical and chromatographic work[3] has shown that cholesterol esters and other lipids, as well as protein and water, are probably present in spherulites of the type described These constituents must therefore be linked in an amphiphilic system within a molecular structure which, at body temperature, is loosely radial and perhaps helical The physicochemical possibilities inherent in such systems have been made explicit in recent work[10,11] on phase-equilibria of soaps, water and sterols, it is highly probable that, in cytology as in physics, some of the particles which used to be called fatty micelles should now be regarded as paracrystalline complexes with this kind of structure which makes them amenable to incorporation in protoplasm while preserving the molecular orientation and penetrability to other lipoids and water, necessary for reactivity Apart from this functional aspect, the importance of a liquid crystalline system in polypeptide solutions[12], and of spherulitic texture in polyethylene fibres[13] suggests, by analogy, that the paracrystalline form may be equally essential as a structural mechanism in certain cells and tissues, especially where complex macromolecules of lipoprotein and steroids are involved

It is a basic property of liquid crystals that their molecules are held together by forces which, though complex, are weaker than those of true crystals. In the case of the liquid crystals described here, the weakness is revealed by the effects of temperature and organic solvents, but the bonds are remarkably resistant to agencies such as ionizing radiation, ultrasonic vibrations and lipolysis, whether by enzymes or detergents, which readily disrupt lipoid complexes in fine emulsions and cell membranes The implications of this peculiar stability, in terms of energy values, are beyond the scope of this article, but it is perhaps clear that a study of this question, coupled with a crystallographic analysis of structure, should be of value in solving a fundamental and largely unexplored problem in molecular biology

The irradiation experiments were carried out at the Research Laboratory of the United Kingdom Atomic Energy Authority at Wantage, Berks , in collaboration with Dr D B Powell

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KINETICS OF REACTION OF THE DECOMPOSITION OF LEAD AZIDE

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It has been reported[1,2] that the decomposition of inorganic azides begins at the crystal surface and that the crystal is soon covered with a thin film of metal formed by the reaction

$$2MeN_3 \rightarrow 2Me + 3N_2 + X \text{ kcal } (Me = \text{metal ion})$$

This reaction has been investigated extensively in the case of silver azide Moskovich and Aleksandrovitch[3] reported the formation of lead atoms at active surface regions during the initial stage of decomposition of α-PbN_6 These lead atoms then act as crystallization centres and cause an autocatalytic acceleration of the decomposition which is accompanied by the accelerated formation of a second solid-phase Pb-PbN_6 We have investigated in detail the reaction kinetics involved in this decomposition of α-lead azide

In ref 4 the isothermal decomposition of α-lead azide at 150°, 175° and 200° C in air was described It was found that at those temperatures the decomposition goes through at least two phases One phase could be isolated and classified as a tetragonal deformed cubic structure but, unfortunately, the other phases could not be isolated According to the chemical analysis of the compound isolated, oxygen could be present only to a small extent These experiments did not show whether the oxygen of the air was important in the formation of these phases during the decomposition, and the question could then be raised as to whether they consisted of basic azides or other oxygen-containing compounds as had been reported in ref 5 These authors reported that basic lead azides have been prepared by hydrolysis of PbN_6 with water, by reaction of PbN_6 with sodium hydroxide, and by precipitating PbN_6 from lead salt solutions via the addition of a mixture of NaN_3 and sodium hydroxide Nine different basic lead azides, including three forms of PbN_6 xPbO, were identified using X-rays

X-ray work on phase 3. It has been mentioned[4] that during the isothermal decomposition of α-lead azide at 175° C in air the last stage of decomposition corresponds to a compound with an approximate formula of ($PbN_{2\ 5}(OH)_n$), herein called phase 3 (The nitrogen is probably present as azide nitrogen) Additional calculations show that some weak X-ray reflexions, which could not be indexed as cubic and which have been ascribed to impurities resulting from other decomposition phases, belong to the structure of phase 3 The crystal structure of this compound is more appropriately considered as a tetragonal deformed cubic lattice On this basis the following data have been calculated

cell dimensions, $a_0 = 12\ 25_4$ Å , $c_0 = 11\ 88_5$ Å , $c_0/a_0 = 0\ 9698$,
volume of the unit cell, $V_0 = 1784\ 66$ Å ^{3},
density (pycnometric) $d_p = 5\ 623$ gm /cm ^{3},
density (X-ray) $d_x = 5\ 633$ gm /cm ^{3}

Debye–Scherrer photographs and X-ray diffractograms of this compound showed the following systematic absences

(hkl) - - - - - absent except $h + k = 2n$
$(hk0)$ - - - - - absent except $h + k = 2n$
$(0kl)$
$(h0l)$ - - - - - absent except $k + 1 = 2n$
(hhl) - - - - - no conditions
$(h00)$ - - - - - no conditions

These extinction data make possible the assignment of the space-groups D_{3h}^{12}, $P4_2/nnm$ and D_{4h}^{7}, $P4/nmm$, with the first of these being the most probable During about thirty experiments this structure was observed within 16 0–16 8 per cent loss in weight of the samples This is approximately the range of homogeneity of this phase

It is interesting to note that phase 3 can also be formed by heating α-lead azide at higher temperatures for shorter periods of time For example, this compound was formed by heating α-lead azide at 360° C for 3 min Longer heating will result in a change in colour from yellow to orange and finally brown.

Decomposition in pure argon and oxygen atmospheres To investigate the influence of oxygen during the isothermal decomposition of lead azide, experiments using thermogravimetric techniques were carried out at 175° C in argon and oxygen While the decomposition in air with 40 per cent relative humidity led to an average of 16 4 per cent loss in weight, experiments in dried argon and oxygen resulted in a 12 per cent loss at the same temperature (Fig 1)

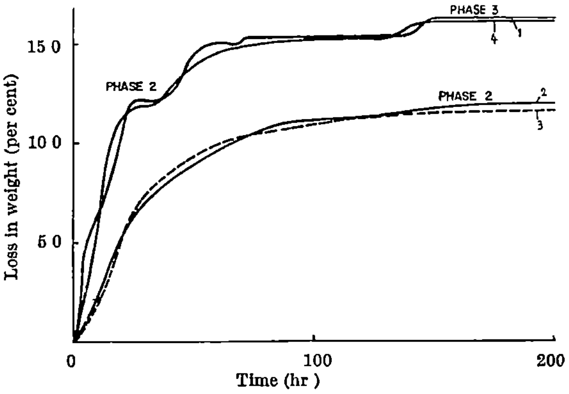

Fig 1 Decomposition curves of lead azide in air with 40 per cent relative humidity (1), in dried argon (2), oxygen (3), and argon saturated with moisture at 26° C (4)

It has been shown[4] that α-lead azide decomposes in air in several steps which must be considered as definite crystal phases During decomposition at 175° C the first step is reached when the sample has lost about 12 per cent in weight However, according to the shape of the curve up to this step, it is possible that there is more than one reaction mechanism involved Up to a 4 6 per cent loss in weight the slope of the curve, that is, the rate of evolution of nitrogen, is equal to 1 3 per cent/hr , whereas the rate of volatilization of nitrogen in the second stage, up to about 9 per cent loss in weight, is 0 36 per cent/hr and, up to 12 per cent loss in weight, about 0 5 per cent/hr These steps can be seen in Fig 1, which indicates a clear break in the curve at 4 6 per cent loss in weight which may be due to the formation of an intermediate state not shown in Fig 6

The decomposition does not follow this course if the experiment is run in dried argon and oxygen While the first step in air containing 40 per cent relative humidity occurs at 175° with 12 per cent loss in weight after about 30 hr. the same result was obtained in dried argon and oxygen after about 100 hr The decomposition in oxygen or argon follows a continuous curve and does not show the changes in the reaction-rate observed in air (Fig 1) However the X-ray diffraction patterns of the products formed in air at ambient conditions, in dried argon and in dry oxygen are the same This indicates that, until this stage of decomposition, oxygen does not affect the reaction It is very probable that the presence of moisture accelerates the decomposition reaction and causes at least a partial hydrolysis

It is believed that subsequent decomposition (greater than 12 per cent loss in weight) is only possible in the presence of moisture. On a weight basis, the residue corresponding to the 12 per cent loss in weight may be identified as phase 2, $\mathrm{PbN}_{3\,5}(\mathrm{OH})x$ This compound can be formed either by heating PbN_6 in air with moisture (relative humidity 40 per cent) at 175° C for 30 hr or by heating in oxygen or argon at the same temperature for 100 hr As expected, phase 2 formed by these three experiments showed identical X-ray diffractograms It is possible that even in the 'dried' argon and oxygen, traces of moisture are present which may contribute to the formation of his phase In Fig 2 the observed reflexions of this phase are compared with those of pure α-lead azide Phase 2 seems to have a higher symmetry than the original α-lead azide but a lower symmetry than phase 3

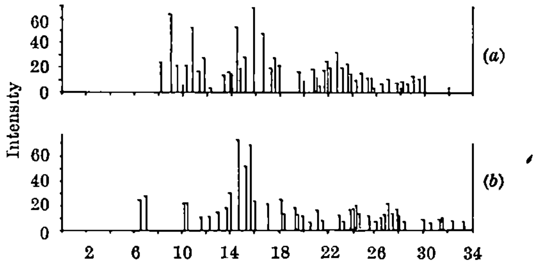

Fig 2. X-ray reflexions of (a) α-lead azide, (b) phase 2

Apparently moisture affects the rate of decomposition, but the manner in which it accomplishes this is not fully understood at the present time It is believed that small amounts of water vapour may hydrolyse the lead azide at elevated temperature[5] If layers of lead were formed during decomposition, one would expect different results because such lead, being very reactive, would immediately form lead oxide in the presence of oxygen Neither the reflexions of lead oxide nor those of metallic lead could be observed on the X-ray diffractograms of phase 2 obtained through decomposition in oxygen, argon or air The formation of phase 3 could not be achieved at the given temperatures in pure argon and oxygen and it was concluded that a relatively high moisture content is necessary for the formation of this phase (Phase 1, previously called α-phase[1], was observed only when heated at 150° C) However, phase 3 was also obtained when α-PbN_6 was heated in argon which had been bubbled through water at 26° C

Comparison of some properties of α-lead azide and phase 3 The ignition time of phase 3 was determined at several temperatures and compared with that of α-lead azide under the same conditions The apparatus used for these experiments is shown schematically in Fig 3

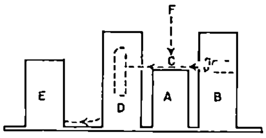

Fig 3 Apparatus for measuring time for ignition *A*, electrically heated plate, *B*, source of light, *C*, beam of light, *D*, photomultiplier tube, *E*, Hewlett–Packard timer, *F*, sample

The instrument consists essentially of a small electrically heated hot plate (*A*) with a centrally located iron-constantan thermocouple for determining its temperature and a photoelectric system for triggering the electronic timer A 1/16-in diameter light beam is focused across the centre of the hot plate (*A*) to a photomultiplier tube (*D*) When the hot plate is at the desired temperature, about 0 003 gm of sample is dropped through the light beam (*C*) on the plate The falling sample breaks the light beam and causes the photomultiplier tube to put out a pulse which starts the counter (*E*). The flash accompanying the ignition of the sample actuates the photomultiplier, which then stops the counter The time until ignition is determined to 0 001 sec and the temperature of the plate to ± 1° C

The results so obtained for phase 3 and α-lead azide are plotted as temperature versus induction-time curves in Fig 4 In both curves (4*a* and 4*b*) every point is the percentage fired out of twenty tests The standard deviation of this method is about ± 5 per cent An attempt was also made to calculate the activation energies of these compounds using the Semenov equation[7] and the plot of this is shown in Fig 4*b* The activation energies of phase 3 and PbN_6 were determined to be 80 and 100 kcal /mole, respectively It should be mentioned that these activation energies are only apparent activation energies[6]

According to these results the compound $\mathrm{PbN}_{2\,5}(\mathrm{OH})_n$ is less sensitive to ignition than the α-lead azide It was noted that below 450° C phase 3

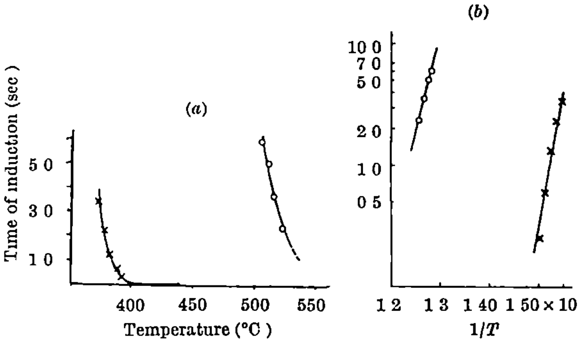

Fig 4 (*a*) Temperature against time of induction ×, α-lead azide, ○, phase 3 (*b*) time of induction against 1/*T* ×, α-lead azide with energy of activation 100 ± 20 k cal /mole, ○, phase 2 with energy of activation of 80 ± 20 k cal/mole was found to undergo slow decomposition, but no ignition was observed at this temperature On the other hand, the lowest ignition temperature for lead azide is about 370° C and its ignition time at 400° C is less than 0 1 sec

To test the electrical sensitivity of phase 3, small 1/4-in diameter plugs of insulating material were coated with a thin gold film to form an electrical bridge and a 0 06 gm pellet of sample was pressed at 3,000 lb /sq in on the gold film The resistance of the loaded plugs ranged from 1 to 3 ohms and their firing characteristics were determined by the discharge of a 1 μF condenser at varying voltages In Fig 5 percentage fired is plotted against the input voltage For comparison, the electrical sensitivity of lead azide is also included in Fig 5 It is apparent that although less sensitive than the untreated lead azide, phase 3 can still be considered as an electrically sensitive material

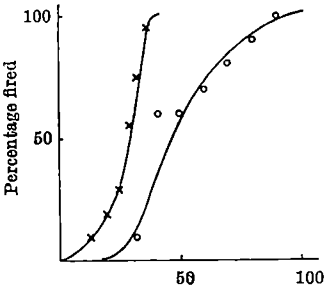

Fig 5 Electrical initiation of α-lead azide (×) and of phase 3 (○)

Investigations with infra-red The infra-red spectrum of phase 3 is slightly different from that of α-lead azide, although the main absorption band at 4 93μ occurs in both samples with the same intensity In Table 1 the observed absorption bands of α-lead azide and phase 3 are listed The pellets used for the infra-red analysis were prepared by pressing 200 mgm. KBr and 2 mgm of PbN_6 or $PbN_{2\ 5}(OH)_n$ at 15,000 lb /sq in The thickness of these pellets was approximately 1 mm

The absorption of both samples was most intense at about 5 0μ This band can best be ascribed to a stretching vibration of the N≡N triple bond As indicated in Table 1, only slight differences between the two samples could be observed so far as their infra-red spectra were concerned In the case of lead azide the most intense absorption band consists of two distinguishable peaks at 4 93μ and 5 05μ respectively, whereas with phase 3 there is only one peak which occurs at 4 95μ Another absorption band of lead azide with a weaker intensity was observed at 7·55μ due to the symmetrical vibration of the azide group In this region the decomposition product (phase 3) showed two absorption maxima at 7 15–7 20μ and 7 63μ The first band is probably due to the N=N double bond while the peak at 7 63μ is possibly caused by an OH bending vibration In the spectrum of both α-lead azide and its decomposition product, an absorption band at 2 95μ could be observed However, the intensity of this band in the spectrum of phase 3 was found to be twice the intensity of the lead azide peak This would suggest that hydrogen bonding is present in both cases Although the samples had been dried very carefully to remove all moisture and water, the spectra showed that this band (2 95μ) was still present It is very probable that the lead azide also contained a small amount of bonded water which could not be removed by the normal drying procedure and which might be considered as a stabilizer (through OH-bonding) of these compounds Apparently even heat treatment with decomposition of lead azide does not remove this water For future investigations it would be of interest to prepare lead azide without using water as a solvent, if possible. Another absorption band was observed in both samples at approximately 6 10–6 20μ which may be attributed to the N—H bond Again, the band due to phase 3 was twice as intense as that of untreated lead azide Finally, a weak absorption band which occurred in both samples with about the same intensity could be observed at 4 30–4 35μ This may be caused by hydrogen bonding in the condensed state One cannot, however, arrive at a final conclusion based on these results at the present moment, but more information may be forthcoming if lead azide can be prepared in a non-aqueous solution

Table 1 Infra-red Spectra

α-Lead azide (PbN_6)			(Phase 3) $PbN \sim 2\ 5\ (OH)_n$		
Absorption band	Int	Remarks	Absorption band	Int	Remarks
2 95μ (3,400 cm $^{-1}$)	4 5	OH bonding vibration	2 95μ (3,400 cm $^{-1}$)	9 3	OH bonding vibration
4 30–4 35μ (2,325–2,300 cm $^{-1}$)	2 0	hydrogen bond	4 30μ (2,325 cm $^{-1}$)	2 5	hydrogen bond
4 93μ (2,030 cm $^{-1}$	25 0	asymmetrical stretching of the azide group	4 95μ (2,030 cm $^{-1}$)	24 0	stretching vibration of the N≡N triple bond
5 05μ (1,920 cm $^{-1}$)	24 5				
6 10–6 20μ (1,640–1,610 cm $^{-1}$)	3 5	N—H bending vibration	6 10–6 20μ (1,640–1,610 cm $^{-1}$)	8 0	N—H bending vibration
			7 15–7 20μ (1,400–1,390 cm $^{-1}$)	9 0	N=N bond
7 55μ (1,325 cm $^{-1}$)	2 8	symmetrical stretching vibration of azide group			
			7 63μ 1,310 cm $^{-1}$	6 5	OH bending vibration
			14 75μ 678 cm $^{-1}$	5 0	not known

Conclusions Based on the results obtained thus far, it appears that the reaction mechanism during the thermal ignition of lead azide in air is dependent on the temperature and time to ignition. Ignition times of less than 0 5 sec have a single mechanism which probably goes through an activated state by which the required energy for a chain reaction is delivered (mechanism I, Fig 6) These activated centres have to reach a certain concentration in a short time-interval in order to deliver enough energy for ignition If, on the other hand, the temperature is not high enough to form this threshold concentration (there may also be a threshold temperature) the available energy cannot be supplied to cause the chain reaction and the decomposition will proceed slowly through

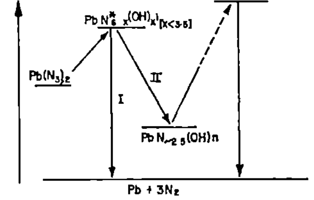

Fig 6 Kinetics of decomposition of lead azide

the different phases (mechanism II, Fig 6) It is possible, however, that within a certain range of temperature both mechanisms are competing with each other For the slow decomposition, a hydrolysis reaction is assumed since the presence of oxygen during this decomposition had no effect on the hydrolysis reaction

We thank Dr J. V R Kaufman for his interest and encouragement during the course of this work, Mr C Campbell for the thermogravimetric work, Mr J Campisi for the X-ray photographs, and Dr N Paraskevopoulos for the infra-red spectra

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PROBABILITY DISTRIBUTION ANALYSER PROGRAMMED FOR NEUROPHYSIOLOGICAL RESEARCH

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THE sensory side of the nervous system is basically 'noisy'. Information supplied by sensory stimulation is transmitted through the brain as patterns of nerve impulses. In the absence of stimulation, however, the trains of nerve impulses do not subside completely but are replaced by a 'spontaneous' discharge which may be quite as striking as the information-rich pattern. It is perhaps surprising that so little attention has been given to an understanding of the nature of this spontaneous discharge There are, however, serious practical difficulties Superficially this discharge of nerve impulses appears to be without any pattern Apart from using devices which indicate the mean rate of the unit discharge, one must undertake a very large amount of tedious manual computational work to derive more comprehensive statistics and this has undoubtedly been responsible for the lack of interest in this phenomenon

Recently a probability distribution analyser was described[1] based on the Hutchinson and Scarrott pulse-height analyser, used extensively in nuclear physics It has been successfully applied to computational problems in the field of stochastic processes and noise We have now programmed this instrument to derive the frequency distribution of intervals between successive impulses of the spontaneous (and light-evoked) discharge of single units sampled at various points in the visual system The distribution is presented as an 'interval histogram' While this work was in progress other workers have independently derived similar statistics from the auditory system[2]

The probability analyser is basically a cathode ray tube electrostatic memory of 64 channels with 15 binary digit capacity a channel. The voltage applied to its input terminal is ordinarily sampled at a fixed (relatively slow) rate of 100/sec , counts being added to the channel corresponding to that input voltage Auxiliary circuitry was developed to convert the time-interval between successive impulses into a voltage proportional to that interval Suitable

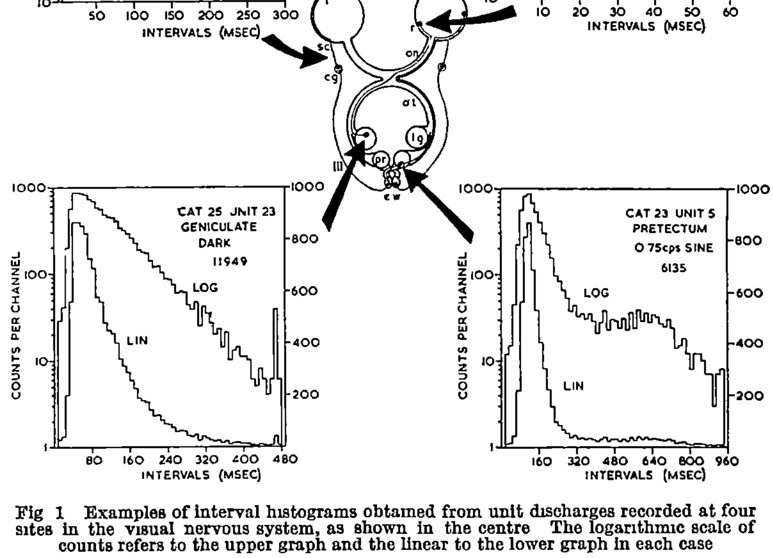

Fig 1 Examples of interval histograms obtained from unit discharges recorded at four sites in the visual nervous system, as shown in the centre The logarithmic scale of counts refers to the upper graph and the linear to the lower graph in each case

gating ensured that the analyser accepted not more than one sample of the voltage corresponding to any interval

Certain limitations of this arrangement were recognized Due to the fixed maximum sampling-rate of 100/sec , there was a probability that a sample would be missed whenever an interval in the discharge was 10 m sec or less The particular sample missed would correspond to the interval preceding the short interval If successive intervals are statistically independent, the missed samples will not affect the shape of the histogram obtained, but only scale down all channel counts by a constant factor, when sufficient intervals have been analysed to smooth out statistical fluctuations However, since there was no prior knowledge that successive intervals in the nerve discharges were statistically independent, we have excluded those units in which missed samples amounted to more than 1 per cent of the total number of intervals examined

The method has been successfully applied to unit activity recorded from four sites in the visual system of the cat retina, lateral geniculate nucleus, pretectal nucleus and short ciliary nerve Some examples of interval histograms obtained are shown in Fig 1 Of immediate interest is the remarkable dissimilarity of the distributions from different regions That of the retinal ganglion cell presented is of asymmetrical Gaussian type whereas the particular geniculate cell distribution seems best compared with a Poisson type On the other hand, the short ciliary unit shows a bimodal distribution. It should be noted that counts due to intervals longer than the range of the analyser have been grouped into the last two channels

Striking changes in the distribution of intervals occur when patterned photic stimulus is presented to the eye This is demonstrated by the pretectal unit when the retina was stimulated by light, the intensity of which was modulated sinusoidally at 0 75 cycles/sec frequency There is a low, second mode in the distribution corresponding to a relative prevalence of long intervals corresponding to a silent period in the discharge during the cycle of light modulation

This is a fast, flexible and accurate method The analyses can be completed approximately 1,000 times more quickly than by conventional methods of photography and measurement of the enlargements As can be seen from the graphs, a sixty-four-fold range of intervals can be examined at any particular setting of the analyser channel-width, and the channel-width can be set to any desired value to accommodate the discharge pattern of the particular unit Large numbers of intervals can be examined (for example, 35,756 in the case of the retinal unit) for the total analyser count capacity is more than 8 million (2^{23}–64), and this is important in smoothing out the effects of statistical variation

A large field has been made accessible and is being opened up by this technique The information abstracted will ultimately form the foundation for the application of a communication theory to the visual system in particular and the nervous system in general

This work was aided by grants from the National Health and Medical Research Council of Australia, the Ophthalmic Research Institute of Australia and the Post-Graduate Medical Foundation of the University of Sydney

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HÆMIN-PROTEIN BINDING IN PEROXIDASE AND METHÆMALBUMIN

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WHEN protohæmin combines with the protein moieties of hæmoproteins its absorption maximum in the Soret region becomes intensified This has been ascribed either to the establishment of an iron-protein bond or to a bond involving the propionyl side-chains of the hæmin

O'Hagan and George succeeded in preparing artificial hæmoglobins[1] and myoglobins[2] with hæmins lacking carboxyl groups They concluded that in these hæmoproteins the essential binding must involve the central iron atom When coupling hæmin or hæm to human serum albumin, however, O'Hagan could demonstrate that the carboxyl groups of the hæmin molecule are involved in hæmin–protein binding[3] Little and Neilands[4] have recently investigated the coupling of hæmins to human serum albumin

Some simple experiments in this field were performed by me a few years ago Since this work was interrupted, the experiments are reported in abridged form Hæmin derivatives were prepared in which either the iron atom was missing (protoporphyrin) or the carboxyl groups were blocked (protohæmin-dimethyl ester) One or the other of these compounds was added to serum albumin or to apo-horseradish peroxidase, and the spectral changes in the Soret region recorded

Human serum albumin was kindly supplied by Dr M Surgenor (then in Boston) in the form of Cohn's fraction V Horseradish peroxidase was crystallized as described in ref 5 Protoporphyrin IX-dimethylester was made according to Grinstein[6] It melted at 221–225° uncorrected Free protoporphyrin IX was obtained by careful hydrolysis of the ester with 25 per cent hydrochloric acid as described in ref 7 and checking the absorption spectrum (peak at 412–413 mμ) Only freshly hydrolysed solutions were used Protohæmin IX-dimethylester was made as in ref 8 The crude product was purified on aragonite columns This crystal form of calcium carbonate proved to be superior to calcite or to mixtures of the two forms (Thanks are due to Prof F Daniels (Madison, Wisconsin) for generous gifts of samples of aragonite and calcite) The columns were prepared with dry benzene A benzene solution of the crude protohæmin IX-dimethylester was added and the column developed with benzene A green zone (*A*, about 5 per cent) travelled fastest, it was followed by a diffuse light-brown band (*B*, about 60 per cent) and a sharply defined dark brown zone (*C*, about 20 per cent) A brown area (*D*, about 12 per cent) stayed on top of the column Benzene eluted *A* (not identified) then *B* (pure protohæmin IX-dimethylester), and benzene/chloroform (1 1) eluted *C* (the monomethyl ester ?) *D* proved to be free protohæmin, elutable by chloroform/acetic acid (99 1)

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Human serum albumin binds only protoporphyrin dissolved in acid aqueous solutions. If the solutions are neutralized or made alkaline, no appreciable spectral change takes place on addition of human serum albumin, possibly due to polymerization Fig 1 shows the Soret spectra obtained on adding 40 μl of a 128 μM protoporphyrin solution in N hydrochloric acid to 2 20 ml of 0·2 M Na_2HPO_4 on one hand, and to 2 20 ml of 3 0 μM human serum albumin in 0 2 M Na_2HPO_4 on the other hand The final molar ratio protoporphyrin/human serum albumin was thus 2 4 1 Rosenfeld and Surgenor[9] had found that human serum albumin forms complexes with ferri protoporphyrin in the ratios of 1 1 and 1 2 When solutions of protoporphyrin-dimethylester or of protohæmin IX-dimethylester in acetone were added to buffered human serum albumin and to buffer alone respectively, no spectral differences between the two resultant solutions were obtained

With horseradish peroxidase, similar, if somewhat less dramatic, results were obtained as with human serum albumin Neither protoporphyrin-dimethylester nor protohæmin IX-dimethylester, both in acetone solution, was bound to apo-horseradish peroxidase The spectra obtained on adding free protoporphyrin to apo-horseradish peroxidase are shown in Fig 2 40 μl of a 79 μM solution of protoporphyrin in 0 2 N hydrochloric acid were added to 2 20 ml. of 0 2 M phosphate buffer of *p*H 6 8, and to 2 20 ml of 1 3 μM apo-horseradish peroxidase in the same buffer respectively The concentration of active apo-horseradish peroxidase was determined by spectrophotometric titration of the protein with protohæmin of known titre The final concentration of protoporphyrin was thus 1 4 μM and the molar ratios protoporphyrin/apo-horseradish peroxidase = 1·1 · 1 0 When the protoporphyrin concentration was chosen at a lower level, spectra intermediate between those shown in Fig 2 were obtained, addition of larger amounts of protoporphyrin to both the blank and the 1 1 protoporphyrin–apo-horseradish peroxidase complex gave no further spectral change

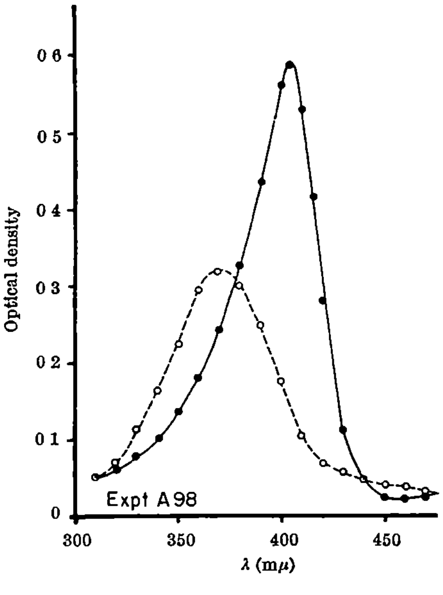

Fig 1 Soret spectra of protoporphyrin IX in phosphate (Na_2HPO_4), in the absence (○ – ○) and in the presence (● – ●) of human serum albumin

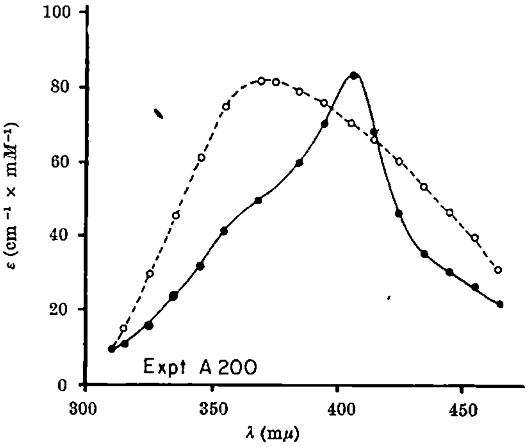

Fig 2 Soret spectra of protoporphyrin IX in phosphate buffer, in the absence (○ – ○) and in the presence (● – ●) of apo-horseradish peroxidase

The maxima of the Soret bands for horseradish peroxidase, protoporphyrin–apo-horseradish peroxidase, methæmalbumin and protoporphyrin–albumin all lie at 403 mμ, irrespective of the presence or the absence of the central iron atom Of more importance is the fact that the extinction coefficients of the porphyrin compounds are equal to or higher than those of the hæmin compounds This is shown in Table 1

Table 1 EXTINCTION COEFFICIENTS, $\varepsilon_{m\mu}$, OF HORSERADISH PEROXIDASE AND METHÆMALBUMIN AND THEIR IRON-FREE COUNTERPARTS AT THE PEAK OF THE SORET BAND AT 403 mμ

Horseradish peroxidase recombined from apo-horseradish peroxidase and protohæmin, *p*H 6 8	81 cm⁻¹m*M*⁻¹
Protoporphyrin–apo-horseradish peroxidase, *p*H 6 8	83 ,,
Methæmalbumin, *p*H 7–10 (cf ref 9)	81 ,,
Protoporphyrin human serum albumin, in Na_2HPO_4	116 ,,

A freshly prepared 1 3 μM solution of the protoporphyrin–apo-horseradish peroxidase complex did not react with methyl hydrogen peroxide as judged by the appearance of the Soret spectrum Protohæmin to a final concentration of 0 87 μM was now added to blank and solution no spectral change occurred Renewed addition of methyl hydrogen peroxide (2 μM) had no influence on the spectrum When protohæmin was added directly to free apo-horseradish peroxidase, complex II was rapidly formed with a Soret peak at 418 mμ The experiment shows that protoporphyrin competes with protohæmin for the same sites at the apo-horseradish peroxidase molecule The results emphasize the importance of the propionyl carboxyl groups of porphyrins in binding with proteins The intensification of the Soret band on binding seems to be mainly due to binding of the carboxyl group to the proteins investigated in the work described here

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TRANSFERRIN DIFFERENCES IN CHIMPANZEE SERA

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TRANSFERRIN exists in a variety of molecular species in man and other mammals[1] Presumably the serum transferrins of the other Primates are similar to those of man While screening blood of Primates for differences in hæmoglobin, transferrin, α_2-globulin and cæruloplasmin, we have been able to test a number of chimpanzee sera and find results both similar to and different from those reported by Boyer and Young[2]

The samples came from four chimpanzees at the Department of Preventive Medicine at Yale ; 19 sera, given to us by Dr Edwin Isacson of Yale University, from animals at the Aero Space Medical Laboratory, Holloman Air Force Base, New Mexico , and 20 sera from the laboratory of Dr Morris Goodman, Wayne State University, Detroit, Michigan. The specific identification of the animals is not certain, though we believe them to be *Pan troglodytes* The variety of taxonomic labels given chimpanzees in the literature is not particularly informative Most of the animals were reported to have come from Senegal, Sierra Leone and the French Cameroons The 20 samples from Dr Goodman came from Orange Park, Florida

Sera were centrifuged at 16,000 r p.m at – 10° C , 1·5 ml of serum was treated for at least 1 hr with 0 5 ml. of ferrous citrate containing iron-59, 10 μc /ml The reaction was carried out either at ambient temperature (19° C.) or at 10° C Serum samples were inserted directly into precast slots in 12–15 per cent starch–gels made with 0 021 *M* borate buffer, *p*H 8·6 The electrophoresis was run in the vertical position at 6 V /cm for 16–18 hr After the run was completed, each gel was sliced in half One half was stained with amido-black dye in methanol/water/acetic acid solvent (5 5 1) The other half was wrapped in 'Saran Wrap' (Dow Chemical Corporation) and placed on a sheet of Kodak No-Screen X-ray film for 24 hr The films were developed in Kodak *D*-8 or *D*-11 developer The bands on the radiograph so produced were compared with various bands found on the section stained with amido-black The serum patterns were compared in repeated runs with the serum patterns of Yale College students and with those of a number of other Primates

A diagram of the transferrin types found is shown in Fig 1. We cannot label our patterns with the same designations that Boyer and Young[2] gave their transferrin phenotypes, since we could compare their patterns with ours only on the basis of the position of band *C* from a human homozygous *TfC* individual We have chosen to number our bands in order of decreasing mobility and name the types with inclusive numbers We found eight separate, distinct transferrin bands on the various radiographs, though never more than five in one individual serum. We name the band with the greatest mobility number 1, the one with lowest, 8 For example, the serum which had five bands, 3, 4, 5, 6 and 7, we named type 3–7 In Fig 1, human type *CC* is shown as well as one of the most frequent transferrins found in the African vervet or green monkey, *Cercopithecus aethiops* Band 3, which corresponds to no other Primates' transferrin that we know, was usually the densest band on the radiographs However, at least one of the eight bands showed up as a dense spot on the radiograph of at least one serum Therefore, we see no reason for distinguishing them on the basis of the extent to which they affect the X-ray film Table 1 shows the frequencies of the various types of transferrins

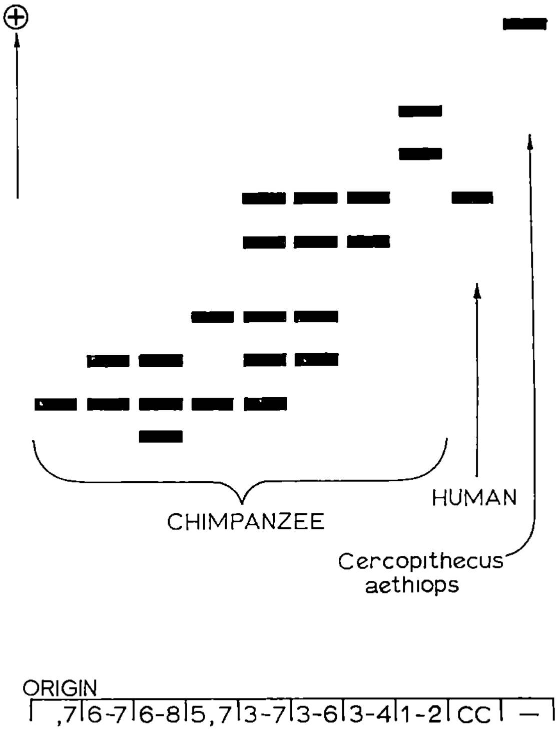

Fig 1. Diagram of electrophoretogram illustrating relative mobilities of transferrin bands in various chimpanzees compared with a human *TfC* homozygote and a common transferrin type from *Cercopithecus aethiops*

Table 1 Frequency of Transferrin Types in Chimpanzees

Type	Total	Number of animals* Group 1	Group 2	Group 3
1–2	1		1	
3–4	2		1	1
3–6 [*AC*]	2		2	
3–7 [*AD*]	7		7	
5, 7	2			2
6–7	14	1	2	11
6–8	12	2	4	6
,7	1		1	
	41†	3	18	20

* Group 1 are chimpanzees from Yale University, group 2 from Holloman Air Force Base, group 3 from Orange Park

† Two sera of the original 43 produced unreadable patterns on each of three runs

Exhaustive experiments in which each sample was saturated with iron-59 could not be undertaken because of the relatively small quantities of serum Nevertheless, where we had enough material and could add a large excess of iron-59, we found that bands which were faint on the original radiograph continued to be faint after addition of an excess of iron-59 The possibility occurs that the degree to which the transferrins are saturated *in vivo* before the blood samples were taken plays a part in the extent to which each band will take up iron-59 *in vitro* (Bearn, A , private communication)

It seems reasonable to identify our 3–7 type with the *AD* type of Boyer and Young, and our 3–6 type with their *AC* The other types do not correspond. The lack of correspondence may be due to species differences Our chimpanzees belong to a group labelled *Pan troglodytes* ; *Pan satyrus*, the name given the chimpanzees from which Boyer and Young received samples, is not known to us and is not recognized as a specific name by Fiedler[3], the name list at present in widest use It is possible that these are the dwarf varieties of the Congo chimpanzee described by Schwarz[4] Boyer (private communication) has stated recently that his animals were identified as *Pan troglodytes* by W. L Straus, jun

Another possibility is that these results are an additional demonstration of the marked polymorphism that chimpanzees show for the transferrin trait. Most of the animals showed the 6–8 and 6–7 patterns The other sera were distributed more or less randomly among the other types. In other words, there are no marked frequency differences between the Holloman chimpanzees and the Orange Park chimpanzees, despite apparent differences in place of origin in Africa

The serum patterns of these chimpanzees were compared with all the other Primates available to us One of our most striking findings is that only *Tupaia glis*, of all the Primates tested, showed iron-binding β-globulins which moved to the same general area on the gels as did those of the chimpanzee All other Primates available to us, except man (we have not been fortunate enough to obtain a sample from *Tarsius* yet), showed iron-binding bands of the β-globulins which have two and even three times the mobility of those of the chimpanzee

We found early in our work that it is best, as Boyer and Young showed, to use borate buffers in the gels and in the electrode trays in order to obtain the fine resolution of chimpanzee transferrin bands necessary to demonstrate multiple zones No other Primates, however, showed more than three iron-binding zones, though many other bands appear in the β-globulin region when macaque sera are subjected to electrophoresis in vertical starch-gels[5]

Unfortunately, it is not possible, at present, to discuss the possible modes of inheritance of this trait in the chimpanzee There are no good pedigrees available now, and it is unlikely that matings critical for working out the inheritance will occur

The multiplicity of the transferrin bands is similar to the situation in cattle β-globulins reported by Ashton[6] It is not clear yet whether all the bands in the β-globulin region of Primates represent separate alleles or whether the mode of inheritance is more complex The situation is made more complicated by the fact that the transferrin bands and the β-globulins do not show a one-to-one correspondence. A more detailed account of the β-globulins in Primates will be published later

I wish to thank Dr Edwin Isacson and Dr Francis Black of Yale University and Dr Morris Goodman of Wayne State University for their co-operation. The work on this project was performed under grant *RG*-6053 of the United States Public Health Service

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MECHANISM OF STREPTOMYCIN ACTION ON BACTERIA: A UNITARY HYPOTHESIS

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THE response of a bacterium to the presence of streptomycin in its environment is genetically determined, and can take three alternate forms, commonly known as sensitivity, resistance and dependence For a sensitive strain, streptomycin is toxic at very low concentrations , for a resistant strain, it is without effect on growth at considerably higher concentrations , and for a dependent strain, it is an essential growth factor Several different states are included under the broad heading of resistance, only one of which will be dealt with here We shall be concerned with the state in which a high level of resistance is acquired as a result of a single-step mutation from sensitivity, a type of resistance that is called indifference by some authors.

In *Escherichia coli*, genetic analysis has shown that sensitivity, dependence and single-step, high-level resistance are determined by multiple alleles of a single genetic locus[1–4] In terms of modern genetic theory, this fact implies that the three phenotypes are ultimately determined by alternate states of a specific protein, or of a regulatory system that con-

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trols the synthesis of a specific protein Hence all the epiphenomena that are specifically associated with each of these three phenotypes should fall into a causal chain, and the three causal chains should all lead back ultimately to three alternate functional states of the streptomycin locus This fundamental logical inference from the genetic data has been ignored in most theorizing about the mode of action of streptomycin Instead, there has been a regrettable tendency to embellish the account of each newly discovered effect of streptomycin on bacteria with yet another topical 'explanation' of the action of streptomycin

Most work on the physiological and biochemical effects of streptomycin on bacteria has been conducted with sensitive and resistant strains This preoccupation with sensitivity and resistance is mirrored by present explanatory hypotheses, which are directed primarily or exclusively towards interpreting the epiphenomena associated with these two states, and do not attempt to account for the epiphenomena associated with dependence One of us (C R S) has recently made a detailed physiological and biochemical study of streptomycin dependence in *Escherichia coli* The hypothesis which we shall present here is based primarily on the resultant findings, but it does have the somewhat novel advantage of explaining, in terms of structural modifications at a single intracellular site, some of the specific epiphenomena associated with sensitivity and resistance

Epiphenomena associated with Dependence on Streptomycin

Full experimental details of our work on streptomycin dependence will be published elsewhere[5] For the present purposes, it is sufficient to describe in summary fashion the rather complex epiphenomena which have been revealed by this work and by the work of earlier investigators

(1) A relatively high external concentration of streptomycin (greater than 100 μgm /ml) is necessary for growth of a dependent strain at maximal rate, when the streptomycin concentration falls below this *critical level,* the growth-rate becomes dependent on, and proportional to, streptomycin concentration[5-8] For any given strain, the absolute magnitude of the critical level varies in response to such factors as the salt content of the medium, the nature of the carbon source and the temperature At very low streptomycin-levels exponential growth can no longer be maintained, growth becomes arithmetic

(2) When the streptomycin-level of the medium is above the critical one, growing cells of a dependent strain have what we shall define as a normal macromolecular composition Under these conditions, the cellular content of protein, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) does not differ significantly from that of the corresponding sensitive strain, growing under identical environmental conditions in the absence of streptomycin[5]. However, when the streptomycin concentration of the medium is below the critical level, the macromolecular composition of the dependent cells becomes abnormal, and the degree of the abnormality is systematically related to streptomycin concentration[5] At progressively lower streptomycin-levels, the RNA content of the cells becomes greater, and the protein content becomes smaller DNA content is slightly sub-normal at low streptomycin-levels The effects of streptomycin concentration on cellular composition are shown in Fig 1

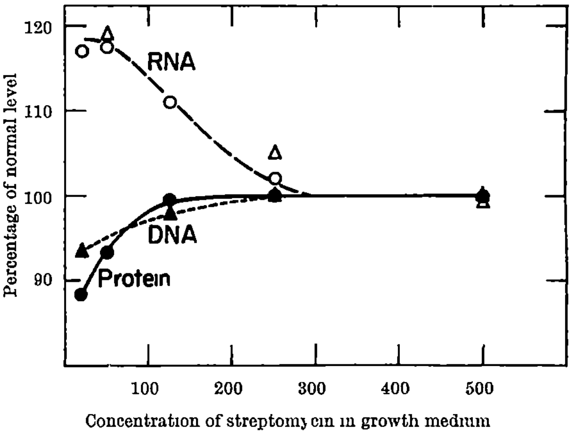

Fig 1 Effect of the streptomycin concentration of the medium on cellular composition of a dependent strain of *Escherichia coli*

(3) If dependent cells which have been grown with a supra-critical concentration of streptomycin are washed and re-suspended in a streptomycin-free medium, they continue to divide for a limited period, the number of divisions is a function of the concentration of streptomycin to which they were previously exposed[1,9] After the cessation of cell division, the cells can elongate[7,9,10], and further nuclear divisions can occur[11] A systematic analysis of this residual growth has revealed other significant facts[5] The cell mass increases exponentially at maximal rate for a short period, the duration of which is directly proportional to the streptomycin concentration of the medium from which the cells were taken The growth-rate then declines progressively over a period of 1–2 hr, after which growth becomes arithmetic Arithmetic growth, accompanied by an approximately six-fold increase of cell mass, continues for 16–20 hr before the stationary phase is finally attained The cessation of cell division coincides roughly with the termination of exponential growth; consequently the increase of cell mass during arithmetic growth is accompanied by a progressive elongation of the cells

(4) Deprivation of streptomycin is not immediately lethal: the size of the viable population, fixed by the cessation of cell division, does not begin to decline until several hours after attainment of the stationary phase[5].

(5) The initial period of residual growth is accompanied by a continuous and systematic change in the macromolecular composition of the cells[5]. In effect, such cells pass through the series of fixed compositional states which occurs in cultures that are grown at different sub-critical levels of streptomycin When the exponential phase of residual growth terminates, the cells have reached a state of extreme compositional imbalance (high RNA content, low protein and DNA contents) which is thereafter maintained throughout the course of arithmetic growth[5]

(6) If a supra-critical level of streptomycin is produced in a population in the course of arithmetical deprived growth, DNA synthesis rapidly regains its normal exponential rate The rate of protein synthesis also increases very shortly after streptomycin is added, but attains the normal exponential level a little more slowly The net synthesis of RNA ceases completely until the RNA-level of the cells has fallen by dilution to its normal value, after which RNA synthesis is resumed at the normal exponential rate[5]

Table 1 EFFECTS OF STREPTOMYCIN DEPRIVATION ON ENZYME SYNTHESIS BY A DEPENDENT STRAIN OF *Escherichia coli*

Functional class	Intracellular location	Enzyme	Specific activity, per cent of normal*
Respiratory	Soluble	Isocitric dehydrogenase	60
	Particulate	DPNH oxidase	60
	Particulate	Succinic dehydrogenase	40
	Gross respiratory activity (Q_{O_2})		45–55
Biosynthetic	Soluble	Threonine deaminase	100
	Soluble	5-Dehydroshikimic reductase	100
	Soluble	Glutamic dehydrogenase	50
	Soluble	Tryptophan synthetase	20
	Particulate	Dihydroorotic dehydrogenase	60
Non-functional under the conditions of cultivation used†	Soluble	Glucose-6-phosphate dehydrogenase	95
	Soluble	β-Galactosidase	5

*The specific activities (on a protein basis) of the enzymes examined remain constant throughout the arithmetic phase of deprived growth, and these values are taken to represent the deprivation-levels The 'normal' specific activity is that found in the cells during their immediately preceding exponential growth in a medium of the same composition, supplied with a supra-critical level of streptomycin

† The strain utilized for these experiments is constitutive for the production of β-galactosidase The medium contained glycerol as the sole source of carbon and energy

(7) Generally speaking, the intracellular state of the RNA in dependent cells growing with a critical level of streptomycin is 'normal', that is, it cannot be distinguished from that in sensitive cells of the same species growing under similar conditions without streptomycin The quantitative distributions of RNA between soluble and ribosomal fractions are closely similar, the sedimentation patterns of the ribosomes are identical, and the isolated ribosomes contain the same amounts of protein and RNA Streptomycin deprivation does not affect the intracellular state of RNA in dependent cells in any of the respects already mentioned Consequently, the increase in the total RNA content of the cells during deprivation simply reflects a very high differential rate of synthesis of *all* forms of cellular RNA[5]

(8) Since deprived cells have an abnormally high content of ribosomes, they also have an abnormally high content of ribosomal protein Hence the cellular content of non-ribosomal protein—broadly speaking, the total complement of enzymes—must fall very substantially as a result of deprivation Analyses of the enzymatic constitution of cells undergoing deprivation show that the decline in level is not uniformly shared by all the enzymes of the cell[5] The synthesis of some is virtually abolished, the synthesis of others is maintained at a rate which is in some cases normal and in some cases reduced, relative to the rate of total protein synthesis A summary of these data (Table 1) shows that the selective effects of deprivation on enzyme synthesis are essentially random No particular class of enzymes is specifically affected, nor is there a specific conservation of those enzymes essential for the maintenance of physiological function

(9) The streptomycin-dependent strain of *E coli* used in our experiments was lysogenic for phage λ, the formation of which can be induced by ultra-violet irradiation Even under conditions of severe streptomycin deprivation (that is, during the arithmetic phase of deprived growth) ultra-violet irradiation of the cells causes induction, with the subsequent lysis of the cells and liberation of active phage particles[5] Hence streptomycin deprivation does not prevent the synthesis by the dependent cell of functional DNA

(10) During the growth of a dependent strain, no change in the streptomycin content of the medium can be detected[5,12] A previous attempt to show streptomycin uptake by the cells[13] gave negative results However, by the use of radioactive streptomycin, we have been able to show that dependent cells bind a small amount[5] This bound streptomycin cannot be removed from the cells by repeated washings with phosphate buffer or with non-radioactive streptomycin The cells were grown in a medium that contained radioactive streptomycin at a concentration which was about double the critical level The amount of radioactivity in the cells was very close to the limits of detection, but with due regard for a large possible experimental error, we can say that under these conditions each cell binds irreversibly some 250,000 molecules of streptomycin Approximately half is extractable by hot water, the remainder is unextractable with cold perchloric acid, but can be completely extracted with hot perchloric acid

Interpretations of the Epiphenomena associated with Dependence

We assume that the dependent cell contains certain specific receptor sites, all of which must be combined with streptomycin for the maintenance of normal cellular function We further postulate that there is a relatively large number of such sites, all of which are identical in structure and in combining power for streptomycin When the medium has a critical streptomycin-level, the streptomycin concentration within the cells is just sufficient to keep all the receptor sites of the growing population combined with streptomycin At sub-critical levels, the internal streptomycin concentration is insufficient to keep all receptor sites combined, and cellular function becomes deranged At supra-critical levels, the streptomycin within the cells is more than sufficient to keep all receptor sites combined, and there is consequently an uncombined pool, the size of which is related to the external concentration This pool is probably loosely held by other cellular constituents, since it cannot exchange with external streptomycin, but can be extracted with hot water

We suggest that the intracellular receptor sites are the ribosomes, and that the firmly bound streptomycin in the cell (that is, the fraction unextractable by hot water) is all attached to ribosomes The number of molecules of streptomycin so bound by each cell is of the order of 100,000, so that there would be relatively few molecules (perhaps only one) bound to each ribosome Let us now see how these assumptions can be used to account for the distinctive epiphenomena which accompany streptomycin deprivation namely, the selective repression of the synthesis of some enzymes, and the over-production of all forms of cellular RNA

According to present concepts about the mechanism of protein synthesis, the ribosomes act as non-specific assembly sites for the manufacture of proteins[14-16] The specific protein which is synthesized at any given time on any given ribosome is determined by the transient attachment to the ribosome of a short-lived 'messenger RNA'[17-21]. The various messenger RNA's are direct gene products, each of which carries in its unique base sequence a copy of the genetic code. This base sequence is presumed to specify the amino-acid sequence of the protein molecule that is assembled on the ribosome.

We propose that mutation to dependence at the streptomycin locus causes a slight modification in the structure of all ribosomes. Although this modification is insufficient to change the gross properties of the ribosomes (as evidence by their normal sedimentation pattern and gross composition), it makes them unable to combine as effectively as ribosomes of the sensitive phenotype with certain messenger RNA's. The defect in combining power can be repaired by the attachment of streptomycin to the ribosome, an event which we shall call activation. The ribosomal structure characteristic of a dependent cell can have three possible effects on the synthesis of specific proteins.

(*A*) If a specific messenger RNA has no affinity for the ribosome of the dependent phenotype, ribosomal activation with streptomycin is indispensable for synthesis of the corresponding protein.

(*B*) If a specific messenger RNA has a reduced affinity for the ribosome of the dependent phenotype, activation with streptomycin is necessary for synthesis of the corresponding protein at maximal rate.

(*C*) If a specific messenger RNA has an unchanged affinity for the ribosome of the dependent phenotype, the rate of synthesis of the corresponding protein is not increased by activation with streptomycin.

After growth with a supra-critical concentration of streptomycin, dependent cells have a fully activated complement of ribosomes, and in addition contain an internal pool of streptomycin, proportional in size to the external concentration. In the absence of external streptomycin, such cells can continue normal growth as long as newly formed ribosomes can be fully activated at the expense of the internal pool. As soon as the pool is exhausted, the rates of synthesis of proteins belonging to categories *A* and *B* begin to fall. This event is translated into exponential growth at ever-diminishing rate, characteristic of the first period of deprivation. The concentration of activated ribosomes in the cells eventually falls to a point at which proteins belonging to category *A* practically cease to be formed, since the probability of the corresponding messenger RNA finding an activated ribosome during its short lifetime has become small. Provided that some of these proteins play an essential catalytic part, growth then becomes arithmetic, as actually observed in the second period of deprivation.

We must now see what interpretation can be offered for the increase in the rate of RNA synthesis which sets in as soon as the streptomycin-level begins to limit the growth-rate. Magasanik and Neidhardt[22,23] have made a detailed study of the control of RNA and protein synthesis during normal bacterial growth. Their findings suggest that growth-rate (which is essentially a reflexion of the rate of total protein synthesis) is determined by the concentration of RNA in the cell. This implies the existence of a feed-back control on RNA synthesis. When a cell is placed under conditions where biosynthetic precursors can be manufactured more rapidly than they can be used for protein synthesis, a larger fraction of the flow of carbon and nitrogen is directed into the synthesis of RNA and of ribosomal protein. This preferential synthesis continues until the limitation placed on the rate of protein synthesis has been overcome, and the cell has achieved metabolic balance under a new set of steady-state conditions.

The growth of dependent cells with sub-critical levels of streptomycin provides the first known instance of an *inverse* relationship between growth-rate and cellular RNA content. How is this apparent anomaly to be explained? As soon as the streptomycin concentration in a dependent cell falls to a level that is no longer sufficient to activate all ribosomes, the rate of protein synthesis begins to fall and biosynthetic precursors begin to accumulate. At this point, the feed-back control mechanism comes into play, and a larger flow of raw materials is directed into the formation of RNA and ribosomal protein. But in the dependent cell under the conditions of streptomycin deprivation, the production of more ribosomes only accentuates the imbalance which it would correct in a normal cell, since it decreases the rate of synthesis of all proteins belonging to categories *A* and *B*. The feed-back control mechanism goes out of regulation, and more and more ribosomes and soluble RNA are made by the cell. The ribosomes can, it is true, serve for the synthesis of proteins belonging to category *C*, but since the proteins of this category constitute an arbitrary array, in terms of their physiological function, the only consequence is to establish a condition of enzymatic imbalance. This affects not only biosynthetic systems, but also the systems responsible for energy-yielding metabolism, as shown by the fact that deprivation can result in a substantial depression of the Q_{O_2} of the cells[5,24].

The ribosomes made during deprivation are potentially fully functional, and the addition of streptomycin to the deprived culture causes their rapid activation. As a consequence, the rate of synthesis of the proteins belonging to categories *A* and *B* immediately rises relative to that of proteins belonging to category *C*. As the enzymatic imbalance in the cells is overcome, exponential growth can be resumed. With the rise in the rate of protein synthesis that follows the re-introduction of streptomycin, the feed-back control on RNA synthesis again comes into normal operation, with the consequence that further net synthesis of RNA is totally repressed until the ribosomal content of the cells has been reduced by growth to a level that once again limits protein synthesis.

The hypothesis which has been outlined here is admittedly complex, and constructed on indirect experimental evidence. Nevertheless, it provides a logically coherent formal explanation for *all* the distinctive epiphenomena associated with streptomycin dependence. It also leads to many explicit predictions, some of which should be susceptible of immediate experimental test.

As emphasized in the introduction, any hypothesis concerning the mode of streptomycin action on bacteria must be deemed inadequate unless it can explain in functional terms the allelism of sensitivity, resistance and dependence. In the remaining section of this essay, we shall accordingly show briefly how this hypothesis can be extended to interpret sensitivity and resistance.

Interpretation of Sensitivity and Resistance

We have postulated that in the dependent phenotype the ribosomes have undergone a slight structural modification which reduces their affinity to a greater or less extent for some messenger RNA's , and further, that a normal affinity can be restored by activation with streptomycin We are therefore led to assume that the sensitive and resistant phenotypes are, in the last analysis, expressions of other structural states of the ribosomes In both these phenotypes, it is obvious that the ribosomes have full affinity for all messenger RNA's in the absence of streptomycin Hence the postulated structural difference between them is of such a nature that its functional consequences are expressed only in the presence of streptomycin We propose that the ribosomes of sensitive cells have a structure that confers on them a very high affinity for streptomycin, and that the resultant combination with streptomycin wholly prevents the attachment of messenger RNA's, thus abolishing protein synthesis The ribosomes of resistant cells, on the other hand, have a structure such that they cannot combine with streptomycin ; hence streptomycin cannot impede the attachment of messenger RNA's, and the synthesis of proteins

The first obvious prediction which can be made on the basis of this suggestion is that sensitive and resistant cells should have markedly different binding capacities for streptomycin This is confirmed by the recent work of Hancock[25] Placed in the presence of a minimal inhibitory concentration of streptomycin, sensitive cells can bind a small quantity irreversibly. The quantity so bound is of the same order as that which we find to be bound by dependent cells placed in the presence of a far higher external streptomycin concentration Resistant cells, on the other hand, bind a relatively insignificant amount of streptomycin[25]. The much lower binding capacity of the resistant cell could, of course, be explained by the assumption that streptomycin cannot enter cells of this phenotype However, if a permeability change is invoked to explain resistance, its allelism with dependence and sensitivity should imply that these two phenotypes are also basically determined by special states of the permeability barrier (presumably the cell membrane) At least for dependence, such a premise is wholly devoid of explanatory value

Another prediction which follows from our hypothesis is that streptomycin should act as a very powerful inhibitor of protein synthesis in cells of the sensitive phenotype, but not in cells of the resistant phenotype This prediction is confirmed by the observations of Erdos and Ullman[26,27]. They have found that in extracts of cells of the sensitive phenotype, low concentrations of streptomycin abolish amino-acid transfer from soluble RNA to protein , this effect of streptomycin is not demonstrable in extracts of cells of the resistant phenotype The demonstration of this differential effect of streptomycin on protein synthesis in cell-free systems is of particular importance, since it reflects a major phenotypic difference between the sensitive and the resistant cell which cannot be attributed to a difference in permeability

One of us (C R S) thanks the National Institutes of Health, U S Public Health Service, for a predoctoral fellowship, during the tenure of which this work was carried out.

Note added in proof Dr D. T. Dubin and Prof B. D. Davis have recently observed that exposure of a sensitive strain of *E coli* to streptomycin results in the intracellular breakdown of the ribosomes (personal communication). This fact is fully consonant with our general hypothesis concerning the primary site of streptomycin action in bacteria, but implies that the special interpretation of sensitivity which we present must be amended streptomycin does not simply inactivate the ribosomes of the sensitive phenotype, it destroys them. Indeed, this amendment makes possible a much more satisfactory explanation of the various epiphenomena associated with sensitivity than does our original interpretation.

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TEMPERATURE CHARACTERISTICS OF BACTERIAL VARIATION IN LEGUME SYMBIOSIS

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THERE is abundant evidence of wide variation within the legume nodule organism *Rhizobium* Cultural[1] and serological[2] characters have been used to define the free-living bacteria, while their behaviour in association with various legumes has been shown to range from non-invasibility[3] or infection but inability to nodulate[4] to all grades of harmony in symbiosis[5–7]

It is usual to describe bacterial variation in symbiosis by comparing the nodulating and nitrogen-fixing properties of various bacterial partnerships of one host genotype under identical growth condi-

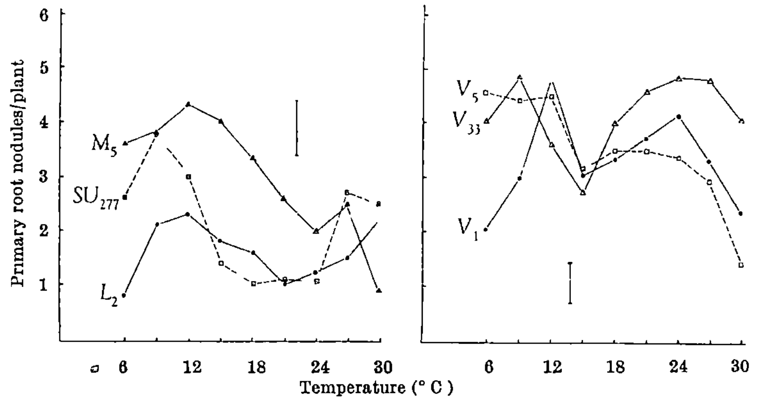

Fig 1 Effect of a range of constantly maintained temperatures on the nodulating performances of selected strains of nodule bacteria on barrel medick (*Medicago tribuloides* Desr) on the left, and purple vetch (*Vicia atropurpurea* Desr) on the right Code numbers refer to strains of *Rhizobium* Plants grown at 400 ft -candles light intensity, 99 per cent confidence limits are included

tions The purpose of this article is to report a new type of symbiotic variation where reaction to a range of environmental conditions is used to determine the physiological characteristics of host plant and invading bacterium

Purple vetch (*Vicia atropurpurea* Desf) and barrel medick (*Medicago tribuloides* Desr) were paired with selected strains of nodule bacteria of Australian or British origin Each host–bacterium combination was grown in culture rooms maintained at a series of constant temperatures (6–30° C) The nitrogen-free sand culture technique was used as described previously[8] Each growth series received 16 hr /day of fluorescent lighting giving intensities of 400, 600, or 800 ft -candles at sand-level in the culture vessels

In work on nodulation response to temperature, the collection of the plants was timed to coincide with the completion of nodulation of the seedling primary root Average nodule number in the 50-plant sample from the 5 replicate pots of a treatment was determined as a measure of initial response to infection Clearing roots in chloral hydrate facilitated the counting of the small nodules developed by some bacterial strains in unfavourable environments Fig 1 illustrates typical nodulation performances in vetch and medick All nodulation curves show optima at or near the extremes of growth temperatures, and comparisons of the relative magnitudes of these pairs of optima would indicate that certain bacteria (for example, V_{33} and L_2) are particularly effective in forming nodules at high temperatures Other bacterial strains (M_5 and V_5) illustrate the opposite effect Several investigations[8–10] have suggested that an existing nodule may exert inhibitory influences on its younger companions, and such a hypothesis might interpret the general depression of nodulation in the middle of the temperature-range as a direct consequence of a more active production of inhibitory substances by the first-formed nodules Similarly, a specific bacterial contribution to the production of inhibitory substances might account for the different temperature characteristics recorded in the nodulation of each partnership of a host plant

Results of a preliminary experiment on the interaction of light and temperature on the nitrogen fixation of bacterial associations of vetch and medick are shown in Fig 2 The values quoted refer to the first 5 weeks of nitrogen fixation, commencing when nodules first acquired visible hæmoglobin pigmentation In both species nitrogen fixation shows a well-defined maximum at 24° C , with symbiosis in barrel medick clearly more tolerant of high temperatures than in purple vetch (compare nodule formation, Fig 1) The higher light intensity benefits fixation particularly at low or medium temperatures The two barrel medick associations offer evidence of a bacterial factor influencing variations in symbiotic activity over the temperature-range Thus the L_2 strain shows significantly greater fixation at lower temperatures, while the reverse obtains at temperatures at or above 24° C These differences are reproduced in both light conditions and, like the initial infection responses, appear to represent minor variations on the general pattern of host-response to temperature

Table 1 records nitrogen analyses of dry weights for typical bacterial associations of vetch and medick harvested after 5 weeks of nitrogen fixation at 600 ft -candle light intensity

A specific inhibition of fixation processes by growth temperatures at or above 27° C is suggested in both associations from the low values for percentage nitrogen in dry weight By contrast, the depression of total fixation at or below 21° C (see Fig 2) is seen to be associated with the higher values for nitrogen percentage, suggesting that growth processes other than nitrogen fixation are retarded in conditions of lower temperatures

The two species studied here may be grouped with those described by Mes[11] (*Vicia sativa*, *Pisum sativum*,

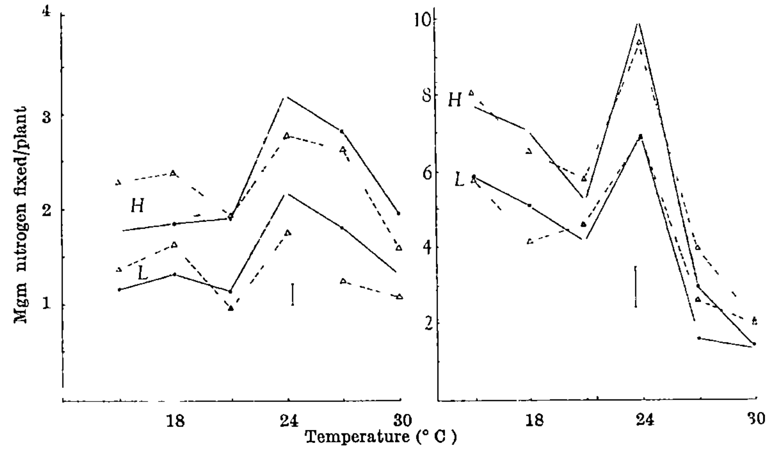

Fig 2 The effects of a range of constantly maintained temperatures and two light intensities on nitrogen fixation by bacterial associations of medick (left) and vetch (right) Code numbers refer to strains of *Rhizobium* 99 per cent confidence limits are derived from the 50-plant sample (5 replicate pots) of each treatment *H*, 800 ft -candles, *L*, 400 ft -candles light intensity, for medick, ●, BM_{101}, , L_2, Δ for vetch, ●, V_{33}, Δ, V_1

Table 1 DRY WEIGHT ANALYSIS OF NODULATED VETCH AND MEDICK PLANTS GROWN IN NITROGEN-FREE CULTURE AT A RANGE OF CONSTANT TEMPERATURES

Growth temp (° C)		15	18	21	24	27	30
Nitrogen fixed as a % of plant dry weight*	Purple vetch—*Rhizobium* V_5	4 4	4 7	4 8	4 1	3 8	2 9
	Barrel medick—*Rhizobium* BM_{104}	3 9	4 3	4 4	3 9	3 7	3 0

* Corrected to exclude nitrogen furnished by cotyledons

Lupinus luteus) and Meyer and Anderson[12] (*Trifolium subterraneum*) as showing a specific suppression of symbiotic fixation at high growth temperatures Mes[11,13] has also described the opposite condition, where nitrogen fixation is specifically depressed by low temperatures (for example, in *Arachis hypogaea*, *Stizolobium deeringianum*, and *Glycine max*) The present work on *Medicago tribuloides* would suggest the possibility of selecting bacterial strains to combat such limitations of symbiosis in unfavourable growth conditions

Further experiments are being conducted to investigate symbiotic activity where host plants are subjected to a range of further environmental factors, including seasonal and diurnal thermo-periodicity and the presence of varied amounts of combined nitrogen in the rooting medium

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CRYSTALLIZATION OF CYTOCHROME b_2 FREE OF THE DEOXYRIBONUCLEIC ACID COMPONENT

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CRYSTALLINE cytochrome b_2 was isolated in 1954 by Appleby and Morton[1,2], who showed that it was identical with L(+)-lactate dehydrogenase of baker's yeast The crystalline enzyme is a flavohæmoprotein[1,3,4] It contains about 5 per cent of a deoxyribonucleic acid (DNA)[5,6] which appears to be homogeneous and which has a molecular weight of 10,000–12,000 as estimated by end-group analysis[7] The DNA-component may be dissociated from the flavohæmoprotein by dialysis against a 70 per cent saturated solution of ammonium sulphate[6,7] without any substantial change in the specific dehydrogenase activity of the enzyme[3,6] The flavohæmoprotein containing the DNA-component is now known as type I-cytochrome b_2, and that free of the DNA-component as type II-cytochrome b_2 (ref 8) As observed by Appleby and Morton[6], the DNA-free enzyme does not crystallize when dialysed for short periods under similar conditions to those used for crystallization of the DNA-containing enzyme

Unless otherwise specified, all procedures described in this article were carried out under essentially anaerobic conditions with oxygen-free solutions in a cold room at 0°–2° C (see ref 3)

To separate the DNA-component from type I-cytochrome b_2, a solution containing about 26 mgm /ml of three-times crystallized protein[3] was dialysed successively (for 15–24 hr in each case) against 50 per cent and 70 per cent saturated solutions of ammonium sulphate at *p*H 8 0 (refs 6 and 7) The precipitated protein was collected by centrifuging and washed with a 70 per cent saturated solution of ammonium sulphate at *p*H 8 0 The combined supernatants contained the protein-free deoxyribonucleic acid component The DNA-free flavohæmoprotein (type II-cytochrome b_2) was dissolved in a minimum amount of 0 5 *M* sodium lactate / 0 1 m*M* ethylenediamine tetraacetate / 0 04 *M* tetrasodium pyrophosphate, *p*H 6 8, centrifuged to remove a small amount of precipitate, and then dialysed against 0 05 *M* sodium lactate / 0 1 m*M* ethylenediamine tetraacetate / 1 m*M* magnesium sulphate at *p*H 6 8 There was no change in the dialysing solution for about 30 hr , at about which time turbidity appeared and increased gradually as dialysis was continued After several days, large pink crystals were observed adhering to the sides and bottom of the 'Visking' cellulose sac After 24 days, the crystals were collected and washed by allowing them to settle under gravity after suspending in 0 05 *M* sodium lactate / 0 1 m*M* ethylenediamine tetraacetate / 1 m*M* magnesium sulphate at *p*H 6 8 The size and density of the crystals created difficulties in collection and transfer ; centrifuging was unnecessary The shape of the larger crystals was recognizable without aid of a hand lens

Whereas the crystals of type I (DNA-containing)-cytochrome b_2 obtained under similar conditions belong to the tetragonal system, and are probably shallow bi-pyramids which appear as square plates when lying flat (see refs 1 and 3), the crystals of type II (DNA-free)-cytochrome b_2 belong to the hexagonal system and consist of hexagonal prisms with pyramids at both ends (Fig 1) Fig 2 shows measurements on average crystals of type I- and type II-cytochrome b_2 The largest crystals of type II-cytochrome b_2 were about three times the size of that illustrated in Fig 2, but they were somewhat fragile and difficulty was experienced in handling them without chipping the faces or the tips of the pyramids Crystals of type I-cytochrome b_2 are weakly and those of type II-cytochrome b_2 strongly anisotropic, especially when oriented in certain directions

A pellet of crystals of type II-cytochrome b_2 appeared darker in colour than a similar pellet of type I crystals, probably due to the difference in size and structure of the crystals Type II crystals

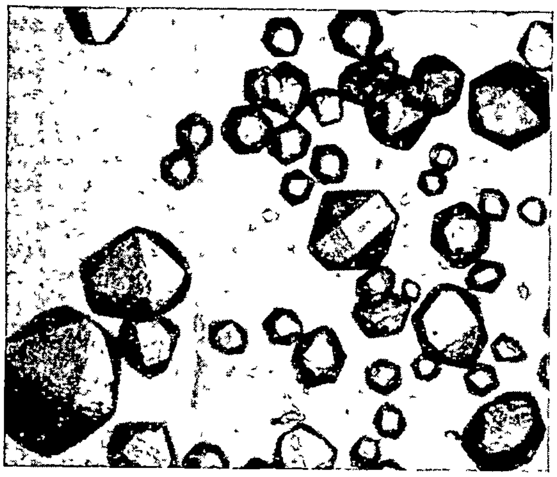
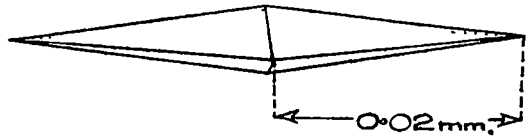
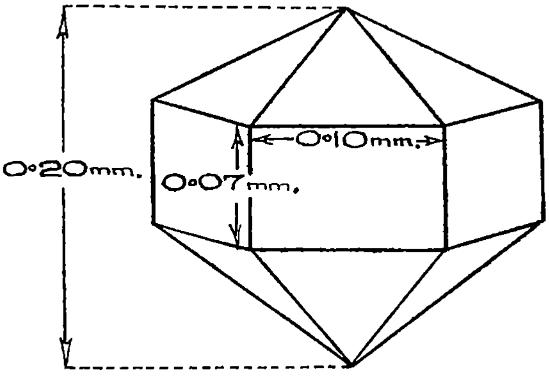


Fig 1 Crystals of type II (DNA-free)-cytochrome b_2 (× 125)



Type I



Type II

Fig. 2 Dimensions of representative crystals of type I (DNA-containing)- and of type II (DNA-free)-cytochrome b_2 obtained under similar conditions

dissolved slowly in 0 5 *M* sodium lactate / 0 04 *M* tetrasodium pyrophosphate / 0·1 m*M* ethylenediamine tetraacetate, *p*H 6 8. On dialysis of this solution (containing about 35 mgm protein/ml), under the conditions used for first crystallization, crystallization was complete in a few hours, the crystals had the same form as in Fig 1, but they were very much smaller All crystals, both in the initial and recrystallized material, appeared to be perfectly formed A number of preparations of crystalline type II-cytochrome b_2 have been produced as described

Table 1 shows comparative properties of solutions of crystalline type I- and type II-cytochrome b_2. The enzymic and physico-chemical properties are very similar, but the absence of the DNA-component from type II material causes a change in the position of the maximum absorption and in the corresponding extinction value in the ultra-violet region of the spectrum The absorption maximum at 269 5 mμ in type II-cytochrome b_2 appears to be due to the contribution of the riboflavin phosphate prosthetic group (which has an absorption maximum at 266 mμ) to the absorption due to the aromatic amino-acids, the flavin-free hæmoprotein derivative has a maximum at 278 mμ (refs 8 and 9)

The two different crystalline forms of type I- and of type II-cytochrome b_2 are distinctive features of the DNA-containing and of the DNA-free enzyme When a solution containing both types of cytochrome b_2 was dialysed as described for crystallization, both tetragonal plates (type I) and hexagonal prisms (type II) appeared in the precipitate The larger and denser type II crystals were separated from type I crystals by forming a layer of a suspension of mixed crystals (in 0 05 *M* sodium lactate/0 1 m*M* ethylenediamine tetraacetate, *p*H 6 8) over 0 5 *M* sucrose / 0 05 *M* sodium lactate / 0·1 m*M* ethylenediamine tetraacetate at *p*H 6 8 and centrifuging at about 100*g* for 5 min The type I crystals were almost completely retained in the uppermost layer, and type II crystals appeared in the precipitate Whereas the solution of mixed type I and type II material had absorption maxima at 265 and 269 mμ, the solutions of the separated crystals showed the distinctive maxima of type I (265 mμ) and of type II (269 5 mμ) material

The two crystal types may also be differentiated by fluorescence microscopy When stained with an appropriate concentration of aqueous acridine orange, type I crystals show a strong green fluorescence due to the presence of the DNA component. The fluorescence is distributed uniformly throughout the crystal. With an excess of dye, an orange-red fluorescence is obtained, as with deoxyribonucleoproteins of the cell nucleus However, type II crystals show no distinctive fluorescence on staining with acridine orange under the same conditions

Type I (DNA-containing)-cytochrome b_2 is regarded as the native, naturally occurring form of the

Table 1 COMPARATIVE PROPERTIES OF CRYSTALLINE TYPE I- AND TYPE II-CYTOCHROME b_2

Property	Type I	Type II
Specific enzymic activities*		
with heart-muscle cytochrome *c*	5,000	5,500
2 6-dichlorophenol indophenol	1,600	1,700
ferricyanide	6,200	6,100
2 6-dichlorophenol indophenol plus phenazine methosulphate	3,800	4,100
Spectral properties of lactate-reduced compound†		
$\frac{\gamma\text{-band (423 5 m}\mu)}{\alpha\text{-band (557 0 m}\mu)}$	6 3	6 3
$\frac{\beta\text{-band (528 m}\mu)}{\alpha\text{-band (557 0 m}\mu)}$	0 5	0 5
$\frac{\gamma\text{-band (423 5 m}\mu)}{\delta\text{-band (328 m}\mu)}$	4 6	4 0
$\frac{\text{ultra-violet band (265 or 269 5 m}\mu)\ddagger}{\gamma\text{-band (423 5 m}\mu)}$	0 86	0 55
Chemical properties		
DNA	5–6%	absent
$\frac{\text{molecules of riboflavin phosphate}}{\text{molecules of protohæm}}$	1	1
$S^{\circ}_{20,w}$ (Svedbergs)§	8 45	8 17

* Enzymic activities were estimated with DL-sodium lactate at 25° C, as described by Appleby and Morton[3], from changes in extinctions measured for the first minute of the reaction The concentration of 2 6-dichlorophenol indophenol was 0 07 m*M*, and of phenazine methosulphate was 0 07 per cent, the reactions with these dyes were carried out at *p*H 8 0 Values are for two preparations prepared and tested under identical conditions, but they are not necessarily the maximum values for either type of preparation

† The ratios are based on extinctions measured at the appropriate absorption maxima with an Optica Model *CF*4 grating spectrophotometer, see Morton[10]

‡ The maximum absorption is at 265 mμ for type I and at 269 5 mμ for type II material

§ $S_{20,w}$ was 7 74 for type I-cytochrome b_2 (55 μ*M* hæm) and 7 40 for type II-cytochrome b_2 (174 μ*M* hæm), the concentration dependence of the sedimentation coefficients are given in ref 11 enzyme A number of derivative proteins have now been prepared[8,9] Type II (DNA-free)-cytochrome b_2 is such a derivative, but the name 'cytochrome b_2' is retained for this material as well as for type I material since both have similar dehydrogenase activity The nomenclature of other non-enzymically active derivatives is discussed elsewhere[8].

We thank the Effront Yeast Co Pty, Ltd, Melbourne, for the gifts of baker's yeast for this work, B Palk for microphotography and R Connolly for assistance in the preparation of type I-cytochrome b_2 and in the determination of the sedimentation coefficients

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POSSIBLE CAUSES FOR PRODUCTION OF ISOLEUCINE BY *STREPTOMYCES RIMOSUS*

By Dr. I. HORVÁTH, I. GADÓ and A. SZENTIRMAI

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It was observed that *Streptomyces rimosus* (strain *BS*21) when cultivated on media containing threonine[1] or α-keto-butyric acid[2] produces both isoleucine and acetylethylcarbinol According to refs 3–5, the biosynthesis of isoleucine and valine appears to proceed along the same pathway In spite of this fact no production of valine could be demonstrated in media containing serine or pyruvic acid Work described here was carried out in order to elucidate the possible causes of this phenomenon

Details concerning the methods and inocula used were published earlier[6,7] Also the media used in the present work were prepared similarly to those used earlier: 1 5 per cent glucose, 0 013 per cent KH_2PO_4, 0 3 per cent sodium chloride, 0 002 per cent 'Tween 80', and 0 5 per cent glycine or any other appropriate amino-acid in an amount corresponding to that of glycine by amino nitrogen content The *p*H before sterilization was 7 0. The media were prepared with tap-water In experiments with washed mycelia two-day-old cultures were used Washing was performed with distilled water The final dilution of mycelia in the incubation mixture was 3–5 mgm /ml dry mycelia

The demonstration of the enzyme synthesizing α-acetolactic acid was as follows 2-ml samples contained 5–10 mgm washed mycelia (dry weight), 0 1 m mole pyruvic acid, 0 02 m mole Na_3AsO_4, 0 3 m mole magnesium sulphate The 2-ml. samples were incubated at *p*H 7 0 for 2 hr at 28° C and the reaction was stopped by the addition of 0 2 ml 50 per cent (v/v) sulphuric acid After standing for 2 hr acetoin was steam-distilled from the mixture and determined as described in ref 8 When washed mycelium walls were used, arsenate was omitted and replaced by 0 02 m mole *p*H 7 0 phosphate buffer

In media where conditions favourable for production of isoleucine were present 0 7 mgm /ml of valine was found to have an inhibitory action on the production of isoleucine This inhibition appeared to be specific for valine as no other amino-acid (in similar concentrations of amino nitrogen) was found to have such an effect Isoleucine itself in concentrations of 0 8 mgm /ml also failed to inhibit production, while the usual amount of the same substance was produced in addition to the level added

When examining the production of isoleucine by washed mycelia, it was found that the rate and extent of production were very much influenced by the medium used for growing mycelia (Fig 1). Mycelia grown on a threonine-containing medium immediately started isoleucine production in the presence of threonine and glucose The production was not influenced by 100 μgm /ml final concentration of chloramphenicol When mycelia grown on a glycine-containing medium were used, the production of isoleucine started only during the fourth hour of incubation and could be completely inhibited by the addition of chloramphenicol (100 μgm /ml).

In incubation mixtures containing serine and glucose the mycelia grown on a threonine-containing medium were found to produce valine The production was remarkably enhanced by the addition of chloramphenicol (100 μgm /ml) (Fig 2) A similar production of valine could never be observed when mycelia grown on media containing any other amino-acid tested were used

Strain *BS*21 of *Streptomyces rimosus* contains a high level of constitutive serine- and threonine-deaminase (dehydrase) The next step of isoleucine synthesis from threonine is accomplished by the enzyme synthesizing α-acetolactic acid[9–16] We succeeded in

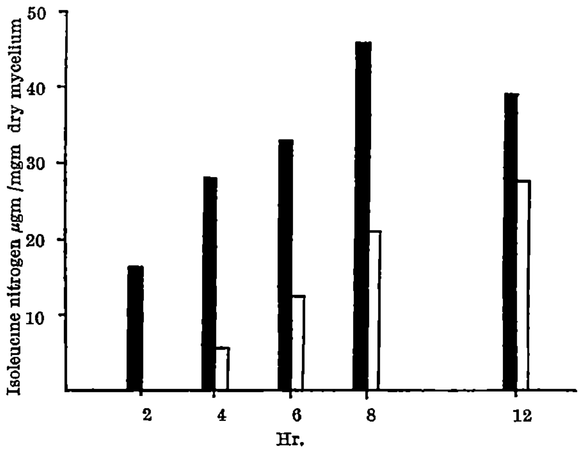

Fig 1 Isoleucine production by washed mycelia
The incubation mixture contained 1 per cent glucose, 0 5 per cent DL-threonine, 0 3 per cent sodium chloride, 0 6 per cent KH_2PO_4 (*p*H adjusted to 7 0 by the addition of sodium hydroxide) Mycelium (dry weight) 4 mgm./ml.

Blacks columns, mycelia grown on threonine-containing medium, white columns, mycelia grown on glycine-containing medium

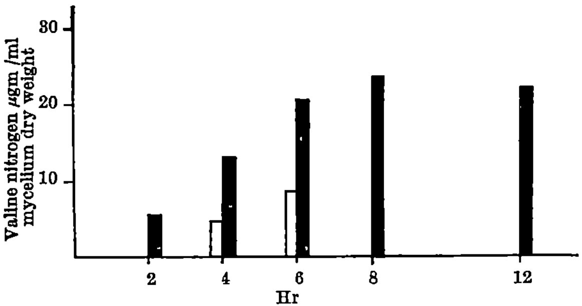

Fig 2 Valine production by washed mycelia
The incubation mixture contained 1 per cent glucose, 0 5 per cent DL-serine, 0 3 per cent sodium chloride, 0 6 per cent KH_2PO_4 (pH adjusted to 7 0 by the addition of sodium hydroxide) Mycelium (dry weight) 5 mgm /ml
White columns, no chloramphenicol, black columns, 100 μgm /ml chloramphenicol added

demonstrating this enzyme in the presence of arsenite or arsenate and we found that the activity of the enzyme exhibited a five-fold increase when the micro-organism was grown on media containing threonine or α-keto-butyric acid (Table 1) Depression of this enzyme by valine in *E coli* and *A aerogenes* has been observed[12,14] In our experiments such an effect of valine could be demonstrated similarly to the observations obtained with *Neurospora crassa*[16] The enzyme was found to be bound to the surface of mycelia and the activity of mycelium walls (washed with 0 3 m.mole of magnesium sulphate, pH 7 0) obtained by ultrasonic disintegration was the same as that of intact mycelia The former, however, was also active in the absence of arsenite or arsenate

Table 1 EFFECT OF THE MEDIUM ON THE ACETOLACTIC ACID SYNTHESIZING ENZYME CONTENT OF MYCELIA

Medium* (per cent)	μgm acetoin/mgm mycelium/hr
Glycine 0 5	3 6 (2 4– 4 8)
Glycine 0 5 + pyruvic acid 0 1	2 4 (1 8– 3 6)
DL-serine 0 7	3 0 (2 4– 4 2)
DL-threonine 0 79	13 2 (11 4–19 2)
DL-threonine 0 71 + DL-alanine 0 06	13 8 (10 2–20 4)
DL-threonine 0 71 + DL-valine 0 08	15 6 (12 0–22 2)
Glycine 0 5 + α-keto-butyric acid 0 1	10 8 (9 6–12 0)

* Basal medium 1 5 per cent glucose, 0 013 per cent KH_2PO_4 0 3 per cent sodium chloride, 0 002 per cent 'Tween 80'

The results obtained can be explained as follows · The α-keto-butyric acid produced from threonine or added to the medium enhances inductively the activity of acetolactic acid and aceto-oxybutyric acid synthesizing enzyme and probably also that of other enzymes involved in valine and isoleucine synthesis, thus resulting in an increased production of these two amino-acids Pyruvic acid did not seem to have a similar effect The cleavage of these two amino-acids with a branching carbon chain is inductively enhanced by valine, resulting in the inhibition of isoleucine production, while chloramphenicol enhances for the same reason the production of valine by mycelia grown on a threonine-containing medium The phenomenon observed, namely, the fact that an intermediary metabolite produced by one of the two amino-acids with similar pathways of biosynthesis or cleavage is able to enhance the biosynthesis or cleavage of both amino-acids is probably one of the general mechanisms controlling amino-acid metabolism

Further details of the present work will be published in *Acta Microbiologica Hungarica*

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A SUBCELLULAR COMPONENT OF *CORYNEBACTERIUM DIPHTHERIAE* HAVING THE CAPACITY TO BIND ANTI-TOXIN

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ATTEMPTS to elucidate the intracellular events occurring during biosynthesis of toxin revealed that a subcellular component having the capacity to bind anti-toxin was formed in toxin-producing diphtheria cells, while no such binding was detectable with a similar component derived from the cells of non-toxinogenic, non-lysogenic strains *C*7 and *C*4 of *C diphtheriae* Examinations of the anti-toxin-binding capacity of various subcellular fractions by using a quantitative absorption test showed that the highest activity was always found in the sediment obtained by centrifugation at 14,500–100,000*g*, but there was no activity in the cell-wall preparation

In this work we used a substrain of toxinogenic Park Williams No 8, the toxinogenic strains *C*7(β) and *C*4(β) of *C diphtheriae* and the *C*7 and *C*4 indicator strains from which they had been derived by infection with β-phage[1,2]

These organisms were grown at 35° C with shaking in Pope's medium[3] under an optimal iron condition (0 08 μgm iron/ml) for production of toxin by the strains of Park Williams No 8, *C*7(β) and *C*4(β) After shaking for 20 hr , toxin content of the culture filtrates of these organisms was found to be 180, 18 and 12 Lf /ml , respectively, while no toxin was detectable in those of *C*7 and *C*4 When shaking was complete, the organisms were centrifuged out of the growth medium, washed in ribonucleoprotein buffer[4] three times, made up into thick suspension in the buffer and exposed to sonic oscillation at 10 kc /s

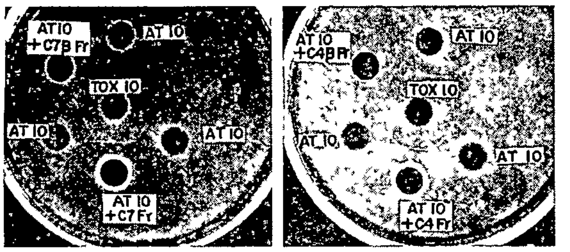

Fig 1 Agar-gel precipitation pattern demonstrating anti-toxin binding by fraction/14,500–100,000 of *C7(β)* and of *C4(β)* of *C diphtheriae*
TOX 10, 0 3 ml of 10 Lf /ml. of crystalline diphtheria toxin, AT 10, 0 3 ml of 10 units/ml of a horse standard anti-toxin, AT 10 + *C7βFr*, *C4βFr*, *C7Fr* or *C4Fr*, 0 3 ml of the supernatants of the mixtures of 0 6 ml of 10 units/ml anti-toxin and of 100 mgm (dry weight) of fraction/14,500–100,000 of each organism

for 30 min The whole disintegrates were then subjected to differential centrifugation as follows the supernatants obtained from the disintegrates by centrifugation at low speed (5,000*g* for 30 min) were centrifuged at 14,500*g* for 30 min and the supernatants thus obtained, which were still slightly turbid, were finally centrifuged at 100,000*g* for 60 min in a Spinco Model L The sediments precipitated at 14,500*g* and at 100,000*g* and washed three times in ribonucleoprotein buffer by centrifugation at the same gravity were referred to as fraction/5,000–14,500 and fraction/14,500–100,000, respectively Purified cell walls were provided by Prof S Kotani, of the Department of Bacteriology, Nara Medical College, these were prepared from toxin-producing cultures of Park Williams No 8 strain by the method described by Kitaura *et al* [5] in his laboratory.

Anti-toxin-binding capacity of these fractions and cell walls of Park Williams No 8 cells were determined, using a quantitative absorption test Thus, appropriate amounts of the materials corresponding to 70–400 mgm of the dry weight were suspended in 15 ml saline containing 15 units/ml of a standard horse diphtheria anti-toxin, incubated at 37° C for 15 min and the supernatants obtained by centrifugation were measured for the residual content of anti-toxin by flocculation with 15 Lf diphtheria toxin Anti-toxin-binding was also observed qualitatively with fraction/14,500–100,000 of each organism, using Ouchterlony's agar double-diffusion technique[6] In this experiment, the supernatants obtained by centrifugation from the mixtures of the fraction and anti-toxin which had been incubated at 37° C for 15 min , or the mixtures themselves were set against crystalline diphtheria toxin (3,300 Lf /mgm) prepared by the method of Katsura *et al* [7]

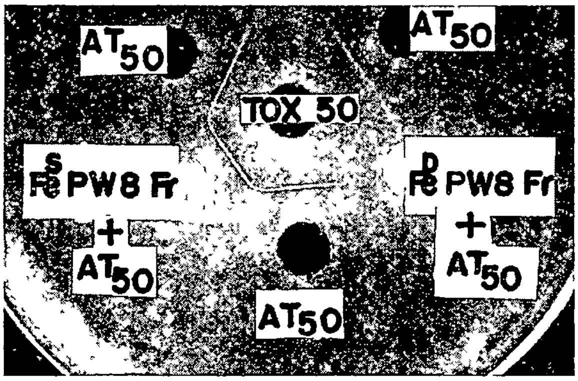

Fig 2 Agar-precipitation pattern demonstrating the absence of anti-toxin-binding capacity in the fraction/14,500–100,000 derived from Park Williams No 8 cells grown in the medium containing sufficient iron to suppress the toxin production
TOX 50, 0 3 ml of 50 Lf /ml of crystalline diphtheria toxin, AT_{50}, 0 3 ml of 50 units/ml of a horse standard anti-toxin, $Fe^{D}PW8Fr + AT_{50}$, 0 3 ml of the mixture of 0 6 ml of 50 units/ml anti-toxin and of 125 mgm (dry weight) of fraction/14,500–100,000 of cells of the cultures grown in an iron-deficiency for optimal toxin production
$Fe^{S}PW8Fr + AT_{50}$, 0 3 ml of the mixture of 0 6 ml of 50 units/ml anti-toxin and of 125 mgm (dry weight) of fraction/14,500–100,000 of cells of the cultures grown in the medium containing sufficient iron to suppress the toxin production

As can be seen from Fig 1, while a slight difference was observed in the appearance of precipitation of toxin with anti-toxin regardless of the presence of *C7* or *C4* fractions, no precipitation lines were formed between the toxin well and those for anti-toxin plus the *C7*(β) or *C4*(β) fractions, indicating an absence or a great decrease of anti-toxin in the supernatants of the latter mixtures

Table 1 shows the anti-toxin-binding capacity of various subcellular fractions obtained from Park Williams No 8 cells It may be seen that fraction/14,500–100,000 can bind as much as 0 68 unit of anti-toxin/mgm of the dry weight No activity was found in the purified cell wall preparation used Of particular interest was the observation that similar fractions from the cells of Park Williams No 8 strain which was grown in Pope's medium to which was added sufficient iron (8 μgm iron/ml) to suppress production of toxin showed no capacity to bind anti-toxin, as illustrated in Fig 2

Table 1 ANTI-TOXIN-BINDING CAPACITY OF VARIOUS CELLULAR FRACTIONS OF A PARK WILLIAMS NO 8 STRAIN OF *C diphtheriae*

Fractions	Anti-toxin absorbed (units/gm dry weight) Deficient iron*	 Sufficient iron†
Fraction/5,000–14,500	256	0
Fraction/14,500–100,000	681	0
Purified cell wall	0	—

* Fractions of Park Williams No 8 cells from the cultures grown in an iron-deficiency for optimal toxin production
† Fractions of Park Williams No 8 cells from the cultures grown in the medium containing sufficient iron to suppress toxin production

Preliminary work showed that 1 mgm (dry weight) of thoroughly washed fraction/14,500–100,000 of toxin-producing Park Williams No 8 has no lethal action in guinea pigs, but gives the intradermal reaction to rabbits which is equivalent to 20 m r d with a standard diphtheria toxin The reaction can be neutralized by anti-toxin Recent experiments have also shown that 0 8–5 0 units of anti-toxin can be formed in rabbits by immunizing with 5 mgm (dry weight) of the washed fraction in incomplete Freund's adjuvants Incidentally, fraction/14,500–100,000 can retain either the antigenicity or the original capacity to bind anti-toxin even after repeated washing (more than five times) in the ribonucleoprotein buffer or in other physiological buffers This clearly indicates that the active principle must be in a bound form Problems of relating subcellular structure to anti-toxin-binding capacity and the significance of the anti-toxin-binding principle in biosynthesis of diphtheria toxin still await further investigation

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LETTERS TO THE EDITORS

GEOCHEMISTRY

Uranium in the Sikhote-Alin Meteorite and its Relation to the Lead Method of Age Determination

The ages of lead of Earth and of meteorites are commonly based on the assumption that when these bodies were formed they contained lead of the same isotopic composition[1,2]. The present differences in the isotopic compositions of the lead in meteorites and also between common terrestrial lead and meteorite lead are due to radiogenic contributions from uranium and thorium since that time.

Recently, Starik *et al.*[3] measured the ratios of lead isotopes and the absolute amount of lead in a number of iron meteorites and found that the iron meteorites can be divided into two groups. One group has lead of 'primeval' isotopic composition, that is, the same as that measured by Patterson *et al.*[4] in the Canon Diablo meteorite. The other group has lead that is rich with isotopes lead-206, -207 and -208, relative to the isotope lead-204; this lead has an isotopic composition quite similar to that of common terrestrial lead (see Table 1). Reed *et al.*[5] have measured the uranium content of several iron meteorites and have found values in the range 10^{-10}–10^{-12} gm./gm. None of these meteorites, with the possible exception of Henbury, has lead enriched in the radiogenic isotopes. There is a dispute over the lead in Henbury[1,3].

Table 1. ISOTOPIC COMPOSITION OF LEAD IN THE SIKHOTE-ALIN AND CANON DIABLO METEORITES

Iron meteorite	Lead concentration	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$
Sikhote-Alin	3×10^{-8}	17·89	15·84	38·19
	3×10^{-8}	17·55	15·60	37·97
Canon Diablo	—	9·46	10·34	29·44

In order to test the assumption that the excess of lead-206 is due to the radioactive decay of the isotope uranium-238, of lead-207 to that of uranium-235, and of lead-208 to that of thorium-232, we searched for uranium-235 in the Sikhote-Alin meteorite, which is a meteorite that clearly[3] has a lead-207 excess. We found less than $1{\cdot}4 \times 10^{-13}$ gm./gm. of uranium-235, corresponding to less than 2×10^{-11} gm./gm. of normal uranium. This amount is more than a factor of one hundred too small to account for the excess lead-207 in the Sikhote-Alin meteorite in 5×10^{9} years.

We irradiated two 7-gm. samples of Sikhote-Alin for 9 hr., and one uranium standard for 2 hr., in a pneumatic tube of the Massachusetts Institute of Technology reactor. The flux was 7×10^{12} neutrons/cm.^{2} sec. We measured the fission product, xenon-135, which has a 9·2 hr. half-life. Since the xenon-135 follows iodine-135, which has a 6·7 hr. half-life in the fission chain, we waited about 12 hr. after the irradiation before processing the samples for xenon. The fission product xenon-133 has been used to measure uranium in stone meteorites[6]. In order to remove possible uranium contamination, we dissolved away 10 per cent of the irradiated samples in nitric acid. We placed the remainder in an alumina crucible in our gas extraction system and melted it by induction heating. The sample was kept molten for 1 hr. in the presence of carrier gas. The carrier used with the first sample and with the uranium standard was xenon, the carrier used with the second sample was a 50–50 mixture of xenon and argon. At the end of the heating, only several small droplets remained in the crucible, the rest of the material had evaporated to the walls of the crucible and the furnace. The hydrogen was pumped away from the gas by its diffusion through a hot palladium thimble. The remaining gas was kept over hot calcium (550° C.). The xenon yield from the first sample and from the uranium standard was 100 per cent. This xenon was taken from the calcium and put into a Geiger counter. For the second sample the gas was removed from the calcium by adsorption on charcoal at the temperature of liquid nitrogen. Argon was removed from the charcoal at 0° C. and xenon removed at 160° C. The xenon yield was 65 per cent; this xenon was added to a Geiger counter. The Geiger counter was removed from the extraction line and placed in an iron shield; the background rate was 15–20 counts/min. The uranium standard consisted of $3{\cdot}0 \times 10^{-8}$ gm. of normal uranium ($2{\cdot}1 \times 10^{-10}$ gm. of uranium-235) in the form of uranyl nitrate evaporated from solution on an aluminium foil. This sample was placed with 7 gm. of iron in an alumina crucible and subjected to the identical experimental procedure that was used with the first Sikhote-Alin sample. The xenon from the standard when first counted 6 hr. after the melting gave 315 ± 5 counts/min.; this activity, which we followed for several days, clearly decayed with a 9·2 half-life to 35 ± 5 counts/min. The amount of long-lived activity agrees with the amount of xenon-133 expected. These results correspond to 420 ± 20 counts/min. of xenon-135 at the time of melting. The gas from the first Sikhote-Alin sample gave 70 counts/min. of a long-lived activity; there was less than 15 counts/min. of 9-hr. activity at the time of melting. This negative result corresponds to less than $2{\cdot}8 \times 10^{-13}$ gm./gm. of uranium-235 or 4×10^{-11} gm./gm. of normal uranium. The long-lived activity was probably argon-37, since this amount of argon-37 could result from 3×10^{-8} c.c./gm. of argon-36 or 5×10^{-7} gm./gm. of calcium in the sample. In the xenon fraction from the second sample the activity was within 5 counts per minute of background. This negative xenon-135 result corresponds to less than $1{\cdot}4 \times 10^{-13}$ gm./gm. of uranium-235 or 2×10^{-11} gm./gm. of normal uranium.

The lead-207 excess in the Sikhote-Alin meteorite is $2{\cdot}2 \times 10^{-9}$ gm./gm. A period of 10^{10} years is required for this lead-207 excess to result from $1{\cdot}5 \times 10^{-13}$ gm./gm. of uranium-235. There is less than $1{\cdot}4 \times 10^{-13}$ gm./gm. of uranium-235 according to our measurements. Such a large age seems unreasonable since it would require that the uranium produced in nucleosynthesis were practically pure uranium-235. The uranium and lead contents of the Sikhote-Alin meteorite are inconsistent with the common assumption made in determining lead ages for Earth and for meteorites.

Starik *et al.* have postulated separate origins, each with a different 'primeval' isotopic lead composition, for the two groups of iron meteorites. This postulate is consistent with the uranium results but makes ambiguous the interpretation of lead ages of meteorites and of Earth

One of us (D E F) is grateful to the U S Atomic Energy Commission for financial support, and to the Smithsonian Astrophysical Observatory for hospitality during the period in which this work was undertaken

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GEOPHYSICS

Palæomagnetism of Hawaiian Lava Flows

PALÆOMAGNETIC investigations of volcanic rocks extruded in various parts of the world during the past several million years have generally revealed a younger sequence of lava flows magnetized nearly parallel to the field of a theoretical geocentric axial dipole, underlain by a sequence of older flows with exactly the opposite direction of remanent magnetization A 180-degree reversal of the geomagnetic field, occurring near the middle of the Pleistocene epoch, has been inferred by many workers from such results[1-3] This is a preliminary report of an investigation of 755 oriented samples collected from 152 lava flows on the island of Hawaii, selected to represent as many stratigraphic horizons as possible (Sampling details are indicated in Table 1) This work was undertaken because Hawaii's numerous thick sequences of lava flows, previously mapped as Pliocene to Historic by Stearns and Macdonald[4], and afterwards assigned ages ranging from later Tertiary to Recent, by Macdonald and Davis[5], appeared to offer an ideal opportunity to examine the most recent reversal of Earth's field

Remanent magnetization measurements using a spinner type magnetometer have now been completed on at least one sample from each of the 152 lava flows sampled, flow averages of all measurements completed to date before demagnetization are shown in Figs 1, 2 and 3 Demagnetization experiments of representative samples in alternating fields up to 800 œrsteds (peak) typically reduce the intensity of magnetization by 90 per cent but do not significantly change directions of magnetization Statistical analyses of the direction of magnetization in the samples, before and after demagnetization, and mineralogical studies are in progress

The preliminary measurements may be summarized as follows (1) The historic flows have directions of magnetization closely grouped about the present geomagnetic field direction on Hawaii, which has changed less than 2 degrees since about 1900 (2) None of the 152 flows sampled has reversed magnetic polarity (3) The scatter in directions of magnetization from flow to flow in a given sequence is much smaller than generally reported in comparable studies elsewhere (4) The average magnetization directions of most of the thick sequences sampled appear to differ significantly both from the present geomagnetic field and from the theoretical field of an axial dipole, moreover, the average directions in different sequences are different from each other

If the geomagnetic field reversal theory is true, then all the flows sampled on Hawaii are post-reversal and hence younger than early Pleistocene Alternative explanations (*a*) that lavas were not extruded when the field was reversed or were afterwards eroded or buried, or (*b*) that self-reversal occurred only in lava flows extruded while Earth's field was reversed, are much less plausible. Moreover, the stratigraphic distribution of the 152 lava flows sampled renders unlikely the possibility that a reversely magnetized sequence is present but was inadvertently missed

The closely parallel directions of magnetization found throughout these rather thick sequences of flows may be interpreted in two ways (1) An entire sequence may have been extruded in a time-interval too short for large amounts of secular variation to have occurred However, rates of secular variation as determined from direct measurements at observatories commonly cause more angular variation in several hundred years than is typically observed in the Hawaiian sequences Accumulation within several hundred years of sequences of more than 50 clearly distinguishable lava flows with total thicknesses up to 450 ft appears rather unlikely (2) That low rates of secular variation prevailed in Hawaii during the time these lava flows were extruded is a more plausible explanation The results from the Historic flows support this interpretation, and the direct measurements back to about 1900 also show less secular variation than is typical elsewhere in the world.

Table 1. SAMPLING DETAILS OF VOLCANIC SERIES EXAMINED

Geological Unit	Age	Flows sampled	Stratigraphic thickness (ft)	Description
Historic flows	Historic	9	—	Extruded at known historical dates between 1750 and 1955, members of Kau, Hualalai, and Puna Volcanic Series are included (Fig 1*a*)
Puna Volcanic Series	Late Pleistocene (?) and Recent	18	373 (I) 64 (II)	From Uwekahuna Bluff on west wall of Kilauea caldera Group II constitutes the lowest 64 ft of the section, and is separated from Group I by an erosional unconformity (Fig 1*b*)
Kahuku Volcanic Series	Pleistocene	29	99 (I) 188 (II) 54 (III)	Stratigraphically, flow Groups I, II and III are progressively lower (Fig 2*a*)
Hamakua Volcanic Series	Late Tertiary and Quaternary	27	135	From south-east side of Laupahoehoe Canyon These flows comprise the lower members of the Hamakua Volcanic Series, as described by Stearns and Macdonald (ref 4) (Fig 2*b*)
Pololu Volcanic Series	Pliocene (?)	50	453	From Waipio Valley These flows are from the lower member of the series, the highly weathered upper member was not sampled (Fig 3*a*)
Ninole Volcanic Series	Pliocene (?)	25	116+	From Kaiholena Ridge The upper 76 ft were sampled on the east side of the ridge, the lower 40 ft on the west, an unknown thickness between these is not exposed (Fig 3*b*)

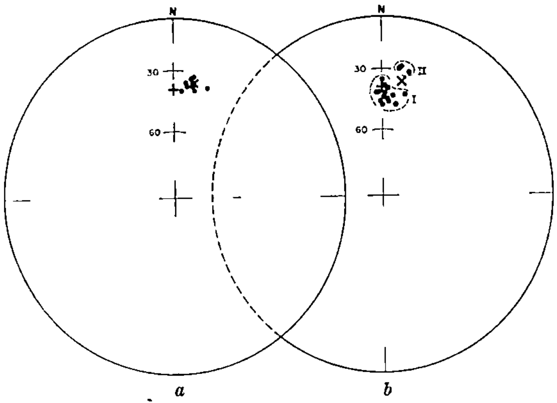

Fig 1 Directions of magnetization of (*a*) Historic lava flows and (*b*) Puna Volcanic Series ●, North-seeking polarity downwards, ○, north-seeking polarity upwards, +, direction of axially symmetric dipole field in Hawaii, ×, present geomagnetic field direction in Hawaii (Lambert equal-area projection with prime circle horizontal)

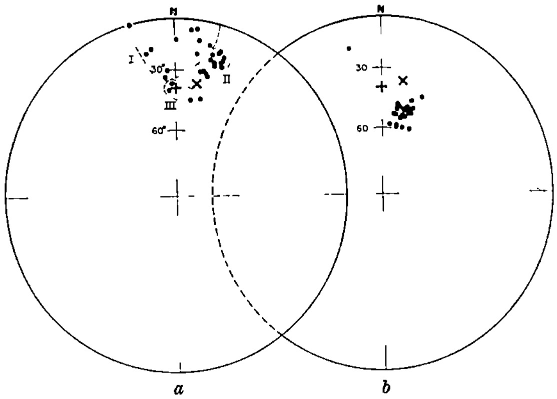

Fig 2 Directions of magnetization of (*a*) Kahuku Volcanic Series and (*b*) Hamakua Volcanic Series Symbols as for Fig 1

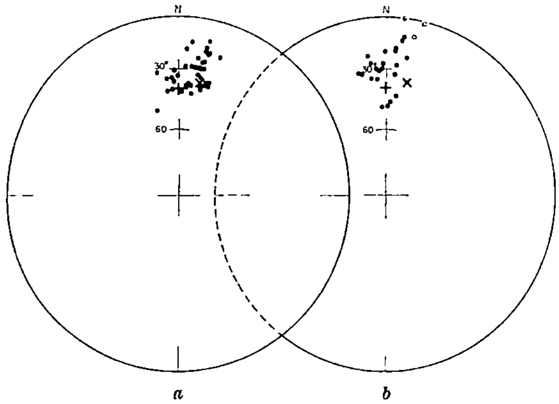

Fig 3 Directions of magnetization of (*a*) Pololu Volcanic Series and (*b*) Nınole Volcanic Series Symbols as for Fig 1

The differences observed between average magnetization directions of the different sequences of flows and the axial dipole field direction do not, in our opinion, indicate that Hawaii has undergone displacements and rotations in the Pleistocene Such an interpretation would require (*a*) lower latitudes than at present for Hawaii during the extrusion of the Pololu and Ninole Volcanic Series, (*b*) markedly higher latitudes during the extrusion of the Hamakua Series; (*c*) lower latitudes during Kahuku Series volcanism, (*d*) the present latitude during extrusion of the Puna Series, substantial rotations of the island of Hawaii since the early Pleistocene would also be required Much more plausible is the explanation that each of these sequences spans a time-interval too short to give an average direction of magnetization parallel to an axial dipole field Although absolute lengths of time cannot be given, it appears from this investigation that stratigraphic sections 450 ft thick and composed of more than 50 flows may accumulate on shield volcanoes in periods of time so short that the non-axial dipole field components do not vectorially add to zero Conversely, the geomagnetic field may have non-axial dipole components for times long enough for the accumulation of these relatively thick sections

Publication of this communication is authorized by the Director of the U S Geological Survey

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PHYSICS

Emission of Ultra-Violet and Visible Radiation from Metals by the Impact of High-Energy Hydrogen Ions

IN a recent communication[1] we reported that ultra-violet and visible radiation is emitted from nickel by the impact of high-energy protons and positive ions, and that the visible part of the spectrum is continuous With a resolved proton beam we observed a flat peak between 4600 and 4900 Å in the distribution curve of spectral intensity

Apart from the radiation resulting from neutralization of positive ions on metal surfaces[2], the point of most significance is to investigate if the radiation observed by us contains spectral lines or the spectrum of the target element on which high-energy positive ions impinge If these lines (or spectrum) are present, their origin remains to be traced

Townes[3] has developed a theory of sputtering of metals under positive ion bombardment based on the assumption of local heating Kapitza[4] has explained in a similar way the emission of electrons from metal surfaces by positive ions This theory is supported by the experimental work of Oliphant and others[5], but some theories[6] of sputtering fail to account for recent experimental results The mechanism of sputtering of metals by positive ions is indeed not yet well understood

We wish to consider the probable mechanism of the emission of radiation from the target metal There seem to be two possibilities for the emission of visible and ultra-violet spectra of the target element (1) the radiation may be emitted from sputtered atoms excited in gaseous phase by positive ions, or by the secondary electrons knocked out of the target, (2) the excited atoms giving out this radiation may originate in the metal itself by the impact

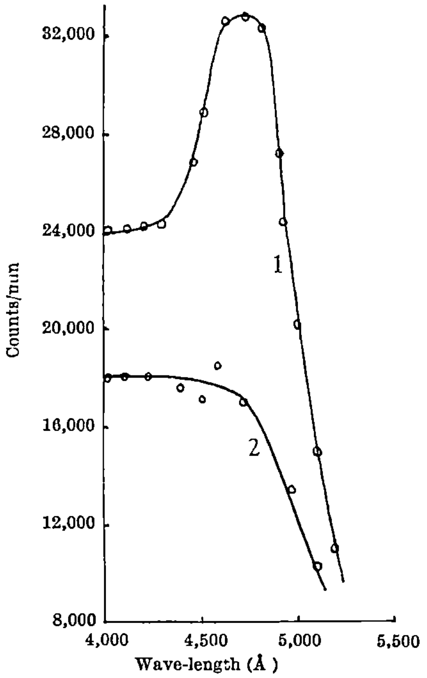

Fig 1 Spectral intensity of distribution curve for radiation emitted from nickel by high-energy positive ions of hydrogen Energy of positive ions = 200 keV , positive ion current = 4 μamp Curve 1, target cooled to low temperature, curve 2, target not cooled

of high-energy positive ions It is difficult to distinguish which of these two processes predominates in the emission of this radiation

If the metal target is cooled to a very low temperature where its thermal conductivity is comparatively high, the local heating effect will be reduced and consequently the sputtering will be less If the radiation observed were due to sputtered atoms excited in the gaseous phase, its intensity would be smaller at lower temperatures of the target Our experimental results, however, show that the reverse is the case.

The target is placed in a special chamber made throughout of glass It houses, besides the target, a copper reservoir filled with liquid air, so that impurities and volatile vapours in the chamber may condense on it The nickel target is degassed with electron bombardment to a bright red heat under a high vacuum for a sufficiently long time to make it clean and free from gas It is then cooled and kept permanently in thermal contact with a metal reservoir maintained at liquid-air temperature during experiments, and radiation emitted from it under the impact of hydrogen ions of energy 200 keV and current strength 4 μamp. is studied with Kodak gelatine filters Filter *2B* is used to cut out the ultra-violet region The distribution curve of spectral intensity shows a pronounced peak between wave-lengths 4600 and 4800 Å , which is a region of strong lines in the arc spectrum of nickel Curves 1 and 2 of Fig 1 show the distribution of spectral intensity obtained with the target cooled to low temperature and without cooling it respectively The visible spectrum is, so far as could be judged from analysis with gelatine filters, continuous within the range studied The intensity of the ultra-violet radiation between 4000 and 3300 Å is greater than that of the visible region as in previous experiments

The presence of the peak in the region of prominent nickel lines in curve 1 leads us to think that this radiation is emitted from the excited atoms which originate in the metal itself under the impact of high-energy positive ions The continuous nature of the spectrum, on the other hand, suggests that some sort of electron plasma oscillations may occur in the metal due to positive ion bombardment on it, and may give rise to this radiation

We believe that, with our experimental arrangement, the chances of accumulation of impurities on the cooled target are small, and consequently the increase in the intensity of the radiation emitted from the cold metal surface cannot be attributed wholly to the excitation of atoms of any dirty film on it.

Details of the chamber and the target cooling arrangement are being described elsewhere

We are grateful to Sir George Thomson for suggestions and advice, and to Sir Mark Oliphant for his interest throughout this work

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High-Speed Direct-Recording System for Electron Diffraction

AN electron diffraction system for the quantitative investigation of rapid phase changes in solids has recently been completed The system will record an electron diffraction pattern of the Debye–Scherrer ring type in 50 msec , giving the electron intensity at the peaks of strong reflexions to an accuracy of 2 per cent The system gives an improvement in speed of direct recording of about four orders of magnitude[1], and is thus attractive for investigating quantitatively rapid changes in solids such as polymorphic transformations, transitions from the amorphous to the crystalline state, diffusion in alloys. It is also suitable for investigating controversial questions in electron diffraction such as the transition from geometrical to dynamic diffraction conditions[2,3]

Fig 1*a* shows a typical record from a polycrystalline gold film about 300 Å. thick. There are in fact two superimposed traces each of 100-msec. duration showing profiles of rings from 220 to beyond 531 The display also shows the profile of the primary beam after it has traversed the film, this gives a continuous measure of the angular resolution of the electron optical system. Once the information in the diffracted beams has been obtained at a sufficient resolution and level it is, of course, easy to magnify the diffracted profiles on the display Thus Fig 1*b* shows the inner rings of the gold together with the main beam in greater detail

Figs 2*a* and *b* show a high-speed amorphous-to-crystalline change in a film of silver in response to sudden heating Fig 2*a* is the envelope of fifty traces, the first of which shows almost no rings while

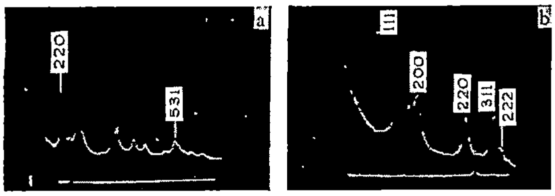

Fig 1

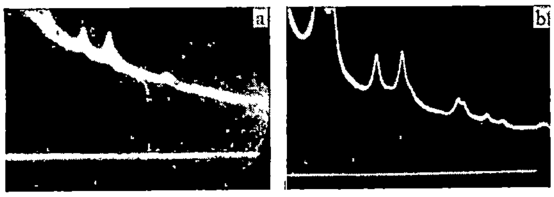

Fig 2

the last trace, 5 sec later, shows the rings considerably developed After 200 sec (Fig. 2*b*) there is little further change

The ultimate speed of recording in such a system is set by shot noise in the diffracted beams. The limitations are the same as those of the scanning electron microscope[4] Measurements show that the intensities at diffracted peaks are about 10^{-9} amp. Thus, for an accuracy of 1 per cent at such a peak, a picture point may be recorded in about 2 μsec One may expect that recording times for complete diffraction patterns of 1 msec are a question merely of improvements in technology.

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Isotopic Composition of Atmospheric Hydrogen and Methane

In a recent communication, Bishop and Taylor[1] express the opinion that the tritium concentration of free hydrogen in the atmosphere has been rising over the past ten years, with a doubling time of approximately 18 months The authors suspect that artificial tritium was released into the atmosphere several years before the Castle test series in 1954, which is commonly assumed to have led to the first pronounced rise in the tritium concentration of terrestrial surface water Bishop and Taylor's communication includes a diagram of the logarithms of all the experimentally determined tritium values in free atmospheric hydrogen plotted against time The plot shows that the values follow a straight line that includes the first value obtained by Faltings and Harteck[2] on atmospheric hydrogen collected in 1948

The question of an addition of artificial tritium into the atmosphere prior to 1954 is of considerable importance in connexion with estimating the natural rate of production by cosmic rays of tritium, which is in general assumed to be of the order of 1 tritium atom per sec per cm ^{2} of Earth's surface

We have been able to secure a sample of atmospheric hydrogen, dating from 1948, contained in 'raw neon' in a regular cylinder, through the courtesy of Mr F R Balcar of the Air Reduction Co, New York This gas gave the following values

T/H = $3\,5 \pm 0\,1 \times 10^{-14} \equiv 35{,}000$ tritium units
δD = $-5\,4 \pm 0\,2$ per cent relative to Lake Michigan water standard

This tritium concentration is very close to that found by Begemann in hydrogen collected shortly before the Castle tests in 1954, and to the value for 1954 represented by the straight line through the points measured by several investigators afterwards It therefore appears unlikely that artificial tritium has influenced significantly the tritium content of atmospheric hydrogen prior to 1954. The deuterium value shows, in agreement with previous findings[3-4], that equilibrium between atmospheric water vapour and hydrogen is not established at ordinary temperatures

A most remarkable observation was recently made by Bishop *et al* [5] The tritium content of atmospheric methane is also very high and only about a factor of 5 below that of present-day atmospheric hydrogen The authors state that they are unable to suggest any satisfactory explanation for the source of this tritiated methane. They suspect an artificial origin

We have independently investigated one sample of atmospheric methane, which was collected in January 1960, by the Linde Co in Gary, Indiana. This sample was large enough to enable us to carry out very accurate, routine carbon-14 and tritium analyses in the La Jolla Radio-carbon and Tritium Laboratories. We are not able to propose any satisfactory explanation for the following experimental findings, but hope that our results, which include deuterium and carbon-13 values, may help in the future in interpreting the observed facts Measurement of carbon-13 was carried out in the laboratory of H Craig (University of California, San Diego), who wishes to discuss his result more extensively in a separate paper A second tritium determination by F Begemann at Mainz gave exactly the same result as ours

T/H = $1\,94 \pm 0\,1 \times 10^{-14} \equiv 19{,}400$ tritium units
^{14}C = $75\,2 \pm 0\,5$ per cent of standard wood, or 60 per cent of atmospheric carbon dioxide in 1960
δD = $-4\,2 \pm 0\,2$ per cent relative to Lake Michigan standard water
$\delta^{13}C$ = $-39 \pm 0\,1$ per mille relative to Chicago Pee Dee Belemnite standard (ref 6)

The tritium value of our 1960 sample is exactly the same as the average of the samples from the northern hemisphere collected by Bishop *et al* during 1958 and 1959, although tritium in terrestrial surface water showed wide variations during this period A comparison of our carbon-14 value with results obtained by W. F Libby on atmospheric methane from 1952 shows no change greater than experimental errors Libby's samples contained 75 per cent and 60 per cent of modern carbon. They were collected in Britain by the British Oxygen Co, at Wembley, and submitted in the form of carbonate by H E Gluckauf We are grateful to Dr Libby for his unpublished results

Agreement of the results is better than might be expected in view of the possibility that artificial inactive methane and hydrogen from city gas, automobile exhaust, and other industrial sources might be present Limits on the amounts of such artificial gases present can be deduced from the measured value of the stable isotopes We are planning to make at least one measurement a year in the future

Support for this work was received from the U S Atomic Energy Commission Division of Biology and Medicine, from the National Science Foundation and

from the Geophysics Directorate of the Air Force Cambridge Research Center.

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Our communication on the growth of the tritium content of free hydrogen in the atmosphere was written before the publication by Begemann and Friedman[1] of detailed measurements for the period 1954–56. These showed considerable seasonal variation, but no pronounced increase during the two years following the Castle test series. Their earliest determination in January 1954 before the Castle test series was $9 \cdot 18 \times 10^4$ tritium units compared with $1 \cdot 66 \pm 0 \cdot 097 \times 10^4$ tritium units found by Grosse *et al.*[2] in the spring of 1952. This obvious increase was concluded by Begemann and Friedman to be due to the Ivy test series of November 1952. Evidence for an earlier increase based on the value $0 \cdot 38 \pm 0 \cdot 125 \times 10^4$ tritium units obtained by Faltings and Harteck[3] in 1948–49 now appears to be refuted by the value of $3 \cdot 5 \times 10^4$ tritium units reported by Bainbridge, Suess and Friedman. It may be noted, however, that this value is more than twice that observed by Grosse in 1952. With three sets of observations indicating a substantial increase prior to the Castle tests, it is difficult to support the conclusion of Bainbridge *et al.* that such an increase is "unlikely".

Our measurements[4] of the tritium content of atmospheric methane collected at Wembley by the British Oxygen Company gave values ranging from $1 \cdot 59 \times 10^4$ to $3 \cdot 36 \times 10^4$ tritium units during the period October 1958 to February 1959, the average value being $2 \cdot 19 \times 10^4$ tritium units. One sample collected on December 7, 1959, had a value of $2 \cdot 85 \times 10^4$ tritium units and another collected on February 8, 1960, a value of $1 \cdot 59 \times 10^4$ tritium units. It is true that the present reported value ($1 \cdot 94 \times 10^4$ tritium units) falls within the range of values obtained by us, but to state that it "is exactly the same" as the average of our samples collected in the northern hemisphere during 1958 and 1959 may give the incorrect impression that short-term fluctuations are very small.

Furthermore, in addition to the considerable short-term fluctuations observed at Wembley, we found that the levels in the northern hemisphere stratosphere and in the southern hemisphere generally were all about $0 \cdot 8 \times 10^4$ tritium units, considerably lower than those at Wembley. The level in the southern hemisphere troposphere has also shown a continuous rise during 1953–58 when our measurements ceased.

It is clear, therefore, that though the future measurements proposed by Bainbridge *et al.* will give some useful information, a more comprehensive sampling programme would be necessary to unravel the origin and distribution of tritiated methane in the atmosphere.

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ENGINEERING

A Novel Electrostatic Machine: the Corona Motor

We would like to direct the attention of readers to a new type of electrostatic motor which may find use in some specialized application. The operation of the motor demonstrates one of the fundamental properties of electrostatic fields, namely, motion of charge under the influence of a voltage gradient.

The motor is shown in Fig. 1 and consists of a disk, made from an insulating material, which is mounted on a spindle, and three pairs of electrodes mounted circumferentially around the disk. The electrodes are alternately connected to the positive and negative terminals of a source of high-voltage direct current. The diameter of the disk is 1·5 in., and a supply of 8–13 kV. is sufficient to operate the motor. The disk rotates either clockwise or anti-clockwise, depending on the direction in which it was started. When running freely in air with the axis turning in jewel bearings, speeds of up to 12,000 r.p.m. are attained.

During running of the motor a corona discharge takes place along the circumference of the disk and extends from each positive electrode towards the next negative electrode against the direction of rotation. The glow discharge increases in intensity and extent with increase in voltage until at a critical voltage flashover occurs between the electrodes.

We surmise that the operation of the motor can be explained as follows (Fig. 2): Consider that the voltage

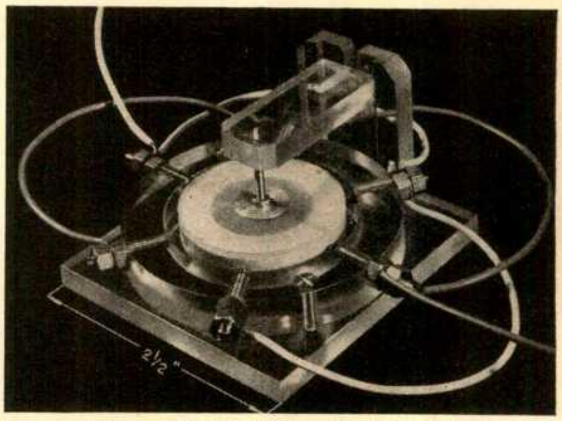

Fig. 1. Photograph of a six-electrode motor

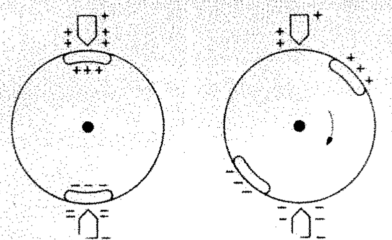

Fig. 2. *a*, Charge is deposited on the rim of the disk by ionization of the air gap. *b*, Disk rotates under action of torque developed by charge dipole

on the electrodes is increased until ionization of the air between the electrodes and the rim of the disk occurs, then a quantity of charge will be deposited on the rim (Fig. 2(*a*)). The charge, being of the same polarity as the electrode from which it emanated, is mechanically in unstable equilibrium with respect to the electric field, and if the disk is slightly disturbed, a couple will act on the disk, which will cause it to rotate, carrying the charge with it (Fig. 2 (*b*)). On reaching the opposite electrode, the charge is neutralized by a further ionized path in the air gap. If the process of charging and discharging carries on continuously, the disk will continue to rotate and will be able to deliver torque.

A possible explanation of why a glow discharge is noticed between alternate pairs of electrodes only is that a negative electrode deposits electrons on the disk, which creates an electric field between the charged area and the positive electrode. When the charged area is carried into the region where the resultant field intensity is large enough, ionization takes place. The positive electrodes, on the other hand, act as collectors of the negative charge. This results in a glow discharge between pairs of negative and positive electrodes only. At low voltages the ionization takes place close to the positive electrode and the region is extended for increased applied voltage until it covers the entire area between positive and negative electrodes. This is in conformity with the mechanism suggested, since the ionization will take place at a given field strength and thus the distance that separates the charged surface from the positive electrode, when ionization starts, will be determined by the magnitude of the charge.

In order to study the wave-forms of current when the motor is running, a small resistance was inserted in the earth lead, and the probe of an oscilloscope was connected across this resistance. It was found that charge transport takes place in short discrete bursts, of approximately 1 µsec. duration. The number of discharges a second depends on the speed of rotation, but a typical value is 2,000 discharges a second. Fig. 3 shows the wave-form occurring in a two-electrode motor.

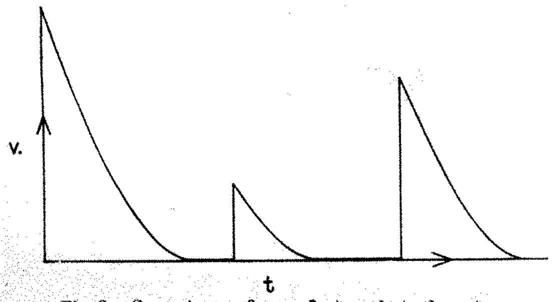

Fig. 3. Current wave-forms of a two-electrode motor

The corona motor is the converse of the Van de Graaf generator. In both cases charge is the active medium, but while in the Van de Graaf generator it is transported against an electric field to supply voltage, in the motor it is allowed to move with the field in order to supply torque.

The simplicity of construction and the absence of magnetic and metallic material might well justify further research to develop the motor into an efficient unit.

We wish to thank Mr. E. Fiedeldey for the mechanical construction of the device.

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Rectifying Action of Non-uniform Fields in Dielectrics

PRELIMINARY work has been done on the breakdown of dielectric specimens under direct and 50 c./s. alternating voltages. With the latter a polarity detector was connected in series with the test specimen and in all cases point–plane electrode systems were used. For air and transformer oil a nickel-plated steel

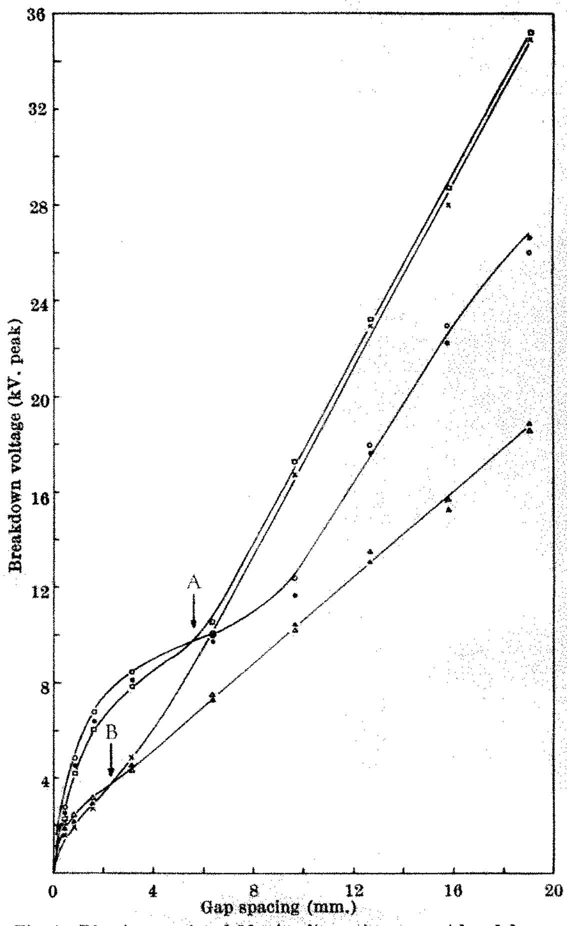

Fig. 1. Direct-current and 50 c./s. alternating-current breakdown voltage characteristics of air. Brass rod: □, negative point (d.c.); ○, positive point (d.c.); ●, 50 c./s. (a.c.). Nickel-plated point: ×, negative point (d.c.); △, positive point (d.c.); ▲, 50 c./s. (a.c.)

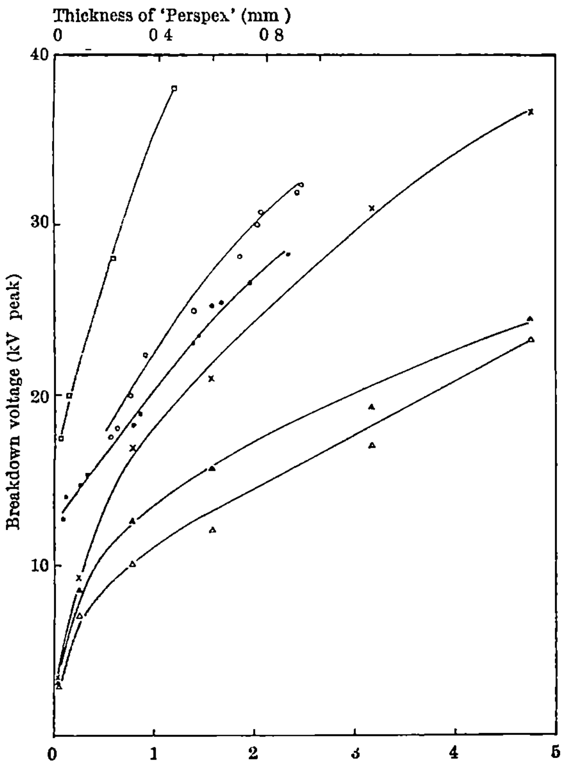

Fig 2 Direct-current and 50 c /s alternating-current breakdown voltage characteristics of 'Perspex' and transformer oil. Electrodes nickel-plated steel point–plane 'Perspex' □, negative point (d c), ○, positive point (d c), ●, 50 c /s (a c) Transformer oil ×, negative point (d c), △, positive point (d c), ▲, 50 c /s (a c)

sewing needle having a radius of curvature of about 2 5μ and a ⅛-in diameter brass rod having a radius of curvature of 1/16 in. were used in conjunction with a brass plate as the plane electrode. For 'Perspex' sheets the point electrode, a sewing needle similar to that described above, was pressed into the specimen at a temperature of 155° C and an aluminium foil was bonded to the other side of the specimen as the plane electrode. This assembly was then immersed in transformer oil All measurements were made under normal temperature and pressure conditions

It was observed that in 50 c /s. alternating fields breakdown of air always occurs when the point is at the positive half-cycle provided that the gap spacing is greater than the critical spacing shown at *A* and *B* in Fig 1 for the two different point electrodes The peak value of the alternating-current breakdown voltage is practically equal to the direct-current breakdown voltage with the point positive Breakdown under alternating voltages of either positive or negative polarity only occurs when the gap spacings are close to the critical value This polarity effect or rectifying phenomenon with large gap spacings is independent of the rate of rise of the applied voltage

This phenomenon also occurs in transformer oil and 'Perspex' as shown in Fig 2 Within the range of gap spacings under investigation the breakdown always occurs when the point is at the positive half-cycle In these two dielectrics other effects apart from the space charges may play an important part in the breakdown mechanisms The fact that the peak value of the alternating-current breakdown voltage in transformer oil is higher than the direct-current breakdown voltage with the point positive may be attributed to the effect of impurities The reversed tendency noted in 'Perspex' may be due to the thermal effect

The consistency of this phenomenon depends on the non-uniformity of the field, and the rectifying action, especially when air is used as the dielectric medium, can possibly be used to rectify high-voltage alternating currents

I wish to thank the Brush Electrical Engineering Co , Ltd , for permission to publish this communication

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CHEMISTRY

Experimental Detection of Auto-Ionizing Transitions in Krypton by Electron Impact

A NUMBER of investigators[1] have discussed the contribution of auto-ionization to the shape of the ionization efficiency curves obtained by electron impact, but, at least in the case of the rare gases, no direct correlation has been found between the structure observed in these curves and the energy-levels known to be strongly auto-ionizing from ultra-violet absorption spectroscopy[2]. I wish to present ionization efficiency curves for the Kr^+ ion which exhibit discontinuities in slope at electron energies that lie quite close to the levels found in absorption. Both the intensity and separation of these levels fall off rather rapidly at higher energies, so that only those that lie directly above the normal $^2P^0_{3/2}$ ionization-level can be expected to be resolved Using the Paschen notation[3] for clearness in the figures; these levels are:

5 S'_1, 14 07 eV 6 S'_1, 14 25 eV 7 S'_1, 14 37 eV
4 S_2, 14 09 eV 5 S_2, 14 27 eV 6 S_2, 14 37 eV

Three independent determinations of the ionization efficiency curve for the Kr^+ ion are shown in Fig. 1 This figure is a photograph of the original recorder traces with the co-ordinate lines filtered out. The curves were obtained by the retarding potential method[4], which had been modified by the addition of a c signal detection, and an x–y recorder to obtain a continuous plot of the 'difference' ion current as a function of the electron energy The terms 'response 2', 'response 3', 'response 4' indicate that successively larger capacitance was introduced between the mechanically rectified output of the a c amplifier and the x–y recorder to improve the over-all signal to noise ratio. The scale for the 'difference' ion current (ordinate) is arbitrary and the divisions are unmarked since the zero of the y-axis amplifier was shifted between determinations to permit all three curves to be traced out on the same sheet of co-ordinate paper The electron energy scale has been shifted approximately 0·3 eV to place the onset of ionization to the $^2P^0_{3/2}$ level at its spectroscopic value of 14 0 (13 996) eV The electron energy was varied by a motor-driven helipot, and a signal proportional to it was impressed on the x-axis amplifier of the recorder A photographic enlargement of Fig 1 showing the onset of ionization and the break in the vicinity of the unresolved 5 S'_1 and 4 S_2 levels is given in Fig 2, and a similar enlargement showing the break near the 6 S'_1 and 5 S_2 levels in Fig 3 No clear break is to be found in the neighbourhood of the 7 S'_1 level on the curves presented in Fig. 1, and the curve labelled response 2 was not enlarged because of the high noise-level.

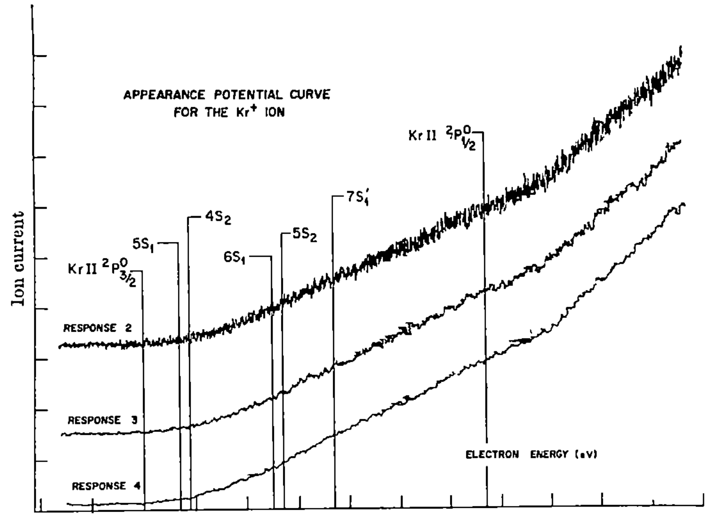

Fig. 1. Appearance of potential curves and auto-ionizing levels for the Kr$^+$ ion

These three curves were obtained a little more than a year ago for use in a paper describing the modification of the retarding potential method, which permitted the ionization efficiency curve to be plotted directly on the recorder paper as a continuous function of the electron energy. The toe of the curve was not examined critically at that time and the departure of the curve from linearity at about 0.15 eV above onset was a source of considerable disappointment. It was attributed to excessive curvature resulting from an abnormally large spread in the electron energy introduced by the square-wave modulation of the electron beam required for a.c. signal detection of the 'difference' ion current. Every effort was made to remove this 'curvature' by adjusting the position of the source magnet, the retarding potential, electrode voltages, etc., but it was not found possible to reduce it below that appearing in Fig. 1.

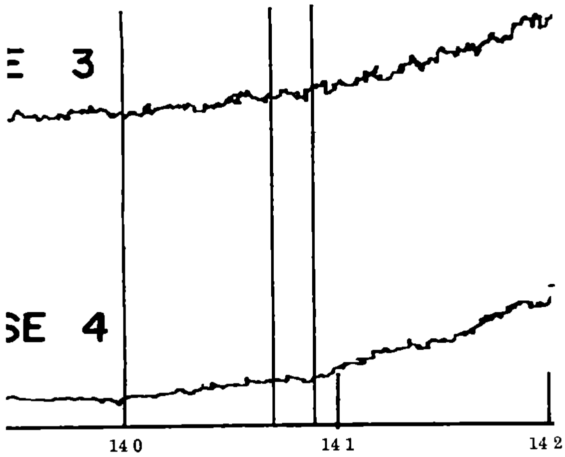

Fig. 2. Enlarged section of Fig. 1 showing the breaks at onset and near the location of the 5 S'_1 and 4 S_2-levels

Following the publication of a paper by Foner and Nall[5] on the structure found in the ionization efficiency curves of the rare gases, the curves shown in the figure were re-examined and the structure discussed earlier was found. Only a few other curves had been preserved, but on nearly all of these a break of some sort could be found near the first two pairs of auto-ionizing levels. These breaks were rather easily identified on five other ionization efficiency curves in addition to those shown in the graphs. Three of these latter showed a fairly strong indication of a break near the 7 S'_1 level. Since the measurements of these curves extended over a period of months and were obtained under different adjustments of the focus of the source, it seems hard to dismiss the agreement between these electron impact results and the spectroscopic data as accidental or instrumental. A more detailed report will be prepared as soon as the mass spectrometer used in this work becomes available again.

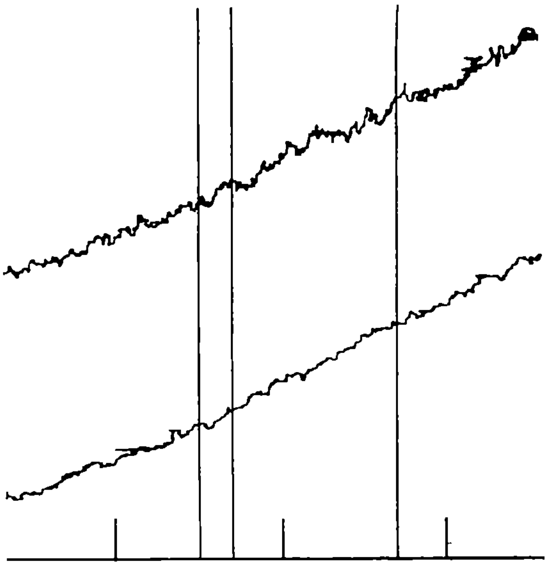

Fig. 3. Enlarged section of Fig. 1 showing the break near the 6 S'_1 and 5 S_2-levels

I thank Prof. W. R. S. Garton and Lady Anne Pery-Thorne for a very helpful discussion of auto-ionizing processes in general during a visit to the Imperial College of Science and Technology last autumn, and to Prof. Garton for pointing out that the auto-ionizing levels listed above as well as many others have been tabulated.

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* Operated by Union Carbide Nuclear Company for the United States Atomic Energy Commission.

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Surface Properties of Silver Iodide

It is a rather curious fact that both low-energy and high-energy surfaces abound, but intermediate energy surfaces of high area are infrequently found and have been little studied In a search of such intermediates, we recently examined the surface chemistry of silver iodide One method is to compare the apparent water specific surface area to the nitrogen area Another result which reflects the surface energy is the measurement of the heat of immersion in water and other liquids of particles of the solid sufficiently high in surface area to give meaningful results

Interest in the surface chemistry of silver iodide increases when one comes to recognize that silver iodide is regarded as hydrophobic[1] in classical colloid chemistry but as hydrophilic in cloud physics[2,3] When the double layer on silver iodide sols is repressed or destroyed, the colloid chemist finds that the particles flocculate quickly and settle out Yet this result may be due only to gravity and might have nothing to do with any hydrophobic character Previous results on the water vapour adsorption on silver iodide are quite contrary Birstein[3] claims some 130 layers of water are adsorbed before P_0 is reached It can be categorically stated that such a reported result is novel Solids adsorb at most 5–10 nominal layers before condensation is reached, as Harkins[4] showed years ago In a recent paper, Dubinin *et al* [5] also concluded that the silver iodide surface is hydrophilic and that it accepts about 3 layers before the condensation pressure

The cloud physicists regard silver iodide as the best 'cloud seeder' yet found presumably because its crystal parameters closely correspond to those of ice Observations of the nucleation and growth of ice crystals on single silver iodide crystals (colour motion pictures taken by Prof B J Mason, Imperial College, London) cast doubt on the concept of epitaxy For one thing, the ice crystals preferentially form at steps in the silver iodide crystal face where epitaxy cannot exist in all directions For another, the hexagonal pattern does not appear until the growing ice crystal has gained some size Finally, many ice crystals are formed on a single silver iodide crystal so that the epitaxy does not extend over the entire face as in the usual case

Some doubt was cast on the other direct measurements of the hydrophilic character of silver iodide by Halsey[7] in a brief note on the matter He concluded that ice and silver iodide surfaces are energetically incompatible although he was not considering cloud seeding at the time He was freezing xenon on to various crystals to attempt to produce energetically uniform adsorbent surfaces

Recent work in this Laboratory supports the contention that the silver iodide surface is rather hydrophobic For one preparation, the value of the ratio of the Brunauer–Emmett–Teller areas for water to nitrogen surface area measurements were $\Sigma H_2O/\Sigma N_2 = 7/100$, for another, $\Sigma H_2O/\Sigma Ar = 37/100$ For still another, $\Sigma H_2O/\Sigma N_2 = 27/100$ These results compare with the $\Sigma H_2O/\Sigma N_2 = 1/1{,}500$ obtained[8] for the almost completely hydrophobic graphitized carbon black, 'Graphon'

Further work is in progress to try to establish the surface characteristics of silver iodide For this purpose, high surface area samples in sizeable quantity are desired These are difficult to prepare, and then to outgas without severe sintering[7]

Our present hypothesis is that the steps in the silver iodide surface possess hydrophilic, possibly oxide, sites On these the water molecules first adsorb in clusters much as on 'Graphon' or graphites Eventually, when the nucleation process is carried out at low temperatures, the ice crystal structure develops

It seems reasonable to suspect higher sticking coefficient for the water on to earlier adsorbed molecules if they are not highly organized and 'busy' interacting strongly with the substrate This situation would probably prevail if water molecules could be adsorbed all over the surface as on an oxide This possible explanation of the efficacy of silver iodide is strengthened by the recent work of Eyring[9] on the sticking coefficient of many atoms and molecules on a number of surfaces He finds that the more ordered the molecules are in the surface, the lower is the sticking coefficient The molecules do not want to pay the 'entropy price' and thus more of them rebound

Possibly clays are good seeders because of the ion exchange sites which also build up water clusters, and proteins, reported by Vincent Schaefer to have excellent cloud seeding properties, are effective because of the separated polar sites at which water clusters can develop

We wish to thank the National Science Foundation for a grant in support of this work

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Production of 'Active' Magnesium Oxide by heating in Vacuum

When samples of precipitated magnesium hydroxide were heated in air for a fixed period of 2 hr at a succession of temperatures ($T°$ C) Gregg and Packer[1] found that the specific surface area S (determined by the Brunauer–Emmett–Teller method with nitrogen at − 183° C) when plotted against T gave the curve I reproduced in Fig 1.

Conducting a similar experiment with starting material similarly prepared and having a similar specific surface (60 m^2 $gm.^{-1}$ as compared with 56 m^2 gm^{-1}), but heating *in vacuo*, we obtained curve II of Fig. 1 the temperature at which the maximal area was obtained has been lowered from *c* 390° C to *c* 300° C and the maximal area has been raised from 190 m^2 gm^{-1} to 340 m^2 gm^{-1}, which is unusually high for 'active' magnesium oxide

This effect of heating in air as compared with vacuum is qualitatively similar to that reported by Razouk and Mikhail[2] working with the mineral brucite, but their areas were considerably lower, and the

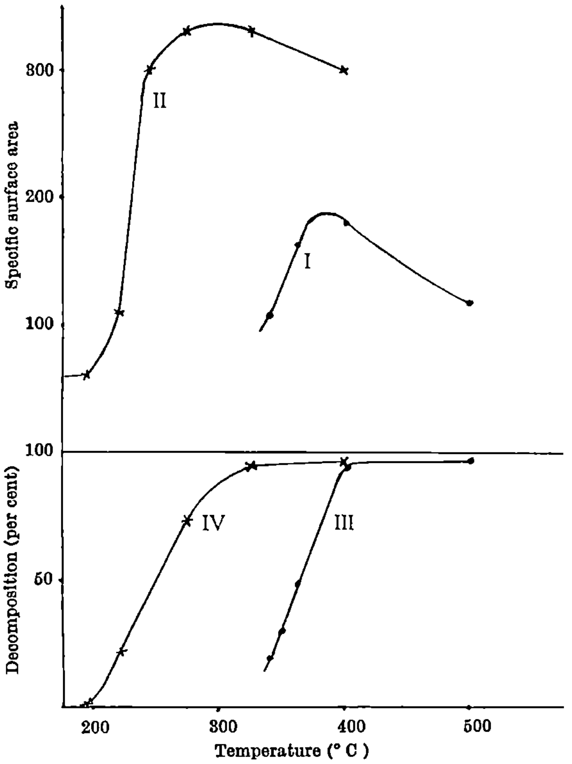

Fig 1 Calcination of magnesium hydroxide in air (curves I and III, see ref. 1) and *in vacuo* (curves II and IV, present work)

maximum was situated at a higher temperature (air, maximal area, 37 m^2 gm.$^{-1}$, at 500° C, vacuum, maximal area, 124 m^2 gm^{-1}, at 350° C) This is possibly due to the larger crystallite size of the mineral brucite as compared with precipitated magnesium hydroxide

An explanation of the superior 'activating' effect of heating in vacuum must await the results of experiments now in progress in this laboratory, but it is probably connected with the higher rate of decomposition *in vacuo*, and the reduction in sintering because of the lower temperature required for nearly complete decomposition (see curves III and IV)

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Catalytic Decomposition of Ethylene on Nickel

The mechanism of the hydrogenation of ethylene on nickel has been studied intensively for at least thirty years; but there is still little agreement as to the exact nature of the intermediates present on the metal surface during the reaction On the other hand, examination of the chemisorption and dissociation of ethylene on nickel in the absence of gaseous hydrogen has been slight and mostly confined to ambient temperatures[1,2] This is surprising as the products and mechanism of ethylene dissociation can play an important part in hydrogenation, exchange and other reactions

The decomposition of ethylene on nickel has been studied as a function of temperature in the range −30° C to 200° C 14 8 gm of high-purity carbonyl nickel were loosely pressed into pellets ¼ in in diameter, reduced in a stream of hydrogen at 400° C for 6 hr and then evacuated at 400° C for 12 hr to a residual pressure of 1×10^{-6} mm Experiment showed that no hydrogen remained on the metal after this treatment. 6 mm of purified ethylene were then allowed to stand over the catalyst for a period of 4 hr at each of the temperatures studied, the volume of the system being about 130 c c. At the end of this period, the contents of the gas phase were condensed in a trap cooled in liquid nitrogen The non-condensable pressure was found to be due to methane The contents of the trap were analysed with a gas-chromatograph, using helium as carrier gas and a silica gel column The results are shown in Fig 1, in which the composition of the gas phase is plotted against the temperature of the catalyst.

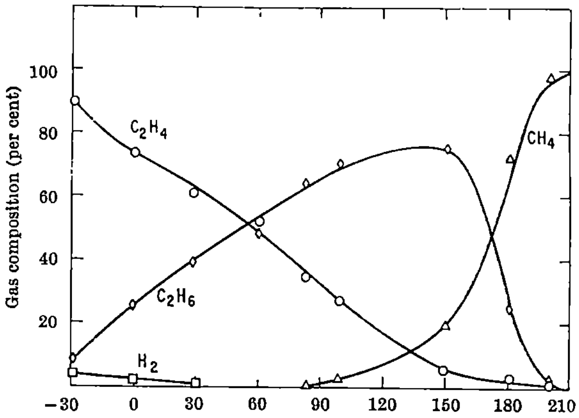

Fig 1 Temperature-dependence of the products of reaction of ethylene (6 mm pressure) with nickel after 4 hr Gas concentrations ○, C_2H_4, ◇, C_2H_6, △, CH_4, □, H_2

At temperatures below 60° C., the products are ethane and hydrogen, obtained by self-hydrogenation of the ethylene This effect has been studied at 20° C by Rideal and Jenkins[1], who suggest that ethylene in the gas phase reacts with chemisorbed hydrogen, obtained by dissociative adsorption of ethylene.

$$CH_2 = CH_2 \rightarrow \underset{*}{CH} = \underset{*}{CH} + \underset{*\,*}{HH}$$

The self-hydrogenation of ethylene was further studied by Beeck[3], who states that at −39° C. no ethane was produced. In the present work, however, appreciable quantities of ethane were found after reaction at −30° C and the rate of self-hydrogenation was found to be measurable down to − 78° C. The quantity of hydrogen evolved on self-hydrogenation was always small and less than 5 per cent of the ethane produced. No C_3, higher hydrocarbons or polymerization products were detected at low temperatures, this result is at variance with that of Morikawa *et al* [4], who found 29 per cent butane among the products at 0° C. Above 60° C, methane began to appear in the gas phase due to the cracking of ethylene and ethane present, the concentration of the latter decreased rapidly above 150° C and, at 200° C., the gas phase consisted entirely of methane.

The overall composition of the adsorbed residues on the surface can be calculated in the form $(CH_n)_x$, as shown in Fig 2 At 0° C, these residues have an

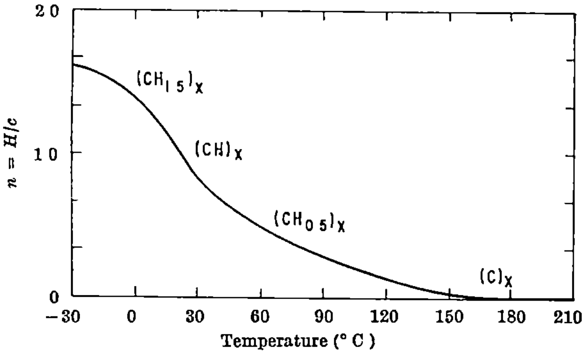

Fig 2 Temperature-dependence of overall composition of surface residues $(CH_n)_x$

average composition corresponding to $(CH_{1\ 5})_x$, but the value of n decreases rapidly with increasing temperature as hydrogen is progressively removed from the surface These observations agree with those of Rideal and Jenkins[1], who found that the residues had an average composition at 20° C approximating to that of acetylene, or $n = 1$ However, Pickering and Eckstrom[5] found no infra-red evidence for the presence of C—H bonds on the nickel surface under these conditions Sabatier[6] found that complete carbonization of nickel occurred with ethylene at 300° C , but it appears from the present work that the metal surface is essentially free of hydrogen at 200° C All adsorbed residues could be completely removed and the metal restored in activity by the reduction and evacuation procedure described here.

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Role of Keto-Enol Tautomerism in the Polarographical Reduction of Some Carbonyl Compounds

DURING some polarographical investigations of certain α-aminoketones[1] and on the elimination of Mannich bases[2] experimental data were gathered, which can be explained by the occurrence of keto-enol transformation Though considerable attention has been paid to the mechanism of polarographical reduction of carbonyl compounds[3], no experimental evidence has been adduced for the participation of the keto-enol tautomerism in the reduction of the carbon–oxygen bond This follows from the fact that, in most cases examined, the keto form is the predominating form, and in buffered solutions used, establishment of keto-enol tautomeric equilibrium is fast However, the decrease of the limiting current of some keto acids has recently been interpreted[4] as being influenced by the keto-enol equilibrium in addition to hydration Experimental evidence for the role of enolization in the behaviour of substances like acetyl and acetonyl acetone[5] is unconvincing On the other hand, it has been discovered that the enolate form participates when anodic waves occur, corresponding to the formation of mercury salts, in alkaline solutions of enolizable ketones (Heyrovský, M , private communication) Keto-enol equilibria were involved also in the complicated and, in some ways, questionable scheme derived[6] for the reduction of thenoyl trifluoroacetone

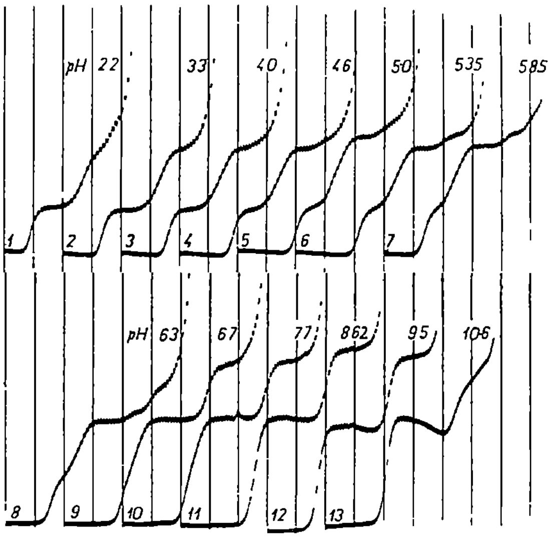
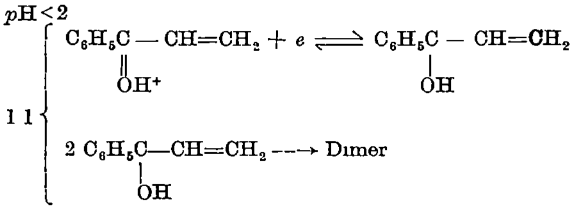


Fig 1 Dependence on pH of waves fo rphenyl vinyl ketone Britton–Robinson buffers, 1×10^{-4} *M* phenyl vinyl ketone Curves starting at 1–6, −0 4 V , 7–11, −0 6 V , 12 and 13, −0 8 V 200 mV (absc), saturated calomel electrode, $h = 56$ cm , $t_1 = 3\ 0$ sec , $m = 3\ 3$ mgm /sec., full-scale sensitivity, 2 0 μamp

Since the conditions for detecting keto-enol tautomerism in solutions are unfavourable, we have focused attention on cases where the carbonyl compound is formed electrolytically at the surface of the electrode

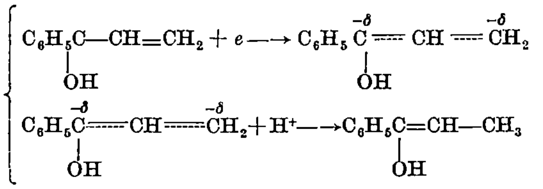
As an example of such an electrode process the reduction of phenyl vinyl ketone is first mentioned In this case (Fig 1) a two-electron reduction occurs first in two one-electron steps in acid solution, followed by waves at more negative potentials. The half-wave potentials of the latter are practically identical with those for propiophenone The participation of keto-enol tautomerism is deduced from the dependence of the height of this more negative wave on pH and buffer composition It is assumed that irreducible enol formed as a reduction product in the first two-electron step is transformed by an acid-base catalysed reaction into the reducible keto form The rate of formation of the keto form from the enol form governs the limiting current of the more negative wave, which acquires a kinetic character The assumed irreducibility of the enol form is in agreement with the behaviour of stilbenes Stilbene itself is reduced at −2 3 V and the introduction of a hydroxyl group could result ($\rho^* = +\ 0\ 30$ V , $\sigma^I_{OH} = +\ 0\ 25$) in a shift of maximum by + 0 1 V.

Hence the mechanism of reduction can be depicted as follows



$$pH<2 \quad 1\ 1 \begin{cases} C_6H_5\underset{\underset{OH^+}{\|}}{C} - CH{=}CH_2 + e \rightleftharpoons C_6H_5\underset{\underset{OH}{|}}{C} - CH{=}CH_2 \\ 2\ C_6H_5\underset{\underset{OH}{|}}{C}—CH{=}CH_2 \dashrightarrow \text{Dimer} \end{cases}$$

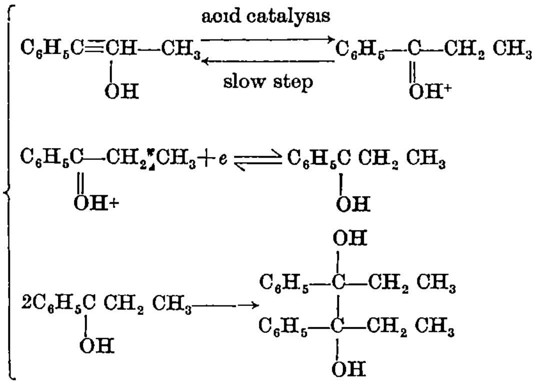
1 2



$$C_6H_5\underset{OH}{C}{-}CH{=}CH_2 + e \longrightarrow C_6H_5\,\underset{OH}{\overset{-\delta}{C}} \cdots CH \cdots \overset{-\delta}{CH_2}$$

$$C_6H_5\underset{OH}{\overset{-\delta}{C}} \cdots CH \cdots \overset{-\delta}{CH_2} + H^+ \longrightarrow C_6H_5\underset{OH}{C}{=}CH{-}CH_3$$

2 1



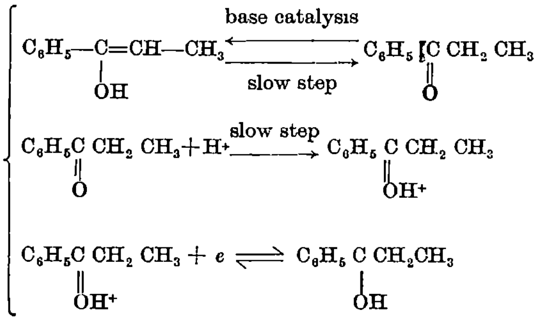
$$C_6H_5\underset{OH}{C}{=}CH{-}CH_3 \underset{\text{slow step}}{\overset{\text{acid catalysis}}{\rightleftarrows}} C_6H_5{-}\underset{\overset{\|}{OH^+}}{C}{-}CH_2\,CH_3$$

$$C_6H_5\underset{\overset{\|}{OH^+}}{C}{-}CH_2\,CH_3 + e \rightleftharpoons C_6H_5\underset{OH}{C}\,CH_2\,CH_3$$

$$2C_6H_5\underset{OH}{C}\,CH_2\,CH_3 \longrightarrow \begin{array}{c} OH \\ | \\ C_6H_5{-}C{-}CH_2\,CH_3 \\ | \\ C_6H_5{-}C{-}CH_2\,CH_3 \\ | \\ OH \end{array}$$

pH 4–6 Waves 1 1 and 1 2 identical as for $pH < 2$

2 1′



$$C_6H_5{-}\underset{OH}{C}{=}CH{-}CH_3 \underset{\text{slow step}}{\overset{\text{base catalysis}}{\rightleftarrows}} C_6H_5\,\underset{\overset{\|}{O}}{C}\,CH_2\,CH_3$$

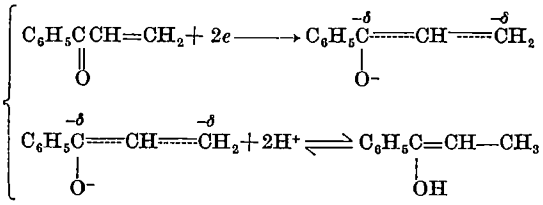
$$C_6H_5\underset{\overset{\|}{O}}{C}\,CH_2\,CH_3 + H^+ \xrightarrow{\text{slow step}} C_6H_5\,\underset{\overset{\|}{OH^+}}{C}\,CH_2\,CH_3$$

$$C_6H_5\underset{\overset{\|}{OH^+}}{C}\,CH_2\,CH_3 + e \rightleftharpoons C_6H_5\,\underset{OH}{C}\,CH_2CH_3$$

and dimerization as in 2 1

pH 6–9

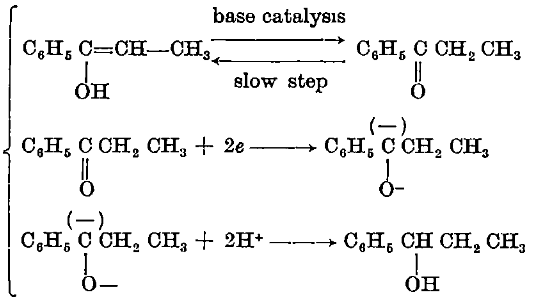
1 3



$$C_6H_5\underset{\overset{\|}{O}}{C}\,CH{=}CH_2 + 2e \longrightarrow C_6H_5\underset{O^-}{\overset{-\delta}{C}} \cdots CH \cdots \overset{-\delta}{CH_2}$$

$$C_6H_5\underset{O^-}{\overset{-\delta}{C}} \cdots CH \cdots CH_2 + 2H^+ \rightleftharpoons C_6H_5\underset{OH}{C}{=}CH{-}CH_3$$

2 3



$$C_6H_5\,\underset{OH}{C}{=}CH{-}CH_3 \underset{\text{slow step}}{\overset{\text{base catalysis}}{\rightleftarrows}} C_6H_5\,\underset{\overset{\|}{O}}{C}\,CH_2\,CH_3$$

$$C_6H_5\,\underset{\overset{\|}{O}}{C}\,CH_2\,CH_3 + 2e \longrightarrow C_6H_5\,\underset{O^-}{\overset{(-)}{C}}\,CH_2\,CH_3$$

$$C_6H_5\,\underset{O-}{\overset{(-)}{C}}\,CH_2\,CH_3 + 2H^+ \longrightarrow C_6H_5\,\underset{OH}{CH}\,CH_2\,CH_3$$

The factors governing the dependence of the limiting current on pH are, in acid solutions, the rate of the acid-catalysed ketone formation, in medium pH-range, either the rate of the base-catalysed ketone formation or of the protonation of the ketone formed, and finally at higher values of pH, base catalysis of the ketone formation.

Essentially the same explanation applies also for the dependence on pH of the limiting currents of acetophenone, when it is formed from $C_6H_5\,CO\,CH_2{-}X$ (X = halogen, NR_3^+, OR, SR, etc) observed by Zuman and Horák[1] and Lund[7] The enolate ion $C_6H_5\,CO\,CH_2(-)$ formed in the first step accepts a proton more quickly on oxygen than on carbon, and the polarographically inactive enol $C_6H_5\,C\,(OH){=}CH_2$ is thus the predominating form in the first reduction step

This enol is transformed either by an acid-catalysed reaction into the protonized form (2 1), when an increase of the one-electron wave with decreasing pH is observed, or by a base-catalysed reaction (2 1′), with subsequent protonation of the ketone formed The rates of ketone formation and protonation being comparable, the resulting current first increases and then decreases with increase of pH (Fig 2*b*). The decreasing part of this dependence, limited by the rate of protonation, is identical with that found for acetophenone Finally, the unprotonized keto form due to reaction catalysed by a base can be reduced in a two-electron step (2 3) at more negative potentials

In accordance with this supposition the dependence on pH of the more negative wave is little affected by

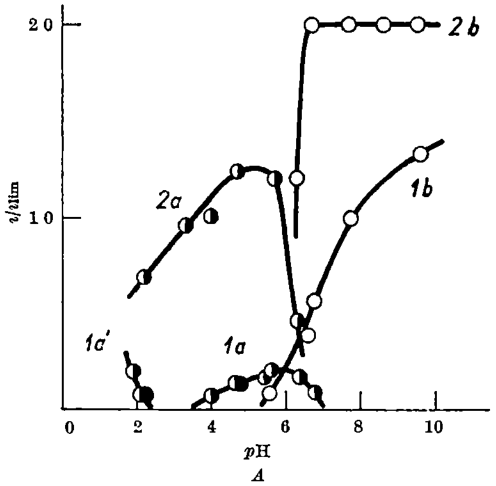


Fig 2 Dependence on pH of limiting currents
The more negative wave for phenyl vinyl ketone (1*a*, 1*b*) compared with waves for propiophenone (2*a*, 2*b*) 1*a*, 2*a*, one-electron wave, 1*b*, 2*b*, two-electron wave

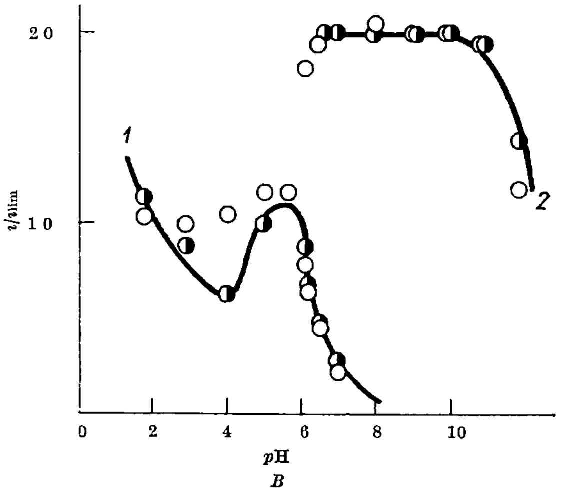


Fig 2 Dependence on pH of limiting currents
The more negative wave for ω-propiophenone methoiodide (halved circles) compared with waves for propiophenone (circles) 1, One-electron wave, 2, two-electron wave

the nature of X in C_6H_5 CO CH_2—X, but depends both on substitution in the phenyl ring and on the methylene group[1,7]

The course of the reduction of phenyl vinyl ketone is also consistent with the idea that the reduction of the protonized form leads to inactive C_6H_5 CH(OH)CH $=CH_2$, while that of the unprotonized form leads to C_6H_5 CO CH_2 CH_3 The increase in height of the wave would be a function of rate of proton transfers only, but this would scarcely explain the increase of the propiophenone wave in acid media

The effect of keto-enol tautomerism on the polarographic curves of ketones formed at the dropping mercury electrode is being investigated further

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BIOCHEMISTRY

Modification of the Properties of Flour Protein by Thiolated Gelatin

Recently, both Hird's group[1] and Mecham *et al* [2,3] have suggested that many characteristic properties of dough may be accounted for by postulating that the flour proteins participate in sulphydryl–disulphide interchange reactions Sokol and Mecham[4] have pointed out, however, that there is as yet no direct evidence that such reactions do occur in dough Thus further evidence bearing on this attractive hypothesis is desirable

As a contribution to this question we have examined the effect of directly adding, to dough, protein containing sulphydryl groups For this purpose we have used gelatin with —SH groups 'built in' by the thiolation procedure of Benesch and Benesch[5], comparing this thiolated protein with normal gelatin which has a negligible content of sulphydryl groups

The effect on some of the physical characteristics of dough was examined by stretching the doughs on an extensometer, thus obtaining load-extension curves (cf Halton[6]) Secondly, using these doughs, determinations were made of the content of hydroxyproline in the gluten, as this insoluble protein complex is primarily responsible for the rheological behaviour of dough Since there is about 14 5 per cent hydroxyproline in the gelatin and none in normal gluten, its presence would indicate an association in the gluten of flour proteins and gelatin

Unyeasted doughs were made by mixing 28 gm. flour with sodium chloride solution (2 5 per cent) Gelatin, thiolated gelatin or other substances to be added were dissolved in the salt solution immediately before adding it to the flour After mixing, the doughs were rested for 1 hr and then stretched on the extensometer These operations were carried out at 26 6° C Glutens were then washed from the doughs with warm water and hydrolysed with acid The neutralized hydrolysates were dissolved in 0·1 *M* citrate buffer, *p*H 2 2, and then passed through a column of 'Zeokarb 225' to separate hydroxyproline from tyrosine, which interferes in the determination Hydroxyproline was determined, in the appropriate fraction from the column, by the method of Neuman and Logan[7]

The extensometer measurements showed that with freshly prepared samples of thiolated gelatin the extensibility of the dough was increased and the elasticity decreased, as compared with the control doughs In the control experiments with normal, unthiolated gelatin no significant changes in the load-extension curves were observed Following Benesch and Benesch, the preparation of thiolated gelatin had been passed through a column of 'Amberlite 400' to remove any *N*-acetyl homocysteine formed during the thiolation reaction It is to be presumed, therefore, that the observed modification of the physical properties of dough were not due to traces of this compound but to protein sulphydryl

As additional control experiments we have also made doughs containing mixtures of normal gelatin with respectively cysteine, homocysteine, partially hydrolysed *N*-acetyl homocysteine thiolactone, and determined the hydroxyproline content of the gluten from these doughs The results in Table 1 show the quantity of gelatin 'bound' per 28 gm flour, as calculated from the content of hydroxyproline in the various gluten hydrolysates

Table 1

Additions to dough (28 gm flour)	Gelatin added to dough (mgm)	Gelatin 'bound' to gluten (mgm)
Normal gelatin	156	5
Normal gelatin	156	5
Thiolated gelatin	160	60
	80	38
Gelatin and homocysteine	156	7
Gelatin and cysteine	156	6
Gelatin and partially hydrolysed	160	6
N-acetyl homocysteine thiolactone	160	8

These results show that while a significant proportion of the thiolated gelatin becomes firmly associated with the gluten, this degree of binding is not observed with gelatin or with mixtures of gelatin and —SH compounds of low molecular weight The evidence at present is insufficient to decide whether this is due to interchange reactions leading to the formation of mixed flour protein–gelatin disulphides or whether it is due to the association of the thiolated gelatin by other mechanisms, for example, by hydrogen bonding, or formation of insoluble disulphide gelatin Experiments are in progress which it is hoped will provide further evidence on this question.

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Separation of Low Molecular Weight Peptides from Amino-Acids on 'DEAE-Sephadex'

METHODS are now available for the separation of amino-acids from peptides, with a molecular weight greater than 1,000, by molecular sieving on cross-linked dextran gels[1,2] However, the separation of peptides of lower molecular weight from all their constituent amino-acids is often difficult because of their similar physical properties Recently I described a method for the separation of neutral amino-acids from neutral peptides of low molecular weight on the anion exchanger 'Dowex-1' with ammonium acetate buffer pH 8 65 (ref 3) At this pH the monoaminomonocarboxylic acids, which all have pK values for the amino group greater than 9 0, are predominantly in the neutral form, whereas many peptides of these amino-acids have pK values less than 8 3 and thus are predominantly in the anionic form (pK values from Cohn and Edsall[4]) This method had the disadvantages that the aromatic amino-acids were strongly adsorbed on the resin, and that traces of amino-acids contaminated the peptide fraction

An improved method is now described using the anion exchanger 'DEAE-Sephadex', with collidine acetate buffer and a pH gradient elution Rudloff and Braunitzer[5] used this buffer and 'Dowex-1' resin for the fractionation of peptides from a partial hydrolysate of hæmoglobin This buffer system has several advantages over the ammonium acetate solution used in the earlier method. It gives better control of pH, is more volatile, and does not interfere with the estimation of amino-acids and peptides by the ninhydrin reagent[6] By using a pH gradient elution it is possible to separate neutral peptides from acidic amino-acids Mixtures of pyridine and acetic acid have been recommended as solvents for separation by molecular sieving on 'Sephadex' and 'DEAE-Sephadex' (ref 2)

There are also advantages in using the diethyl-amino-ethylether (DEAE) derivative of cross-linked dextran gel rather than the quaternary ammonium derivative of polystyrene DEAE exchangers have a reasonably high capacity in the pH 8 region[7] With 'DEAE-Sephadex' the functional groups are mainly inside the gel grains, and thus when they are charged, ions of opposite charge will be repelled from the grains Porath and Lindner[2] showed that aromatic compounds, which are strongly adsorbed on polystyrene resins and dextran gels, can be effectively fractionated on 'DEAE-Sephadex', since intimate contact with the strong adsorption centres is avoided. Hence, a separation of aromatic amino-acids from peptides should be possible on this material.

To illustrate the use of this method, a mixture of a typical tripeptide, DL-leucylglycylglycine, and twelve common L-amino-acids was fractionated and the result is shown in Fig 1 'DEAE-Sephadex' A25 (Pharmacia, Uppsala, Sweden, Lot No *To*7871M, particle size 100–270 mesh) was cycled with 0 5 M hydrochloric acid and 0 5 M sodium hydroxide and converted to the acetate form with 0 5 M acetic acid A column 20 cm × 1 cm diameter was thoroughly equilibrated with 0 1 M collidine (2,4,6,-trimethyl-pyridine) acetate buffer pH 8 55, prepared from water free from carbon dioxide The amino-acids were each approximately 2 5 mM and the peptide 5 0 mM in the buffer with the pH adjusted to 8 45 Cystine, which would be eluted with the peptide, was first partially oxidized to cysteic acid by treating 1 0 ml of the mixture with 0 05 ml 30 per cent hydrogen peroxide and evaporating to dryness, *in vacuo*, in less than 10 min at room temperature The mixture was transferred to the column with 1 5 ml buffer A total of 20 ml of buffer was placed between the top of the column and the mixing chamber, which contained 150 ml buffer Elution was started and a gradient in pH established using 0 1 M acetic acid until fraction No 45, when 1 0 M acetic acid was used Fractions of 2 5 ml volume were collected at a rate of 11/hr The temperature was 30° C (room temp) From each fraction 0 5 ml was analysed by the quantitative ninhydrin reagent[6], and the results expressed as leucine equivalents (μmoles per fraction) The pH of each fraction was determined (some absorption of carbon dioxide occurred in the more alkaline fractions while they were in the collector, causing a slightly low value for the pH) The amino-acid composition was determined by paper chromatography

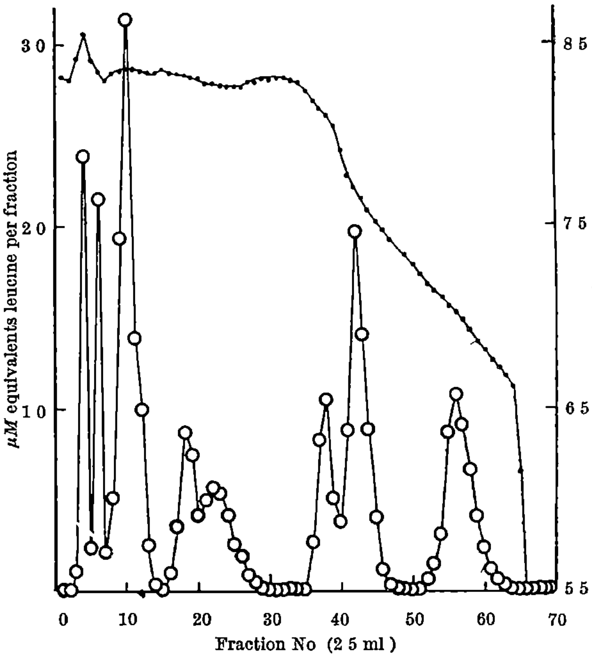

Fig 1 Separation of DL-leucylglycylglycine from L-amino-acids on a column of 'DEAE-Sephadex' A25 (for details see text) ○, total ninhydrin colour of fraction expressed as μM equivalents of leucine, ●, pH of fraction, Peak 1, lysine, 2, proline (as μM proline), 3, alanine, glycine, valine, leucine, 4, serine, 5, phenylalanine, 6, tyrosine, 7, leucylglycylglycine, cystine, 8, glutamic acid, aspartic acid

The amino-acids were eluted from the column in the order expected from the pK values of their amino groups[4] The aromatic amino-acids were adsorbed to some extent but not so strongly as on the 'Dowex-1' resin Only fraction No 40 contained both tyrosine and leucylglycylglycine Their separation would be improved by re-running and taking smaller fractions. As the pH value of the eluant fell below 7 9 the peptide began to emerge. Cystine, because of its slightly acidic character, emerged with the peptide. It was possible to remove only 46 per cent of the cystine by the oxidation procedure used More vigorous oxidation may damage other amino-acids[8]. However, cystine can be easily removed from most peptides by partition chromatography When some other neutral peptides

DL-alanylglycine, DL-alanyl-DL-alanine, DL-alanyl-DL-methionine, DL-alanylglycylglycine, glycylglycine, diglycylglycine, glycl-L-leucine and glycyl-L-tryptophan) were chromatographed on this system, they also emerged when the *p*H value of the eluant fell below 8 0 No difficulty was experienced with the aromatic peptide glycylphenylalanine

With the earlier method[3] carbon dioxide was found to interfere with the separation, and the formation of carbamino-acids was suggested to explain the presence of amino-acids in the peptide fraction However, using 'DEAE-Sephadex' it was possible to separate leucine from glycylglycine with 0 1 *M* collidine carbonate buffer *p*H 8 5 This matter is being investigated in greater detail Recently the separation of amino-acids and peptides on DEAE-cellulose with carbonic acid was reported[8].

I wish to thank Prof T F Dixon for his criticism of the manuscript, Dr R L M Synge for his interest and encouragement of this work, and S Kanason for technical assistance

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Demonstration and Some Properties of Peroxidase in Pig Thyroid Gland

ALTHOUGH it has been postulated by various authors[1,2] that the thyroid contains a peroxidase which plays an essential part in the synthesis of organically bound iodine, the available data do not seem to be sufficient to prove its presence This communication submits distinct evidence for the presence of peroxidase in pig thyroid tissue, especially in its microsomes

In this work an assay[3] employing guaiacol instead of iodide as a hydrogen donor was used, since this method is not only sensitive and convenient to carry out but also makes it possible to obtain the initial rate of reaction, which is difficult to measure using iodide as a hydrogen donor Peroxidase activity in the preparation was expressed in terms of specific and total activity (see Table 1)

It was found by this assay method that thyroid homogenate always revealed fairly strong peroxidase activity although the activity varied considerably from preparation to preparation The activity in the homogenate disappeared entirely after boiling for 2 min.

Table 1 INTRACELLULAR LOCALIZATION OF PEROXIDASE IN PIG THYROID

Pig thyroid slices were washed repeatedly with 0 9 per cent sodium chloride solution, blotted with filter paper, and a 10 per cent tissue suspension was homogenized in a 0 25 *M* sucrose solution using a Waring blender Sediments obtained by differential centrifugation were dispersed uniformly in phosphate buffer, *p*H 7 0 The enzyme assay system contained 1 0 ml of 0 1 *M* phosphate buffer, *p*H 7 0, 1 0 ml of 0 1 *M* guaiacol an adequate amount of the tissue fraction, 0 027 ml of 0 03 *M* hydrogen peroxide, and water to a final volume of 3 03 ml The over-all reaction was followed by a Beckman spectrophotometer at 20° C by measuring the absorbance at 470 mμ (ref 3) One unit of peroxidase activity is defined as an increase in absorbance of 1 0 per sec under the conditions specified above Specific activity refers to the number of units per mgm of nitrogen

Fraction	Preparation	Specific activity (milli-units per mgm nitrogen)	Total activity (milli-units per gm wet tissue)
Homogenate	*a* *b*	12 5 9 5	250 102
Sediment 700*g*, 10 min (nuclei)	*a* *b*	19 0 9 5	24 0 31 0
Sediment 5,000*g*, 10 min (mitochondria)	*a* *b*	16 3 23 6	12 1 11 3
Sediment 80,000*g*, 30 min (microsomes)	*a* *b*	77 0 68 1	58 5 63 0
Supernatant 80,000*g*, 30 min	*a* *b*	2 2 0 9	17 5 6 1

a refers to experiments that started from pooled glands of seven pigs, *b* from ten pigs

The distribution of peroxidase activity in the subcellular fractions is given in Table 1, which shows that both specific and total activity are greatest in the microsomal fraction Appreciable activity was also found in the nuclear and mitochondrial fractions, but this is thought to be attributable to unbroken cells and concomitant microsomes, respectively The activity due to contaminating hæmoglobin in the particulates was estimated to be negligibly small on the basis of the measurement of the amount of hæmoglobin contained in the preparations and of the peroxidative activity of crystalline horse hæmoglobin under the same conditions

The microsomal peroxidase was found to have the following properties (1) The Lineweaver–Burk plot at a constant concentration of guaiacol (33 m*M*) was linear in the range of the concentration of hydrogen peroxide, 0 3–0 03 m*M* Beyond a concentration of hydrogen peroxide of 0 4 m*M*, distinct inhibition was observed, increasing with the increase in the hydrogen peroxide concentration The Lineweaver–Burk plot at a concentration of hydrogen peroxide of 0 27 m*M* was linear in the range of the concentration of guaiacol studied (64–0 9 m*M*) (2) The *p*H optimum was about 8 6 under the conditions of the standard assay system except in the case of *p*H. (3) The enzyme was inhibited by potassium cyanide, sodium azide, and sodium fluoride, in such a way that the percentage inhibition versus the logarithm of the concentration of each reagent represented a sigmoid curve of the first order The concentrations for 50 per cent inhibition were $2\ 2 \times 10^{-6}$ *M*, $1\ 3 \times 10^{-5}$ *M* and $5\ 8 \times 10^{-3}$ *M*, respectively The inhibition by cyanide was caused, on the basis of kinetic study, by the competition with hydrogen peroxide for the enzyme These facts suggest that the enzyme is probably a hæmoprotein, as are other known peroxidases (4) The enzyme was completely inhibited by thiouracil and 3-amino-1,2,4-triazole at a concentration of 2 m*M*, whereas it only partially inhibited by the same concentration of potassium perchlorate and potassium thiocyanate

It was found that the microsomal peroxidase was rendered soluble by treatment with 1 per cent digitonin for 40 min at 2° C This enzyme was further purified about two-fold by salting-out with ammonium sulphate between 40 and 60 per cent saturation. The purified enzyme revealed practically the same properties as the one bound in the microsomes.

The partially purified enzyme preparation was able to catalyse, in the presence of enzymically generated hydrogen peroxide, the incorporation of substrate amount of iodide into thyroglobulin as efficiently as thyroid particulate fraction did[4] It was also possible (unpublished observations of Dr M Suzuki of this Institute) for the enzyme preparation to catalyse the iodination reaction of tyrosine under the experimental conditions of Alexander[2] These results seem to show that the thyroid peroxidase dealt with in this communication catalyses the peroxidation of iodide and probably plays an important part in the hormone synthesis in thyroid tissue

We thank Dr M Suzuki of this Institute for carrying out one of the experiments

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Transaminase Activity in Arterial Tissue of the Rabbit

Rinehart and Greenberg[1,2] observed a marked thickening of the intima and deposition of lipid in pyridoxine-deficient monkeys Though the role of pyridoxine as an essential co-factor in fat and protein metabolism has been well established, the precise mechanism of atherogenesis in pyridoxine deficiency has not been elucidated Goswami and Sadhu[3] have adduced evidence tending to show that the hypercholesterolæmia of pyridoxine deficiency is not related to impairment of formation of arachidonic acid. The possibility that atherogenesis may be a consequence of defective formation and maintenance of collagen and elastic tissue brought about by decreased transaminase activity therefore gains added importance However, while the transaminase activities of several tissues, both under normal and pathological conditions, have been examined, very little work has been reported on the transaminase activity of arterial tissue The present communication reports the demonstration of significant transaminase activity in arterial tissue

Adult male rabbits weighing approximately 1 kgm were killed, and different segments of arterial tissue dissected out, homogenized in a Potter–Elvehjem homogenizer at 0° C with disodium phosphate (0 1 M) buffer of *p*H 7 4, and centrifuged at 2,500 r p m for 10 min in the cold The supernatant was examined for transaminase activity Portions were incubated with different L-amino-acids (glutamate, aspartate and alanine) as amino donor and α-keto acids (α-ketoglutarate, oxaloacetate and pyruvate) as amino acceptor. The incubation mixture for the assay of enzyme activities consisted of 1 0 ml of crude extract (0 3 mgm nitrogen/ml), 0 2 ml of 0 1 M amino donor and 0 2 ml of 0 2 M amino acceptor The samples were incubated for 1 hr at 37° C The protein was precipitated by boiling the samples for 10 min , cooling and then adding acetone to 5 ml volume The samples were centrifuged and 0 04 ml of the supernatant fluid was taken for paper chromatographic determination of amino-acid in the reaction product (Table 1)

High transaminase activity was noticed with aspartate + α-ketoglutarate and with glutamate + oxaloacetate No transaminase activities were noticed with glutamate + pyruvate, aspartate + pyruvate, alanine + α-ketoglutarate or alanine + oxaloacetate

Table 1 TRANSAMINATION OF DIFFERENT L-AMINO-ACIDS WITH α-KETO ACIDS

Amino donor	Amino acceptor	Amino-acid formed
Aspartate	α-Ketoglutarate	Glutamate
Alanine	α-Ketoglutarate	Nil
Glutamate	Oxaloacetate	Aspartate
Aspartate	Oxaloacetate	Nil
Aspartate	Pyruvate	Nil
Glutamate	Pyruvate	Nil

The variation in transaminase activity within the *p*H range 4 0–10 0 was examined using aspartate and α-ketoglutarate The optimum *p*H was found to be between 7 0 and 8 0, maximum transamination of the keto-acid was observed after 30 min of incubation, with no further increase thereafter. Ageing of the extract at room temperature (30° C) for 4–6 hr or in the refrigerator for 4–5 days did not lower the transaminase activities appreciably

The transaminase activity was found to be inhibited after pre-incubation of the extract (at 37°) with *p*-chloromercuric benzoate (10^{-4} M), mercuric chloride ($5 \times 10^{-5} M$) and silver nitrate (5×10^{-5} M). But when incubated with cysteine, *p*-chloromercuric benzoate could not inhibit the transaminase activity Enzyme activity was unaffected by pre-incubation with deoxypyridoxine (10^{-4} M), potassium cyanide (10^{-4} M), hydroxylamine hydrochloride (10^{-4} M), 2,4-dinitrophenyl hydrazine (10^{-4} M), 8 0 oxiquinoline (10^{-4} M) and penicillamine (10^{-4} M)

Detailed work dealing with the co-factor requirements, purification, kinetics and other properties of the transaminase system in normal and atherosclerotic arterial tissues will be reported elsewhere

I am grateful to Dr J C Ray, director of this Institute, and to Dr A Narayanaswami for their interest in this work

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Interaction of ^{14}C-Œstrone with Tryptophan in the Presence of Peroxidase

IN 1955, Stotz *et al* [1] discovered an œstrogen-sensitive peroxidase in rat uterus and later these workers[2] found that those tissues which had a high rate of cell renewal also had high peroxidase activity This enzyme may therefore play a significant part in cell proliferation and growth, and it was thought of interest to investigate its effect on œstrogens, using initially the very stable and more readily available horseradish enzyme In preliminary experiments[3] it has already been shown that œstrone and other phenolic œstrogens could interact with protein and certain amino-acids when incubated with peroxidase under aerobic conditions, and the present communication deals with this reaction involving tryptophan

(16–^{14}C)œstrone (4,000 c p m in 10 μgm) dissolved in 5 ml phosphate buffer *p*H 7 4 was incubated under oxygen with tryptophan (3×10^{-3} M), manganese chloride (10^{-5} M) and horseradish peroxidase (10 μgm), for 5 hr at 37° C with constant shaking, and the amount of radioactivity remaining in the aqueous medium after extraction with ether was determined This was repeated under various experimental conditions In addition, each ethereal fraction was examined for œstrone or its metabolites by chromato-

Table 1 PERCENTAGE CONVERSION OF ^{14}C-ŒSTRONE TO WATER-SOLUBLE ETHER-INSOLUBLE PRODUCTS BY PEROXIDASE IN THE PRESENCE OF TRYPTOPHAN UNDER VARIOUS EXPERIMENTAL CONDITIONS

Experimental conditions	Percentage of added radioactivity remaining in aqueous medium after extraction with ether
Normal incubation*	61 5 68 5
$MnCl_2$ omitted	4 8 5 5
Tryptophan omitted	1 1 1 0
Peroxidase solution boiled	1 6 1 2
Anaerobic incubation	0 8
Incubated with KCN (10^{-3} M)	0 5
Incubated with sodium azide (10^{-2} M)	14 5
Incubated with sodium iodoacetate (10^{-}[illegible] M)	57 3
Incubated with catalase (100 μgm)	1 1
Kynurenine substituted for tryptophan	5 5
$MnCl_2$ omitted and incubated with H_2O_2 (2×10^{-5} M)	14 2
$MnCl_2$ omitted, kynurenine substituted for tryptophan and incubated with H_2O_2 (2×10^{-5} M)	13 8
Tryptophan added after incubation and solution immediately extracted with ether	2 2
^{14}C-Œstrone added after incubation and solution immediately extracted with ether	2 0

* Incubation mixture peroxidase, 10 μgm , œstrone, 10 μgm , $MnCl_2$, 10^{-5} M, tryptophan, 3×10^{-3} M, in 5 ml

graphy[4] followed by autoradiography[5] The anaerobic incubation was carried out in an evacuated Thunberg tube.

The results in Table 1 show that horseradish peroxidase, in the presence of tryptophan, is able to convert œstrone to water-soluble ether-insoluble metabolites in high-percentage yields No such products were formed in the absence of this amino-acid and only unchanged œstrone could be detected in the ethereal fraction, showing that tryptophan was directly involved in the reaction and was not merely acting as a carrier No water-soluble compounds were formed when tryptophan or œstrone was added only after incubation and the solutions immediately extracted with ether, and there is evidence that the tryptophan molecule was altered during the reaction This was indicated by the yellow colour of the solution after incubation and the change in ultra-violet spectrum, and was confirmed by chromatography

The reaction was dependent on manganese ions and was abolished under anaerobic conditions or on boiling the peroxidase solution Cyanide and azide, which are known to affect iron-containing enzymes, were also found to be inhibitory, but iodoacetate, which combines with sulphydryl groups, had no effect

The marked inhibition by catalase suggests that hydrogen peroxide is involved in the reaction and although added hydrogen peroxide in the absence of manganese was not very effective, even at concentrations above 2×10^{-5} M, the results agree with the findings of Knox and Mehler[6], who showed that only slowly generated peroxide could be utilized efficiently for the breakdown of tryptophan by liver peroxidase-oxidase Kynurenine, an oxidation product of tryptophan, which is not known to generate hydrogen peroxide with peroxidase, gave only very low results, but with added peroxide the yield of water-soluble products was as high as with the parent amino-acid

These results suggest that horseradish peroxidase, acting as an aerobic oxidase, can utilize tryptophan to generate hydrogen peroxide and that afterwards, behaving like a true peroxidase, it converts œstrone to metabolites which interact with this amino-acid or its oxidation products However, the final proof for this hypothesis must await the isolation and identification of the water-soluble complexes and it is also hoped to determine their physiological activity

We wish to thank Prof R L Noble and the National Cancer Institute of Canada for their support of this work

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Auxin Activity in Ether-insoluble Fractions of Extracts of Rhubarb Petioles

DURING the past four years efforts have been made in this laboratory to evolve methods suitable for the purification of coleoptile growth promoters known to be present in water-soluble, ether-insoluble fractions of plant extracts In previous work[1] ethanolic extracts from which ether-soluble compounds had been removed were chromatographed directly In the present work large quantities of elongating rhubarb petioles were extracted, and it was necessary to purify these 'final ethanol extracts' further Two of the most promising procedures are described here

(1) *Alumina column purification* Very clean preparations are obtained when the 'final ethanol extract' is run through an alumina column It has not been possible to specify the exact conditions of experiment necessary to give an active extract Fig 1 shows some of the properties of an active preparation The general trend of the results has been confirmed in independent experiments, and is similar in many ways to other work on so-called 'interconvertible' auxins[1,2] Four identical chromatograms were prepared , one was assayed directly (*a*), and selected zones on the others were eluted in ethanol and then re-chromatographed (*b*–*e*) Since the activity is highly labile, distilled water was chosen as the chromatographic solvent, because of its neutrality and speed of running Relative to the original chromatogram, the eluates show greater activity, except in the case of the R_F 0 55–0 7 eluate (*c*), which lies between two sources of promoter(s) on the original strip Elution without re-chromatography does not give a comparable release of activity. Thus, when segments of a chromatogram are placed in separate vials and treated with ethanol for 2 hr (as in the standard elution procedure), then the ethanol evaporated off and bioassay medium added, there is no enhancement (*f*) If the whole chromatogram is eluted and re-chromatographed, there is enhancement (*e*) It may be suggested that losses during manipulation lead to an increase in promotion by diluting the original quantities to less than supra-optimal concentrations Alternatively, release of promoter(s) from precursor(s) may be involved.

(2) *Tert -butanol chromatography* Active preparations quite distinct from those described above can be obtained consistently by applying the 'final ethanol extract' to Whatman No 3 paper as a strip and chromatographing at 30°–35° C using *tert* -butanol as solvent. Much anthocyanin and other material is retained in the R_F 0–0 5 region, while some stimulators are washed farther down the paper (Fig 2*a*).

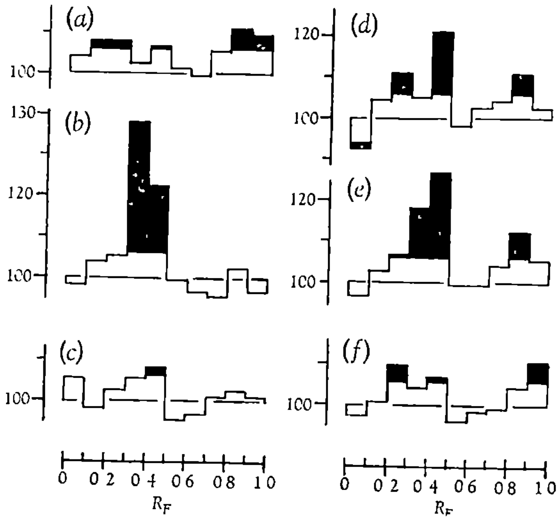

Fig 1 *Avena* coleoptile straight-growth activity on chromatograms (method as in ref 1) Vertical scales represent section length as percentage of controls Responses outside fiducial limits of controls are blacked in Each chromatogram represents 10 gm fresh weight of petioles *a*, Assayed directly, *b*, *c*, *d* and *e*, assayed after zones 0 2–0 5, 0 55–0 7, 0 8–1 0 and 0–1 0 respectively had been eluted and re-chromatographed, *f*, see text All assays were carried out on the same occasion

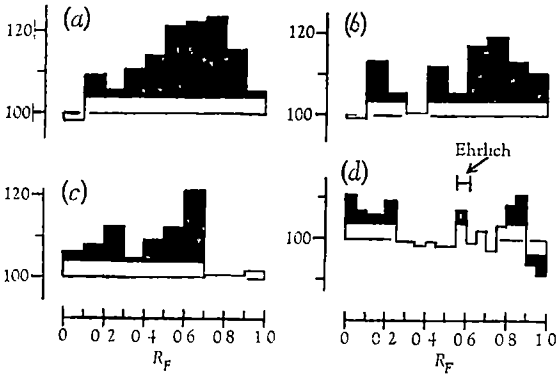

Fig 2 *a*, *Tert* -butanol chromatogram of final ethanol extract, *b*, *c* and *d*, are re-chromatographed eluates using distilled water, *b*, isobutanol/methanol/water, 80 5 15, *c*, and isopropanol/0 880 ammonia/water, 8 1 1, *d*, as solvents *d* represents 16 gm, the others 10 gm fresh weight of petioles

This active zone can then be eluted and re-chromatographed with other solvents on Whatman No 1 paper Histograms of activity for three solvents that have been commonly used in investigations of ether-insoluble auxins[1,2] are shown in Fig 2*b*, *c* and *d*.

Identification of two of the spots resolved by isopropanol / ammonia / water was achieved using an extract of 8 kgm of petioles.

Spot (i) R_F 0–0 25. Elementary analysis, titrations, ion-exchange chromatography, and infra-red spectrum identified the material as the half ammonium salt of malic acid. Malic acid itself is not a growth stimulator in the assay system used (the medium contains citrate as part of the buffer solution), but the half ammonium salt, and ammonium (as phosphate) are slightly active This particular spot may therefore be an artefact of ammoniacal chromatography

Spot (ii) This was located by its Ehrlich reaction (Fig. 2*d*) rather than its weak growth activity It was eluted and shown to co-chromatograph with 2·7 × 10^{-3} μc aliquots of carbon-14 labelled tryptophan in a series of 21 different one-dimensional solvent systems

When this *tert* -butanol method of purification was used checks were made to show that the 'alumina auxins' were absent There are other indications that the two methods yield quite different substances For example, the '*tert* -butanol' group do not show R_F shifts, or release of growth activity when zones of chromatograms run using water as solvent are eluted and re-chromatographed Again, they are stable, and do not pass through an alumina column

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PHYSIOLOGY

Peroxide-like Substance in Alarmed Mice

WHILE studying the production of an oxidizing agent which could be extracted with butanol (hereinafter called 'apparent peroxide') in X-irradiated mice, we found that some was produced without irradiation, merely by transferring the mouse somewhat roughly to the small irradiation chamber (Work on irradiated and dummy-irradiated mice will be submitted to the *International Journal of Radiation Biology*) As this phenomenon may be of physiological interest we have made a few further observations on it

The apparent peroxide was estimated with anaerobic leuco brilliant cresyl blue, approximately as previously described[1]. The temporal sequence of experimental and control mice was randomized to eliminate secular effects Table 1 shows that merely lifting mice by their tails with rubber gloves (to avoid possible transfer of peroxide from the hands) produced a statistically significant difference in peroxide between six experimental animals and six controls. Table 2 shows the larger effect of lifting by the scruff of the neck with polythene-covered forceps. Fig 1 shows that the increase was maximal within 1 min after lifting with forceps and that it fell to half the maximum in about 97 min

Table 1

Micromoles of peroxide/gm of mouse

	Not handled	Lifted by tail
	0 13	0 24
	0 19	0 28
	0 23	0 25
	0 24	0 27
	0 28	0 25
	0 20	0 27
Mean	0 21	0 26

The difference in means would occur by chance about 1 in 50 times and is therefore significant

Table 2

Micromoles of peroxide/gm of mouse

	Not handled	Lifted by scruff of neck with forceps
	0 23	0 35
	0 13	0 39
	0 27	0 33
	0 25	0 36
	0 24	0 37
Mean	0 22	0 36

The difference in means would occur by chance less than 1 in 1,000 times and is therefore significant

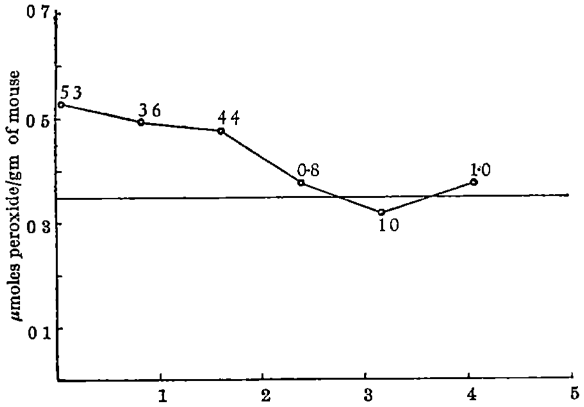

Fig 1 Peroxide in alarmed mice as a function of time The figure beside the points are a value of the statistic *t* between each experimental group (wavy line) and the untreated group (horizontal straight line) The 5 per cent and 1 per cent levels of *t* are 2 18 and 3 05 respectively The biological half-life of the peroxide is estimated by interpolation to be 97 min Each point represents a mean of seven mice The times were randomized in a Latin square

The identity of the peroxide-like substance or substances concerned is unknown. Adrenochrome, a possible though doubtful metabolite of adrenaline, has the necessary chemical properties ; but Table 3 shows no mean difference between mice injected intraperitoneally with adrenaline and with saline, though both increase the apparent peroxide presumably through alarm from the injection

Table 3

Micromoles of peroxide/gm of mouse

	Injected with saline	Injected with adrenaline
	0 29	0 38
	0 29	0 34
	0 36	0 25
	0 33	0 22
	0 38	0 44
Mean	0 33	0 33

With adrenocorticotrophic hormone (Table 4) only a small mean difference above saline, statistically insignificant with groups of five mice, was found.

Table 4

Micromoles of peroxide/gm. of mouse

	Injected with saline	Injected with adrenocorticotrophic hormone (0 05 unit/mouse)
	0 25	0 38
	0 30	0 33
	0 25	0 24
	0 35	0 37
	0 24	0 31
Mean	0 28	0 33

The difference in means would occur by chance about 1 in 5 times and is not significant

It is therefore possible that the present phenomenon depends on some hitherto unknown component of the total response to alarm, involving perhaps autoxidation of unsaturated substances such as linoleic acid to form hydroperoxides

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Evidence for Independence of Voltage of the Membrane Conductance of Rabbit Ventricular Fibres

AT the present time the available evidence indicates that the prolonged repolarization phase of the cardiac action potential is associated with a drop in the membrane conductance to potassium ions (g_K) which occurs during, or shortly after, the upstroke of the action potential and persists for the major part of the repolarization phase Noble[1] has put forward a mathematical model which reproduces Purkinje-like action potentials In this model, g_K is an instantaneous function of the membrane potential and falls when the membrane is depolarized

We have previously shown[2,3] that the displacement of membrane potential produced by hyperpolarizing current applied during the repolarization phase of rabbit ventricular action potentials bears a linear relationship to the magnitude of the applied current This finding suggests that the ionic conductance of the membrane does not change with the value of the membrane potential, as it should were it to conform with Noble's model

The following experiment further affirms the independence of voltage of the ionic conductance of membrane action potentials of rabbit ventricular fibres The tissue was stimulated at a rate of 3 4 sec^{-1} Double-barrelled microelectrodes were used together with a current clamp and associated apparatus previously described[4] Hyperpolarizing current was passed through the cell membrane via one barrel of the electrode, the other barrel recording the resulting potential change The current was started approximately 50 msec before the upstroke of every fourth propagated action potential and terminated during the early half of the repolarization phase. A typical result is shown in Fig 1 A fairly accurate estimate of the hyperpolarization produced at any time is afforded by superimposing the crests of the two action potentials and measuring the potential displacement of the normal action potential and that occurring during flow of current[4].

If the ionic conductances were dependent on voltage then, during the hyperpolarization, they would assume new values, so that, at the break of the current pulse, the subsequent time course of potential

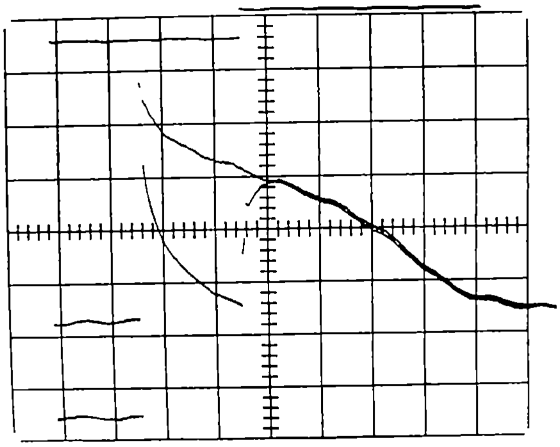

Fig 1 Top trace current (hyperpolarizing), calibration, 0 25 μamp per large division Lower trace upper of the two, an action potential recorded before the application of current, lower trace action potential recorded during current flow The current pulse was started 50 msec before the upstroke of the action potential and terminated, as shown, during the repolarization phase Calibration 25 mV and 20 msec per large division

should differ from that of a normal action potential. The outcome of the experiment is clearly shown in Fig. 1, where the voltage displacement is close to 50 mV. just prior to the break of the current. After the break of the current the membrane potential returns within 10 msec to a time-course identical to that of the preceding normal action potential. During this 10-msec. interval, a brief voltage spike may occur. Its amplitude depends on the value of the current and the time of break of the current. We believe that this spike is the result of a localized, non-propagated increase in sodium conductance subsequent to 'reactivation' produced by the preceding hyperpolarization. However, even in these instances, the membrane potential returns to its normal time-course after the spike.

These facts are in accord with the hypothesis that a sodium-carrying mechanism dependent on voltage exists in rabbit ventricular fibres, but that the subsequent decrease in g_K, which normally follows the activation of such a sodium-carrying mechanism, is independent of voltage.

This investigation was supported by the Life Insurance and Medical Research Fund of Australia and New Zealand.

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Effect of L-Glutamic Acid on the Weight of Embryonic Chick Tibiotarsi cultivated *in vitro*

IN a previous communication[1] the dose-response line relating the final length of embryonic chick tibiotarsi cultivated *in vitro* and the concentration of L-glutamine in the medium was described. Furthermore, it was shown that the presence of 25 mgm per 100 ml. L-glutamic acid in the medium had no effect on the position of the L-glutamine dose-response line. From this result it was argued that L-glutamic acid does not act as a precursor for L-glutamine in this system. The purpose of the present communication is to present additional results on the wet and dry weights of the same bones. These results were not included in the first communication since their discussion was irrelevant to the point at issue, and could have created confusion.

The experiment was a 2×3 factorial design: 1, 5 and 25 mgm per ml. L-glutamine and 0 and 25 mgm per 100 ml. L-glutamic acid were incorporated in all six treatment combinations in the chemically defined medium *BGJb*[2]. The experimental error was reduced by utilizing a balanced incomplete block design for comparing six treatments in blocks of two, thereby exploiting the uniformity of the pair of bones from each embryo. The bones were cultivated, using the floating lens paper technique, at 37° C. for six days in an atmosphere of humidified 5 per cent carbon dioxide in air. The medium was renewed every second day. At the end of the experiment the wet weight and dry weight of each bone were determined on a Cahn electric microbalance using the techniques described elsewhere[3]. The analyses of variance were done after all the data were converted to logarithms.

Table 1. ADJUSTED MEAN LOG WET WEIGHTS (MGM.) AND MEAN LOG DRY WEIGHTS (μGM.) OF SEVEN-DAY OLD EMBRYONIC CHICK TIBIOTARSI AFTER CULTIVATION FOR 6 DAYS ON MEDIUM *BGJb*, IN THE PRESENCE OF VARIOUS COMBINATIONS OF L-GLUTAMINE AND L-GLUTAMIC ACID. EACH MEAN IS BASED ON FIVE OBSERVATIONS

	L-Glutamine (mgm. per cent)					
L-Glutamic acid (mgm. per cent)	log wet weight 1	5	25	log dry weight 1	5	25
0	0·032	0·088	0·305	1·932	1·979	2·177
25	0·102	0·230	0·403	2·062	2·104	2·259

Analyses of variance
(Calculated using the methods of Kramer and Bradley, ref. 4)

Source of variation	D.F.	Mean squares log wet weight	Mean squares log dry weight
Between embryos (unadj.)	14	0·0175	0·0164
Between treatments (adj.)	(5)		
L-glutamic acid	1	0·0481*	0·0566*
L-glutamine			
Linear	1	0·2480‡	0·1463†
Quadratic	1	0·0105	0·0176
Interaction	2	0·0020	0·0011
Intrablock error	10	0·0055	0·0093

*$0·05 > P > 0·01$; †$P < 0·001$; ‡$0·01 > P > 0·001$.

The adjusted means are shown in Table 1, together with the analyses of variance. As with the results on length[1] there are highly significant linear regressions of log wet weight and log dry weight on the logarithm of the concentration of L-glutamine in the medium. The mean squares for L-glutamic acid are both significant at the $P = 0·05$ level. Inspection of the adjusted means shows that the presence of L-glutamic acid has increased the wet and dry weights of the bones at all levels of L-glutamine. Since the interaction mean square is not significant in either analysis of variance we may assume that the L-glutamine dose-response lines for both responses, in the presence and absence of L-glutamic acid, are parallel. The vertical distance between these lines is an estimate of the logarithm of the ratio between the weight of a bone in the presence of L-glutamic acid and the weight of a bone in the absence of L-glutamic acid. These ratios, and their fiducial limits of error ($P = 0·05$), have been calculated from the data in Table 1, and are found to be: wet weight, 1·27 (1·06–1·52), dry weight, 1·30 (1·03–1·64). The corresponding ratio for the data on length is 1·03 (0·90–1·18). The difference between the results for length and wet and dry weights is therefore considerable. Further statistical analysis showed that L-glutamic acid and L-glutamine had no significant effects on the dry weight/wet weight ratios of the rudiments.

The results demonstrate that the weights of embryonic chick tibiotarsi are increased by supplementing medium *BGJb* with L-glutamic acid. The increase is 27 per cent in wet weight and 30 per cent in dry weight. Medium *BGJb* contains twelve essential amino-acids and L-glutamine[5]. Although L-glutamic acid can be synthesized by these rudiments from glucose[6] the amount produced in this medium appears to be insufficient to produce a maximal response. In earlier work on the effect of amino-acid deficiencies on the growth in wet weight of chick tibiotarsi[7] it was shown that L-glutamic acid could be omitted from medium 858, which contains 20 essential and non-essential amino-acids, without affecting the response in wet weight. It seems likely, therefore, that when L-glutamic acid is omitted from medium 858, some other non-essential amino-acid can substitute for it. This could be either by its conversion to L-glutamic acid, or through its having an identical physiological activity.

We should not be dismayed that the results on length and the weights of the bones do not agree, since there is increasing evidence[8] that the elongation of the chick tibiotarsus is caused largely by hypertrophy of the diaphyseal chondrocytes, and that mitosis and the secretion of matrix by the chondrocytes play only a minor part. Thus L-glutamic acid probably produces its effects on the weights of the rudiments by stimulating mitosis and/or the secretion of matrix, and has little connexion with the mechanism of hypertrophy. The activity contrasts sharply with the effect of L-glutamine, which seems to be important for elongation as well as growth in weight

Work on the nutritional requirements of whole animals has shown that L-glutamic acid is not required for adult rats, men, and roosters , it is required, however, for the optimum growth of young chicks, rats and mice, and for egg production by hens[9]. In all these investigations the basal diet contained a minimal number of amino-acids The results described in this communication on the nutrition of an organ growing *in vitro*, on a chemically defined medium containing a minimal number of amino-acids, are remarkably similar to the results on whole animals.

This investigation was supported in part by U S Public Health Service research grant *C*–161–60 from the National Cancer Institute, Public Health Service.

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Effects of Intraventricularly Injected Anti-N. Caudatus Antibody on the Electrical Activity of the Cat Brain

It is well known that anti-tissue antibodies, when injected into an animal of the donor species, may exhibit the selective affinity for the homologous organ and produce certain modifications in its morphological and functional state[1] The damaging effects of anti-tissue antibodies have been demonstrated in different organs[2] However, no information could be found in the literature available concerning the physiopathological events associated with an *in vivo* collision between anti-brain antibodies and the corresponding nervous tissue Bearing in mind the high complexity of morphological and functional organization of the brain, a series of immuno-electrophysiological experiments has been undertaken to investigate the possible differential reactivity of various cerebral structures when exposed to the action of different brain region anti-sera The present communication, as a part of these investigations, is a preliminary report concerning only some of the effects of intraventricularly administered anti-n. caudatus antibody on the electrical activity of various parts of the cat brain

Cats operated under 'Nembutal' anæsthesia were used. Surface and depth, 'Teflon'-insulated silver and stainless steel electrodes (0 005–0 01 in in diameter), respectively, were implanted into various regions of the brain (frontal cortex, frontal white matter, n caudatus, hippocampus, hypothalamus, thalamus and brain stem reticular formation). Needle electrodes for the interior of the brain were introduced by means of a Horsley-Clarke stereotaxic instrument All electrodes were implanted into the left cerebral hemisphere, while the cannula was inserted into the right lateral ventricle, according to the method of Feldberg and Sherwood[3] Electrical activity was recorded throughout the experiment using a 12-channel Reega-Alvar electroencephalograph Details regarding recording and stimulation technique have been published elsewhere[4]

The cat n caudatus was carefully dissected, homogenized, mixed with Freund adjuvant and injected subcutaneously into rabbits (1 0 gm of fresh tissue per kgm. body-weight) The γ-globulin from the immune rabbit sera was separated by the method of Basset *et al* [5] Immune rabbit γ-globulin was investigated by diffusion-gel[6] and complement fixation reaction[7], in which the saline extract of n. caudatus tissue was used as antigen The reaction between n caudatus antigen and the corresponding immune rabbit γ-globulin revealed 2–3 precipitin lines in the agar and a titre of 1/128–1/256 in the complement fixation test Normal rabbit γ-globulin, taken prior to the immunization, did not show any serological activity with antigen prepared from the n caudatus tissue

The cats were divided into three groups the first (experimental group) was treated with immune rabbit γ-globulin, the second (control group) received normal rabbit γ-globulin, and animals from the third (control group) were injected with physiological solution in order to evaluate the possible influence of volume of injected fluid Two to three injections of 0 3 ml daily, with an interval of 60 min between the injections, were administered on four successive days Total protein content of immune rabbit γ-globulin injected into an animal of the first group was 46 08 mgm , while that of normal γ-globulin administered to an animal of the second group amounted to 60 24 mgm

Some of the results are illustrated in Fig 1. In both control groups no essential change in electrical activity of various cerebral structures investigated could be noticed Records made in the course of normal γ-globulin application, as well as the records taken at intervals within the period of one month following the last injection, were found to be fairly comparable with the activity recorded prior to the γ-globulin administration (Fig 1*A*) However, in the experimental group of animals, intraventricular application of anti-n caudatus γ-globulin was followed by pronounced modification in electrical activity The evolution of electrographic disturbances seemed to pass through three consecutive stages The first stage was characterized by the appearance of spikes and high-voltage sharp waves localized in the n caudatus. These transient irritative abnormalities become apparent on the third day of antibody administration, and disappeared two to three days afterwards The second, also transient stage was characterized by slight general accentuation of background activity and by sporadic appearance of diffuse abnormalities consisting of bursts of 4–6/sec high-voltage waves, in all leads. Such activity outlasted the disappearance of spikes and sharp waves in n caudatus for several days The

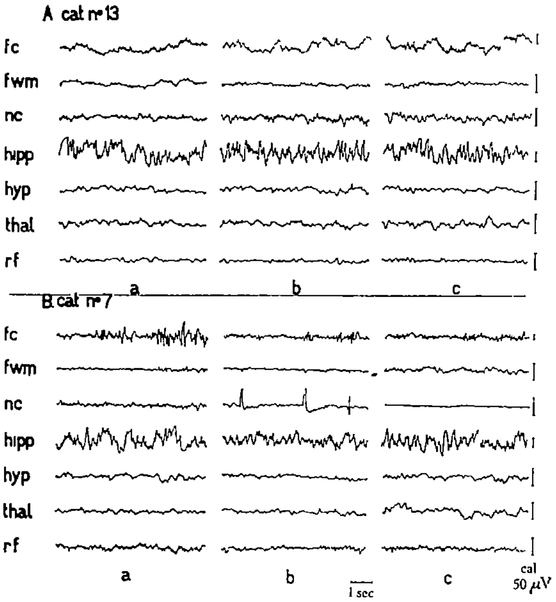

Fig 1 *A*, Records made (*a*) before administration, (*b*) following the third day of injecting, and (*c*) eight days after the last injection of normal rabbit γ-globulin *B*, Records taken (*a*) prior to antibody application, (*b*) after the third day of injecting and (*c*) seven days following the last intraventricular injection of anti n caudatus γ-globulin *fc*, frontal cortex, *fwm*, frontal white matter, *nc* n caudatus, *hip*, hippocampus, *hyp*, hypothalamus, *thal*, thalamus, *rf*, brain stem reticular formation, for explanation, see text

third stage was characterized by gradual slowing down, progressive decrease in the amplitude and almost complete disappearance of spontaneous electrical activity of the caudate nucleus within a month after antibody administration (Fig 1*B*). Such an evolution of electrographic abnormalities was reflected in modification of potentials in n caudatus evoked by acoustic stimuli, as well as in the change of activity propagated to n caudatus during seizures induced by electrical stimulation of the hippocampus It should be emphasized that no change in electrical activity of the caudate nucleus could be observed in animals treated with anti-hippocampus antibody (unpublished experiments).

In a previous paper[7] it was found that lipids from various regions of cat brain lack individual specificity but are all highly organ-specific. That is why, in the present experiments, the saline extract of n caudatus containing mainly protein components has been used as antigen in preparing the corresponding anti-serum N. caudatus was chosen because it forms the wall of the lateral ventricle and could be, therefore, easily attacked by the intraventricular route and thus brought into prolonged contact with antibody injected into the cerebrospinal fluid This, of course, could not be assured adequately if the antibody were administered by a systemic route[8] Diffuse electrographic abnormalities reported here were also observed following intraventricular administration of some other anti-brain region sera, such as anti-hippocampus, and should be, therefore, considered as nonspecific manifestations However, the initial irritative phenomona, followed by a progressive decrease in electrical activity, strictly confined to n caudatus, strongly suggest that anti-n caudatus antibody has definite affinity towards the homologous nervous tissue.

This work has been supported in part by a grant from the Yugoslav Foundation for Scientific Research, contracts No 490/1 and No 202/1.

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Effect of Colchicine on Rat Liver Xanthine Oxidase

Colchicine has been shown to inhibit deoxyribonuclease and de-phosphorylation and de-amination of deoxyribonucleotides[1]. The beneficial effect of colchicine in gout is possibly due to the effect of this drug on purine metabolism, since human serum nucleotidase and guinea pig liver xanthine dehydrogenase are inhibited by the alkaloid—the last-named only when given in large doses[2]. The purpose of the present communication is to investigate the effect of colchicine on rat liver xanthine oxidase, and on the purified enzyme

Male Wistar white rats on a well-balanced diet were used throughout this work Liver xanthine oxidase was determined from the oxygen uptake in a conventional Warburg apparatus at 37° C, using hypoxanthine as a substrate and air as the gas phase[3] The xanthine dehydrogenase activity was measured using the anaerobic reduction of the colourless dye triphenyltetrazolium chloride in a Thunberg vacuum tube as suggested by Villela[4] Pyrophosphate buffer (0 015 *M*), *p*H 8 6, was used in both determinations The results were expressed in mm ^{3} oxygen/gm dry weight/60 min for xanthine oxidase and in μgm formazan/gm dry weight/30 min for xanthine dehydrogenase activity

Colchicine (0 1 mgm /100 gm. body-weight) was administered by intraperitoneal injection of an alcoholic solution (6 8 per cent) containing 20 mgm

Table 1 Liver Xanthine Oxidase and Xanthine Dehydrogenase of Rats injected with Colchicine and with Thiocolchicoside

Conditions	No. of rats		Xanthine oxidase* (mm ^{3} O_2/gm dry weight in 60 min)	Xanthine dehydrogenase* (μgm formazan/gm dry weight in 30 min)
	Xanthine oxidase	Xanthine dehydrogenase		
Normal	4	11	1,656 ± 152	227 ± 86
Alcoholic solution (control)	16	15	1,208 ± 328	159 7 ± 45 1
Colchicine (0 1 mgm)†	13	12	739 ± 231	56 6 ± 22 5
Thio-colchicoside (0 2 mgm)†	2	3	1,539	250
Thio-colchicoside (0 4 mgm)†	2	3	1,343	242

* Average values ± S D, S D = $\sqrt{\Sigma(d^2)/n-1}$
† Per 100 gm body-weight

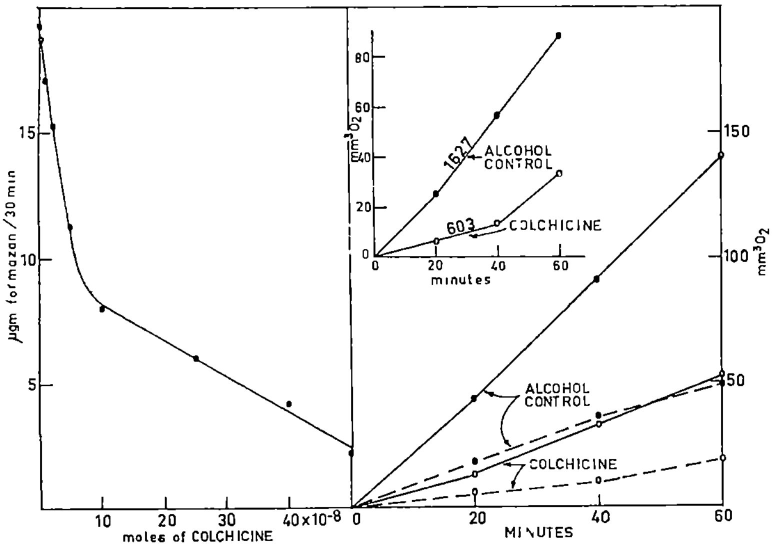

Fig 1. Rat liver xanthine oxidase and xanthine dehydrogenase inhibition by colchicine. Left, *in vitro* inhibition of purified xanthine dehydrogenase activity. Right, *in vivo* inhibition of liver xanthine oxidase activity. The graph in the upper part represents values of net oxygen uptake in mm³ oxygen/gm dry weight/60 min

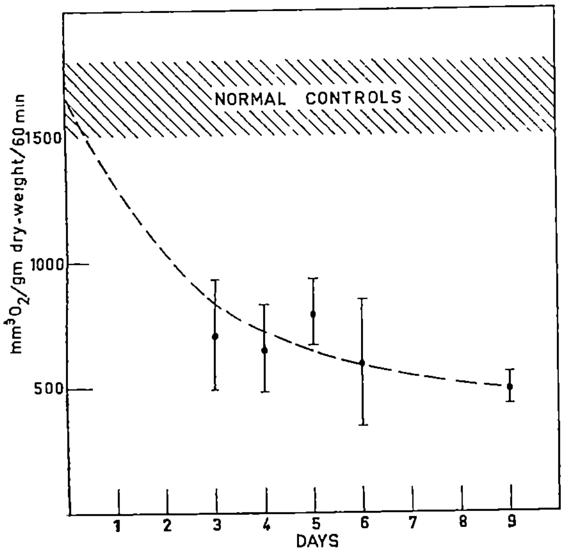

Fig 2. Rat liver xanthine oxidase inhibition by colchicine in diet. Dotted line of the curve represents the decrease of activity. Abscissa: Time in days in which the rats were fed with colchicine

of colchicine per 100 ml. Blanks were performed by injecting a 6·8 per cent alcoholic solution. Colchicine was administered orally *ad libitum* by adding colchicine (Houdé) to the diet (5 mgm /100 gm).

For *in vitro* experiments purified liver xanthine oxidase was prepared by solubilization of the enzyme with butanol[5] after the digestion of rat liver homogenate (in 0·2 *M* Na_2HPO_4) with crude trypsin at *p*H 7·3. After precipitation with ammonium sulphate between 30 and 60 per cent saturation, the enzyme was adsorbed in $Ca_3(PO_4)_2$ and eluted with 0·1 *M* pyrophosphate buffer, *p*H 8·6.

Experiments *in vitro* with 5×10^{-7} mole of colchicine did not show any statistically significant difference in the activities of xanthine oxidase or xanthine dehydrogenase when rat liver homogenate in pyrophosphate buffer was used. However, with purified enzyme, an inhibitory effect was obtained even with 10^{-9} mole of colchicine (Fig 1, left).

Values for normal rats and controls injected with alcohol as well as rats fed with colchicine are reported in Table 1. A statistically significant inhibitory effect was observed with colchicine for the liver enzyme activity (for xanthine oxidase, $t = 4·50$, $P < 1$ per cent, for xanthine dehydrogenase, $t = 7·75$, $P < 1$ per cent) (Fig 1, right). When the drug was administered orally the inhibition was noted in the first days (Fig 2).

Thio-colchicoside ($C_{27}H_{33}O_{10}NS$), a sulphur-containing analogue of colchicine without antimitotic activity, was used in some other preliminary experiments but appeared to be without any inhibitory effect (Table 1).

The results reported indicated that colchicine has an inhibitory effect on liver xanthine oxidase and xanthine dehydrogenase activities when given to rats orally or in the peritoneum. Although the liver homogenate protects this effect *in vitro*, the purified enzyme is inhibited with small doses of the drug.

This investigation was supported in part by grants (to O. R. A. and E. M.) from the Conselho Nacional de Pesquisas.

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Effect of Hydrocortisone on Uptake of Iron-59 in Incubated Rat Red Cells *in vitro*

Hydrocortisone added to blood containing reticulocytes in a suitable substrate did not alter the rate of synthesis of hæmoglobin and no evidence was obtained that it influenced the rate of ripening of reticulocytes. This applied to various dosage-levels *in vitro* and to plasma obtained from animals which had been injected with large doses of hydrocortisone. It was shown, however, that preliminary incubation of reticulocytes with hydrocortisone before the addition of iron-59 reduces the amount of iron-59 which enters the reticulocytes.

Reticulocytosis was induced in laboratory rats by intraperitoneal injection of phenylhydrazine hydrochloride. Five days later, blood was removed by cardiac puncture and collected in heparin. Iron-59 uptake was measured by incubating 1-ml portions of blood with hydrocortisone sodium succinate (supplied by courtesy of the Upjohn Chemical Co.) in physiological saline. About 0·4 µc of iron-59 as ferric chloride was added at various times, the cells incubated for a further 2 hr., washed 10 times with physiological saline and assayed with an EKCO scintillation counter.

In all instances the uptake of iron-59 in the presence of hydrocortisone was compared with the uptake in the presence of sodium succinate, because it has been shown that preliminary incubation itself reduces uptake[1]. It was found that the amount of iron-59 entering the reticulocytes was not altered by preliminary incubation for up to 5 hr in the presence of hydrocortisone at concentrations between 0·0083 and 0·500 mgm./ml. of blood. However, an increase in the concentration to 1·0, 1·5 and 2·0 mgm./ml. resulted in a reduction of uptake of iron-59 which was proportional to the concentration of hydrocortisone.

This effect of hydrocortisone is shown in the following experiment in which reticulocytes were incubated for different times with varying concentrations of hydrocortisone. Blood samples of 1 ml. were incubated for 2 and 5 hr. in the presence of 0·5 or 1·0 mgm. hydrocortisone. Iron-59 was added and the uptake observed at 2, 4 and 6 hr. Hydrocortisone did not influence the amount of iron-59 in the cells with only 2 hr. incubation. It was also without effect, compared with the controls, when the cells were incubated with 0·5 mgm./ml. for 5 hr. There was, however, a marked decrease in the total uptake of iron-59 after 5 hr. incubation with 1·0 mgm. hydrocortisone and this effect was still further increased when the cells were suspended in saline instead of their own plasma.

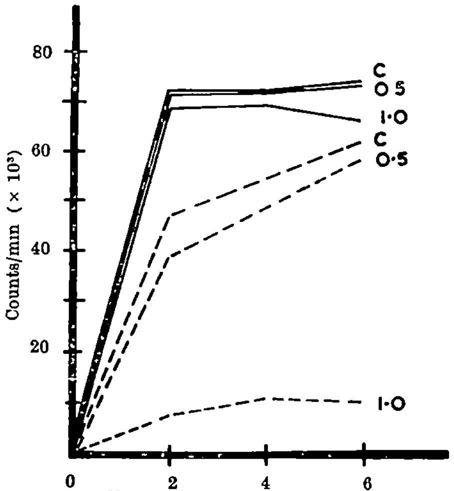

Fig. 1. Uptake of iron-59 in pre-incubated rat reticulocytes. ——, 2 hr. pre-incubation; ----, 5 hr. pre-incubation

Warburg experiments on 2-ml. blood samples showed a slight decrease in oxidative respiration in the presence of hydrocortisone sodium succinate, but a similar decrease was found in sodium succinate. Incubation with 1·0 mgm./ml. hydrocortisone resulted in a marked increase in the rate of outward movement of potassium, but incubation with 0·5 mgm./ml. hydrocortisone did not produce a significant increase.

Hydrocortisone in high concentration thus increases the pre-incubation effect already observed by Clark and Walsh[1], although its action on the reticulocytes is slow. It also causes a change in certain permeability properties but no change in oxidative respiration. It is not known whether hydrocortisone operates by means of the same mechanism as pre-incubation, or whether some other mechanism is involved. It may produce its effect (*a*) by causing the reticulocytes to mature more rapidly, thus decreasing the iron-59 requirement, or (*b*) by altering some components of the reticulocytes so that less iron can be accepted. A reduction in oxidative respiration would have been expected if the effect were an increase in the rate of maturation of reticulocytes, because such maturation is characterized by loss of the oxidative pathway. On the other hand, there is evidence that hydrocortisone affects the permeability of reticulocytes.

Reticulocytes therefore differed from rat liver cells[2] in their reaction to hydrocortisone because (*a*) hydrocortisone must be used in high concentration before any effect is observed with reticulocytes, and (*b*) oxidative respiration of reticulocytes is not affected by hydrocortisone even in high concentrations. These differences are probably related to differences in metabolism and structure of the two types of cell.

This work was supported by a grant from the National Health and Medical Research Council of Australia.

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Action of Methyl Hydroxybenzoate on Nervous Conduction

METHYL hydroxybenzoate and its sodium derivative are recommended by the B.P.C. and by Martindale[1] as preservatives for creams, emulsions, lotions and ophthalmic solutions, and they are used to preserve local anæsthetics. During an investigation of the action of phenol on nerve fibres[2,3], we examined the action of methyl hydroxybenzoate and its sodium derivative on conduction in fibres of the spinal roots of the cat, and also on the vagus and sympathetic nerves.

These substances, dissolved in Krebs's solution to form 0·1 per cent and 0·2 per cent solution, when applied directly to the spinal roots or to the vagus and sympathetic bundle in the neck, block conduction in nerve fibres of all sizes, myelinated and unmyelinated. The effect was typical of a local anæsthetic in that small fibres were blocked before large and that the conduction block was reversible. The blocking effect of a 0·1 per cent solution of methyl hydroxybenzoate was found to be similar to that of a 0·05 per cent solution of procaine.

The substances are recommended as preservatives, as, according to Martindale, they are "inert and non-toxic". The results mentioned above were obtained with the weakest solutions recommended by the B.P.C., the concentrations advocated being 0·1 and 0·2 per cent methyl hydroxybenzoate and 0·1–0·3 per cent of the sodium derivative.

The fact that in acute experiments conduction returned in fibres which had been blocked, following removal of the substance and washing the root or nerve bundle, cannot be taken to mean that no subsequent degeneration of the affected nerve fibres takes place. Exactly this situation occurs with phenol in non-aqueous solution. Although electrophysiological techniques have shown recovery of conduction in fibres which have been blocked by phenol, there is histological evidence of subsequent massive degeneration among these fibres[4]. If methyl hydroxybenzoate causes similar effects, it is possible that the degeneration of a proportion of nerve fibres following the injection of local anæsthetics in various painful states may account for the prolonged or permanent relief of the pain that sometimes occurs Whether this is the explanation of the prolonged therapeutic effect of local anæsthetics or not, it remains important to note that such recommended preservatives are not inert and that their presence should be taken into account both in experimental and in therapeutic situations

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HÆMATOLOGY

Alkali-resistant Hæmoglobins in Prosimian Primates

PROSIMIAN Primates are a group of animals which share characteristics of behaviour, anatomy, biochemistry, cytogenetics, and physiology with the monkeys on one hand and the more primitive Insectivora on the other[1] We have been examining the hæmoglobins of a number of these animals One of our most interesting discoveries is the relatively high level of alkali-resistant hæmoglobin found in these animals

Blood samples were taken from the femoral veins of anæsthetized or tranquillized animals. Hæmolysates were prepared as described by either Jonxis and Huisman[2] or Singer *et al* [3]. The alkali-denaturation tests were carried out by the method of Singer *et al* [3] All samples were read in a Coleman Junior spectrophotometer at 540 mμ Fresh and stored samples gave essentially identical results

The results given in Table 1 include those from tests on individual adult and juvenile animals from a variety of genera of Primates These results are of some comparative interest, since we have found no significant amounts of alkali-resistant pigments in adult animals in the Anthropoidea There is a difference in average amount of alkali-resistant pigment among the three major groups of Prosimians tested The Lemuriformes appear to have the highest average levels, 41–75 per cent, the Lorisiformes have levels between 8 4 and 52 per cent, and the single Tupaiiformes sample showed 9 per cent alkali-resistant hæmoglobin The age of the animals apparently is not related to the amount of denatured pigment, since several in each group, except *Tupaia glis*, were infant or juvenile animals This is in marked contrast to the higher Primates which have been examined Singer *et al* [3] found, in new-born human beings, a high level of alkali-resistant hæmoglobin (80 per cent), the level afterwards fell to the negligible value of 1 7 per cent at the age of 36 months Similar values have been found in *Macaca mulatta*[4]

Electrophoresis on starch-gels of hæmoglobin from the animals studied here, with Poulik's discontinuous buffer system[5], demonstrated at least two components in all the Lorisiformes and in most of the Lemuriformes One of these components may correspond to the alkali-resistant fraction we found A detailed account of the hæmoglobins of these Prosimians is in preparation

Table 1

Animal	No tested	Alkali-resistant hæmoglobin (per cent) Average	Range
INSECTIVORA			
Elephantulus rufescens	1	0 4	
PRIMATES			
Prosimii			
Tupaiiformes			
Tupaia glis	1	9 1	
Lemuriformes			
Lemur mongoz	2	75 5	68 1–82 3
L fulvus	9	67 1	50 9–86 4
L catta	6	61 8	51 1–71 5
L variegatus	1	44 0	
L macaco	2	41 5	39 5–42 7
Hapalemur griseus	1	67 0	
Lorisiformes			
Nycticebus coucang	2	52 5	49 5–55 5
Perodicticus potto	17	31 9	21 1–52 6
Galago crassicaudatus crassicaudatus	18	36 5	25 3–59 1
G crassicaudatus agisymbanus *	5	36 0	32 0–46 8
G senegalensis senegalensis	4	8 4	7 2–10 1
Anthropoidea			
Erythrocebus patas	1	2 1	
Papio comatus	9	0 3	0 1–0 5
P doguera	40	0 2	0–6 5
Macaca mulatta (fœtus)	2	82 0	75 9–87 6
M mulatta (infant)	1	50 9	
M mulatta (adult)	5	0 3	0–1 4
Cebus apella (infant)	1	10 8	
C apella (adult)	1	2 6	
Lagothrix lagotricha	2	2 6	2 4–2 7
Ateles spp	2	0 6	0 5–0 7
Pan troglodytes	1	0 2	

* Species identification in doubt The animals appear to be of the *G crassicaudatus* group, however, some authorities have called them *G senegalensis zanzibaricus*

One elephant shrew (*Elephantulus rufescens*) of the order Insectivora was examined According to Polyak[6] *Tupaia* is closely related to this species. A level of about 0 4 per cent alkali-resistant hæmoglobin, which is negligible, was found in our sample from an old animal

The question of the nature of this alkali-resistant hæmoglobin in the Prosimians is raised There is little doubt that in higher Primates, including man, this hæmoglobin is the principal fœtal pigment. The term fœtal would be misleading, if not incorrect, when applied to the alkali-resistant fraction of Prosimian hæmoglobin. A possibility is that this hæmoglobin in the Anthropoidea was originally a major component of the respiratory pigment, as in the Prosimians, but gradually became confined to a shorter period in the animal's life, until, in the highest Primates, it is present only in the fœtus and during the first few months of life This hypothesis requires an investigation into the physiological function of the alkali-resistant pigment in Prosimians

Our work was supported by Research Grant 6053 of the United States Public Health Service, Research Grant 12331 of the National Science Foundation, and the research fund of the Department of Anthropology, Yale University It is with pleasure that we acknowledge the assistance and encouragement of colleagues in various countries who helped us obtain the live animals used in this work

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PATHOLOGY

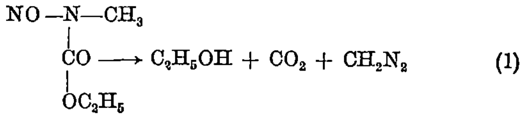
Interaction of the Carcinogenic *N*-Methyl-Nitrosourethane with Sulphydryl Groups

In a preliminary communication I recently reported the production of lung adenoma in mice and squamous lung carcinoma in a rat, which was exposed to gaseous diazomethane. Among other rats given *N*-methyl-*N*-nitrosourethane (NMU) by stomach tube, a carcinoma of the stomach developed after a single dose of this compound[1].

Since this communication was published, more stomach tumours have been observed in the rats given NMU by stomach tube[2]. Stomach tumours have also been reported in rats given NMU in drinking water continuously until death[3]. Moreover, when NMU was administered to adult rats by a short mouse-stomach tube, the animals developed œsophageal squamous carcinoma[2]. Subcutaneous injections into mice gave rise to local sarcomata. It is evident that NMU acts at the site of application and that it is able to produce tumours in various tissues. This would qualify it to be considered a 'local' carcinogen.

However, the evidence described here indicates that the effective entity is not NMU but diazomethane, which is formed when NMU interacts with tissues. In 1895 von Pechmann[4] suggested that the toxic effects of NMU, which he observed on himself, might be due to diazomethane, formed from NMU in contact with tissues.

That this is really the case was qualitatively confirmed in the following way. NMU was added to tissue homogenates at 37° C. and the mixture was covered with a layer of ethyl ether. Rapid evolution of gas occurred; the gas bubbled through the layer of ether. This indicated that the gas is not soluble in ether, hence it is not diazomethane. Evidently, the diazomethane formed according to reaction (1) reacts immediately with tissue constituents, with evolution of nitrogen according to reaction (2).



$$\begin{array}{l} NO-N-CH_3 \\ \quad\;\; | \\ \quad\;\; CO \longrightarrow C_2H_5OH + CO_2 + CH_2N_2 \\ \quad\;\; | \\ \quad\;\; OC_2H_5 \end{array} \qquad (1)$$

$$CH_2N_2 \longrightarrow CH_2 + N_2 \qquad (2)$$

Expecting this to be an enzymatic reaction, it appeared convenient to study it in the Warburg apparatus, the likely final product being nitrogen. The other gaseous product of the reaction, carbon dioxide, is conveniently absorbed by 10 per cent potassium hydroxide, placed in the usual way in the middle well of the vessel. Tissue homogenates were prepared in Sorensen's phosphate buffer, *p*H 7.2 (1 : 10), and introduced into the main compartment of the Warburg flask (3 ml.). Solutions of NMU (1 : 100) were placed in the side-arm (0.5 ml.). (The solutions of NMU were freshly prepared from commercial *N*-methylnitroso-urethane by dissolving it in 50 per cent ethyl alcohol, 1 : 20; this gave a clear solution which was then diluted further with distilled water 1 : 5.) The effect of the buffer on NMU and of 10 per cent alcohol (0.5 ml.) on tissue homogenates was tested concurrently each time. The contents of the side-arms were added to the main compartment after 15 min. equilibration at 38.5° C. With tissue homogenates rapid gas evolution immediately followed the addition of NMU and was almost complete in 15 min.; the decomposition of NMU in buffer solution was very slow and slight gas evolution persisted for many hours.

When heparinized rat blood and plasma were tested in this way, rapid evolution of gas from NMU occurred only in the presence of blood corpuscles. The specific agent responsible for the decomposition of NMU is evidently cellular. The reaction was not affected by cyanide, azide or fluoride; it was, however, abolished by copper, indicating that SH-groups may be involved.

Indeed, when 1 per cent solutions of cysteine or glutathione in phosphate buffer at *p*H 7.2 were substituted for blood or tissue homogenates, the evolution of gas from NMU took place in the same way immediately after mixing. This proved that the reaction is chemical rather than enzymatic. Biotin, having sulphur in the ring, cystine, thioacetamide, or some representative cell constituents, such as glycine, tyrosine, guanine, pantothenic acid, etc., had no effect on NMU under the conditions tested.

The question arises: Are some of the SH-cellular compounds directly involved in the carcinogenic action of NMU, diazomethane and possibly of certain other alkylating agents? Diazomethane is known to be a very versatile reagent; it can methylate not only SH-groups but also any acidic or phenolic hydroxyls, amino- and imino-groups; it can add to double bonds, enlarge ring and carbon chains, form methyl branchings. It can conceivably form intra- and intermolecular methylene-bridges.

Which of the multiplicity of possible reactions is related to the carcinogenic action of this compound? The fact that decomposition of the strongly carcinogenic NMU takes place at the SH-groups supports the already existing evidence[5] that these may be involved in the carcinogenic process. However, if carcinogens combine with certain SH-compounds *in vivo*, the type of their binding is likely to differ fundamentally from that of non-carcinogenic agents, many of which are excreted as mercapturates. The action of carcinogens is irreversible and compatible with the survival of the affected cells; the interaction products possibly remain irreversibly *in situ*, and prevent the physiological replacement of some specific SH-compounds, the action of which they block.

Since this communication was submitted for publication, Emmelot and Mizrahi[6] reported that cysteine can counteract the action of dimethylnitrosamine. As the latter can act as a methylating agent (probably through the release of diazomethane after enzymatic oxidation)[7], these results confirm the view based on the more direct evidence here reported, that certain sulphydryl compounds are involved in the action of diazomethane and probably also of other carcinogens.

I wish to thank Miss A. Mackinnon for valuable technical assistance.

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Antagonism between Antibacterial Drugs

WHEN two antibacterial agents are combined, the effect of the combination may equal the sum of the antibacterial effects of its components (addition), it may exceed this sum (synergism), or it may be smaller (antagonism)

According to Jawetz and Gunnison[1,2], the antibiotic and chemotherapeutic drugs can be divided into substances the effect of which is mainly bactericidal (penicillin, streptomycin, neomycin, and bacitracin) and substances that generally have only a bacteriostatic action (chloramphenicol, tetracycline, erythromycin and sulphonamides) Combinations of bactericidal substances often show synergism, but are never antagonistic Antagonism is also extremely rare when two bacteriostatic agents are combined But there is a chance of this type of interference when a bactericidal substance is combined with a bacteriostatic one

Recently a more up-to-date schema, which also provides data about the latest accessions in the field of antibacterial substances, was developed in our laboratory As a criterion of antibacterial potency the bactericidal action has been used This criterion was applied by means of replica tests[3-7], the standard of measuring the bactericidal action of the separate drugs and their combinations being not the diameter of the replica inhibition zones, but the number of colonies developing in these zones The results have been arranged in the form of a schema

The schema is based on numerous results obtained with some dozens of strains of *Staphylococci*, *Enterococci*, *Salmonellae* and *coli*-bacteria. More than 90 per cent of all strains investigated showed reactions as laid down in the schema With streptomycin, bacitracin, novobiocin and the neomycin-group of antibiotics we noticed a slightly higher frequency of deviations Our experiments confirm the conclusion arrived at by Jawetz and Gunnison that a fairly sharp distinction can be made between drugs acting *in vitro* mainly bactericidally (in the diagram enclosed by circles) and bacteriostatic drugs (in the quadrangles) Experiments with combinations of the drugs mentioned in the schema revealed that it is possible to distinguish four groups of agents Group 1 (bactericidal substances) nitrofurans (nitrofurantoin, furazolidone, furaltadone), polymyxins (including colistin), streptomycin, neomycins (including kanamycin and framycetin), and bacitracin, Group 2 (bactericidal substances) penicillin, novobiocin, vancomycin, ristocetin and amphomycin, Group 3 (bacteriostatic substances) tetracyclines, chloramphenicol and the erythromycin group of antibiotics (erythromycin, magnamycin, spiramycin, oleandomycin), Group 4 (purely bacteriostatic substances) the sulphonamides and cycloserine

With regard to the combinations of these substances the following points are worthy of note

As a rule a substance belonging to one and the same group (that is, drugs in the same circle or quadrangle in the diagram) can be combined with another one of the same group without any risk of antagonism

Drug antagonism is also only rarely encountered combining one of the bactericidal substances of group 1 with one of the substances of groups 2, 3 and 4 In these combinations the bactericidal agent generally strongly predominates and several of them even present clear cases of synergism (for example, streptomycin + penicillin, kanamycin + penicillin, and polymycin + chloramphenicol)[8-11] Generally speaking, therefore, any drug from group 1 combines well with any one drug from the three other groups.

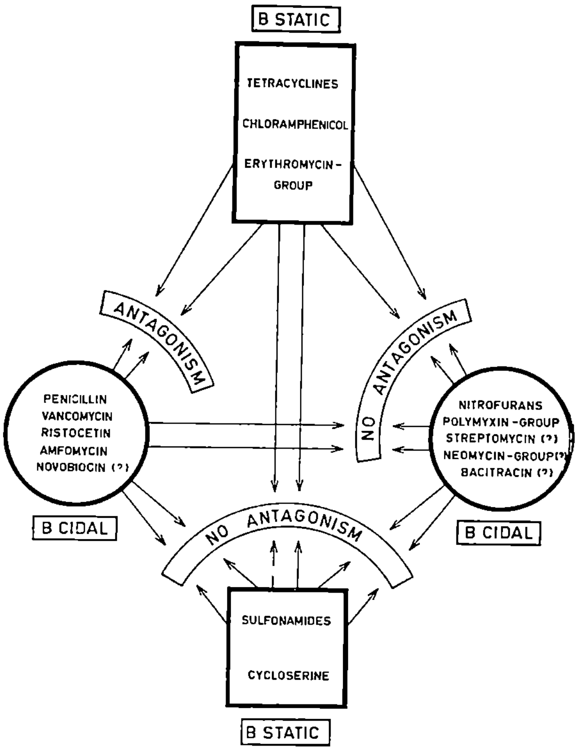

Fig 1 Schema showing presence and absence of drug antagonism in pairs of antibacterial substances

A quite different reaction occurs when a bactericidal antibiotic of group 2 is combined with a bacteriostatic drug of group 3 In these combinations the weaker, that is the bacteriostatic, drug dominates, so here there is antagonism No such antagonism occurs when the drugs of group 2 are combined with those of group 4. Here the bactericidal drug predominates and sometimes there is even synergism (for example, penicillin + a sulphonamide[12])

The different behaviour of the two groups of bactericidal substances (groups 1 and 2) is probably due to the different action of the drugs of these groups on growing and resting bacteria It is a well-known fact that penicillin can only destroy bacteria when they are in the growing stage The drugs of group 3 cause bacteriostasis and thus render penicillin ineffective That the sulphonamides and cycloserine, which act also bacteriostatically, do not neutralize penicillin may be accounted for by the fact that a comparatively long space of time elapses before these drugs cause bacteriostasis. This interval lasts a few hours[13], which is amply sufficient to enable the relatively quick-acting penicillin to inflict irretrievable injury on the bacteria[14].

It is fairly safe to say that the bactericidal action of streptomycin, polymyxin and the nitrofurans is not to any large extent tied down to the growing stage of the bacteria[13,15,16] Consequently, the bactericidal effect of these drugs is little impaired by bacteriostasis caused by one of the substances of groups 3 and 4

On the other hand, the impairment of antibacterial action that occurs when a drug belonging to group 2 is combined with one from group 3 is not so negligible The extent to which it affects the bactericidal action of penicillin in combination of this antibiotic with chloramphenicol, tetracycline, and erythromycin, was made the subject of a closer investigation. This revealed that, with regard to *Staphylococci*, the antagonism between penicillin and the other antibiotics manifests itself over a wide range of concentrations, from at least 0 1 to 10 units of penicillin per ml. Varying the concentration of the bacteriostatic antibiotics from 1 to 100 times the minimum inhibitory level did not cause the antagonism to disappear What we also found was that the antagonism only affects the bactericidal potency of penicillin, the relatively weak bactericidal potencies of chloramphenicol, tetracycline, and erythromycin remaining unimpaired. In these cases, therefore, the type of antagonism encountered is strictly unilateral and only manifests itself in the reduced antibacterial action of one of the two components of the mixture

In the above instances the antagonism results in the antibacterial potency of the stronger component of the combination being destroyed or lowered Sometimes, however, it is the weaker component that is robbed of its effect by the other agent This type of antagonism occurs when, for example, procaine penicillin is combined with a sulphonamide Procaine, which is antagonistic to sulphonamide, cancels the action of this bacteriostatic, and, therefore, weaker agent But on the other hand, a sulphonamide cannot impair the effect of the bactericidal, and therefore stronger, substance penicillin Hence this weaker form of antagonism is also unilateral

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New Renal Tubular Amino-Acid Transport System and a New Hereditary Disorder of Amino-Acid Metabolism

WE have investigated the metabolism of amino-acid in a kindred with familial nephritis and deafness Renal hypoplasia and photogenic epilepsy, both familial, and a third independent familial disorder affecting L-proline metabolism were also found in this kindred Familial nephropathy with deafness was recently reviewed[1], but its occurrence with these disorders was not described

Chromatographical methods were used for qualitative[2,3] and quantitative[4,5] analyses of plasma and urine amino-acids in the father, mother, and six offspring including the male propositus, one male and four female sibs The following results were obtained·

Plasma Fasting L-proline concentration was significantly elevated above the accepted normal range (1 5–3 4 mgm per cent)[6,7] on one or more observations, in the propositus and three affected female sibs (Table 1), consistent changes in the concentrations of other amino-acids were not detected in these subjects Plasma amino-acids were normal in other kindred patients investigated

Urine Three subjects with abnormal concentrations of plasma L-proline also exhibited an amino-aciduria involving glycine, hydroxyproline and proline, the latter two amino-acids are not present in normal urine after the first few months of life[8] A patient, with less abnormal plasma proline concentration, excreted only glycine in excess (Table 1) The mechanism of the amino-aciduria has been investigated Fasting amino-acid renal clearance-rates were determined in one affected female sib by the methods described by Cusworth and Dent[6], before and after intravenous infusion of L-proline (71 mgm /kgm.)

Glycine and hydroxyproline clearance-rates were greatly increased while their own plasma concentrations remained normal, their clearance-rates were higher still when plasma proline concentration was further increased (Table 2) Urinary proline excretion, on the other hand, required an elevated plasma proline concentration

Table 1 FASTING PLASMA AND URINE PROLINE, HYDROXYPROLINE AND GLYCINE CONCENTRATIONS IN SIBLINGS WITH HYPERPROLINÆMIA

Subject	Sex	Age (yr)	Plasma proline* (mgm per cent)	Urine amino-acids (mgm amino-acid/100 mgm total nitrogen†)		
				Proline	Hydroxy-proline	Glycine
C M	F	13	6 6	0	0	2,010
			6 4	—	—	—
S M	F	12	11 3	—	—	—
			20 1	1,875	389	4,910
F M (propositus)‡	M	5	7 8	406	42	489
M M	F	3	9 9	307	100	2,350
			7 5	0	0	613
			15 5	235	28	1,700

* Range of normal values (plasma) (refs 6 and 7) proline, 1 5–3 4 mgm per cent

† Range of normal values (urine) (ref 8) proline 0, hydroxyproline 0, glycine 537–1,371 Adult values valid for children beyond infancy.

‡ Patient has renal failure with reduced filtered load of amino-acids

These results imply that glycine and hydroxyproline re-absorption in the renal tubule was impaired and that increased proline re-absorption (secondary to excessive filtration) was a determining factor for the amino-aciduria other amino-acid clearance-rates were not significantly affected in this work

Six normal subjects were also investigated Fasting amino-acid clearance-rates were determined before (30-min periods) and after (consecutive 15-, 30- and 30-min periods), separate acute (3 min) intravenous infusions of equimolar amounts of L-proline (71 mgm /kgm , five subjects), hydroxy-L-proline (82 mgm /kgm , two subjects), and glycine (48 mgm /kgm., three subjects) Table 2 also presents results on one of the subjects who received all three infusions L-proline or hydroxy-L-proline infusion increased the clearance rates of the other two members of the triad Glycine infusion produced similar but less impressive results Clearance-rates of other amino-acids were relatively unaffected. Complete results on normal and affected subjects will be presented elsewhere

These results suggest that glycine, L-proline, and hydroxy-L-proline share a common transcellular

Table 2 FASTING AMINO-ACID INFUSION INVESTIGATIONS

Subject	S M hyperprolinæmic		C S normal		C S normal		C S normal	
Infusion	L-Proline (71 mgm /kgm)		L-Proline (71 mgm /kgm)		Glycine (48 mgm /kgm)		Hydroxy-L-proline (82 mgm /kgm)	
$\frac{uv}{p} = \frac{\mu gm /min /1\ 73\ m^2}{\mu gm /ml}$ *	Before (control)	After (45–75 min)	Before (control)	After (0–15 min)	Before (control)	After (0–15 min)	Before (control)	After (0–15 min)
L-proline $\frac{uv}{p}$	$\frac{11\ 4}{113\ 1}$	$\frac{948\ 9}{184\ 9}$	$\frac{0}{14\ 3}$	$\frac{(—)}{139}$	$\frac{0}{20\ 7}$	$\frac{5\ 7}{22\ 5}$	$\frac{0}{21\ 0}$	$\frac{56\ 4}{26\ 0}$
Glycine $\frac{uv}{p}$	$\frac{197}{15\ 2}$	$\frac{467\ 2}{16\ 2}$	$\frac{53\ 0}{18\ 3}$	$\frac{323}{17\ 1}$	$\frac{88\ 0}{20\ 2}$	$\frac{(—)}{121\ 7}$	$\frac{80\ 0}{13}$	$\frac{231\ 2}{16\ 0}$
Hydroxy-L-proline $\frac{uv}{p}$	$\frac{17\ 4}{0}$	$\frac{61\ 9}{0}$	$\frac{0}{0}$	$\frac{414}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{(—)}{165\ 0}$

* Normal range (ref 6), proline $\frac{0}{15–34}$, glycine $\frac{49–116}{10–22}$, hydroxyproline $\frac{0}{0}$, (—) = immeasurable peak (infused amino-acid)

transport system in the renal tubule, the system has higher preference for the two amino-acids than for glycine

The cause and significance of the hyperprolinæmia in the affected patients is under investigation; investigations of the full pedigree indicate its inheritance by a recessive mode, independent of the other familial disorders

This work was supported by grants from the United States Public Health Service (*RG*–6742), and the National Heart Institute (*H*4706), from the United Cerebral Palsy Research and Educational Foundation, and the John A Hartford Foundation One of us (C R S) was a McLaughlin Travelling Fellow and performed some of these investigations in the laboratory of Dr C E Dent, University College Hospital Medical School, London

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BIOLOGY

Serological Relationships between the Nine-spined and the Three-spined Stickleback

IN zoological literature we can find some publications[1,2] in which the nine-spined stickleback, *Pungitius pungitius* (L), is considered as belonging to the genus *Gasterosteus* L But most contemporary zoologists consider that this species belongs to *Pungitius* Coste. This difference in attitude has encouraged me to undertake investigation on serological relationships between the three-spined stickleback, *Gasterosteus aculeatus* L , and the nine-spined stickleback, *Pungitius pungitius* (L)

In my investigations I have applied the method proposed by Taliev[3,4] based partly on his own observations and partly on the results of other work

To get the antigen and the antiserum, I used sticklebacks collected from the Olechówka River (near Łódź on July 20, 1960) The whole length of the body of fifty-six specimens investigated was 42–57 mm The sticklebacks, without food for 24 hr , were ground into a mass after they had their heads cut off and their intestines and ribs cut out I used this method because the small body of the stickleback does not permit one to get sufficient quantity of blood[5] necessary to produce the antigen and the antiserum I fixed the mass with Ringer–Lock's tincture in the proportion 1 . 3 ; then I filtered it through infusorial earth I used the product of filtering as an antigen and a vaccine to immunize rabbits After the immunization I took blood directly from the heart of the rabbits Having cleared plasma[6] from the morphotic elements, I fixed it with Ringer–Lock's tincture in the proportion 1 : 1, thus obtaining the antiserum In a similar way I obtained the antigen from the nine-spined stickleback. The nine-spined sticklebacks (25 in number) were caught in the Bobrówka River, district of Łowicz, on 23 9 1960. The length of their bodies was 40–61 mm. In applying serology to establish the relationships among species[7], I also prepared the antigens from grown three-spined sticklebacks collected from the Bobrówka River (23 9 1960), the length of body being 45–53 mm , and from the young three-spined sticklebacks of this year (from the Olechówka River, 19 7.1960). the length of body being 25–30 mm

In my work I applied the ring precipitation technique I diluted the antigens as well as the antiserum with Ringer–Lock's tincture I used the antiserum in constant concentration, 1 · 5 To interpret the results obtained I applied Boyden's method in which the positive reaction of the lowest concentration of an antigen on an antiserum obtained for it is accepted as 100 per cent

As seen from Table 1, the serological distance between *Gasterosteus aculeatus* L and *Pungitius pungitius* (L) is great and it amounts to 56 3 per cent The results obtained by me are approximately the same as those obtained by Taliev when examining the degree of serological relationship of many genera in the Cottoidae and the Salmonidae[3]

Table 1

	Antigens from	Antiserum against *Gasterosteus aculeatus* L from the Olechówka River in the concentration of 1 5	
		The lowest concentration of the antigen with positive reaction	Percentage scale Boyden (per cent)
1	*G aculeatus* L from Olechówka River	1 800	100
2	*G aculeatus* L from Olechówka River, juv	1 300	37 5
3	*G aculeatus* L from Bobrówka River	1 800	100
4	*P pungitius* (L) from Bobrówka River	1 350	43 7

The fact that identical results are found for the same species taken from different fishing places supports the application of serology to taxonomic investigations The non-typical reaction with antigen of young three-spined sticklebacks is also in agreement with work already published

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Biotin, a Growth Requirement for Four Soil Amœbæ

SUBSEQUENT to the publication of Adam's defined medium for the growth of Neff's *Acanthamoeba* sp [1], I attempted to grow *Hartmannella rhysodes* in this medium slightly modified in the concentrations of amino-acids[2] A further modification of Adam's medium was also tried in which all the amino-acids used were natural isomers (L forms), except threonine, valine and serine which were used as racemic mixtures (DL forms), and the methionine concentration was halved (13 5 mgm /100 ml) All organic nutrients were obtained from the California Corporation for Biochemical Research (Los Angeles) , the amino-acids used were 'A grade' except DL-isoleucine ('C grade') The culture methods have been described previously[3] Both modifications of Adam's medium failed to support growth of this hartmannellid amœba past the second or third serial culture using either glucose or sodium acetate as the carbon source When biotin was added (0 5 μgm /100 ml), satisfactory growth was obtained in serial cultures The average maximum yield for duplicate cultures in the defined medium, containing 0 1 *M* glucose and grown at 30° C. on a rotary shaker, was 5 76 × 10^6 organisms/ml and the mean generation time was *c* 40 hr This is comparable to growth in the proteose peptone (Difco, Detroit)–glucose medium[3] Identical results have been obtained with Neff's *Acanthamoeba* sp , *Acanthamoeba castellanii* and *Mayorella palestinensis* in both modifications of Adam's medium with either glucose or sodium acetate as the carbon source Therefore all four soil amœbæ appear to require biotin in addition to the vitamins already present in Adam's medium (thiamine and B_{12})

In order to establish whether biotin is a true requirement for the growth of these amœbæ or an artefact peculiar to the defined medium in my laboratory, I used 'Avidin' to bind biotin[4] in the proteose peptone–glucose medium (PPG) described previously[3] 'Avidin' (Nutritional Biochemicals Corporation, Cleveland, containing *c* 1,000–2,000 units/gm) was added to the PPG medium in a concentration of 4 mgm /100 ml The 'Avidin'-treated medium (PPG–A) and the controls were filter-sterilized All four soil amœbæ mentioned above were carried in serial cultures of the PPG–A medium Both the PPG medium and the PPG–A medium, supplemented with enough biotin to saturate the added 'Avidin' (10 μgm /100 ml), were used as controls At each subculture of the serial cultures explants were made into both control media The PPG–A medium failed to support the growth of all four soil amœbæ past the second or third serial culture, whereas growth occurred in the explant controls The experiments were carried out in duplicate on a shaker[3] and in stationary, slanted test-tube cultures at 30° C

At present it is not possible to explain the apparent lack of a biotin requirement in the defined medium described by Adam[1,2] for Neff's *Acanthamoeba* sp The observations given in the present report indicate that, in my laboratory, Neff's *Acanthamoeba* sp , as well as *Hartmannella rhysodes*, *Acanthamoeba castellanii* and *Mayorella palestinensis*, require biotin for growth in the defined medium and in the undefined proteose peptone–glucose medium

This work was supported by grants from the Research Corporation (Brown-Hazen Fund) and the National Research Council of Canada

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Effect of Ribonuclease on Shell Regeneration in *Ariophanta* sp.

IT is supposed that the process of shell growth in molluscs consists of two phases. During the first step there is the elaboration of a protein membrane, and in the second mineralization of this membrane takes place[1,2]. Wagge has suggested that in *Helix aspersa*, which can rapidly repair its shell if this be damaged, the regeneration of the shell is brought about by the amœbocytes, which first lay down an organic framework that later on becomes calcified Since ribonucleic acid is concerned with synthesis of proteins[3,4], which in this case form an integral part of the shell, we have tried to assess its role in the regeneration of the shell in the snail *Ariophanta* sp (Pulmonata)

For experimental work adult snails were chosen, and into each was injected 0 5 ml. of distilled water containing 2 mgm of ribonuclease (Armour, crystalline) Prior to injection, the enzyme solution was placed in a water-bath at 70° C for 20 min so as to destroy the proteolytic activity present in the ribonuclease preparation. The control specimens received 0 5 ml of distilled water only Pieces of shell (slightly less than 1 cm ^{2}) about 2 cm away from the mouth of the shell were removed Eighty snails were used, of which 40 served as controls After injection, the animals were placed in glass bowls with wet filter papers at the top and bottom of the bowl so as to saturate the air with water vapour. They were not fed during the period of regeneration Observations were made on the nature of the regenerating pieces at intervals of 24, 48 and 72 hr , after which parts of the shells with the regenerating pieces in the centre were cut from the specimens treated with ribonuclease and from controls They were wiped dry and dehydrated in an air oven. The area of the regenerated piece was measured by an occular reticule Later, the regenerated piece was carefully cut out and weighed From this, the weight of the regenerated piece per 1 cm.^{2} was calculated for both the treated and the control specimens.

Examination of the regenerated shell showed that the process of calcification was considerably inhibited in specimens which had been treated with ribonuclease when compared with the controls even at the end of 72 hr The dry weight of the regenerated piece at the end of 72 hr in treated snails was 5 63 ± 0 16 mgm per cm^{2}, whereas in the controls it was 10 72 ± 0 40 mgm per cm^{2}

According to Brachet[3] and Casperson[4], there exists a correlation between ribonucleic acid and protein synthesis, the former author[5] has shown that ribonuclease inhibits the incorporation of labelled phenylalanine into the proteins. Therefore, it is possible that ribonuclease, by acting on the ribonucleic acid in the amœbocytes, inhibits the synthesis of proteins which form the essential constituent of the organic framework of the shell

The mineralization of this membrane is brought about by the amœbocytes which transport the calcium ions from the digestive gland and the shell The inhibition in the calcification of the regenerating piece in the specimens treated with ribonuclease may be due to the inability of the amœbocytes to transport the necessary amount of calcium ions to the region of repair, since ribonuclease, by acting on the ribonucleic acid of the amœbocytes, prevents the binding of calcium ions at their cell surface A similar inhibition in the uptake of calcium ions by *Elodea* cells in the presence of ribonuclease was observed by Lansing and Rosenthal[6].

It is suggested that ribonuclease, by inhibiting the synthesis of proteins which form an essential constituent of the shell and also by preventing the binding of calcium ions at the surface of the amœbocytes which normally transport calcium ions to the area of repair, slows down the process of regeneration

Grateful acknowledgment is made to the Department of Atomic Energy, Government of India, for financial assistance

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Action of a Detergent (Dodecylbenzenesulphonate) on the Gills of the Trout

In the course of our work on the detrimental effect of surface-active agents on fishes we examined changes induced in the gills of the trout by sodium dodecylbenzenesulphonate (DBS) We found a reduction of the epithelium accompanied by a loss of the mucous cells on the top of the gill lamina starting at a concentration of 5 mgm DBS/l The same amount causes the respiratory folds of the lamina to stick together An increasing destruction of epithelial cells follows the rise of concentration, until at 20 mgm DBS/l. after 1 hr the epithelium is destroyed to such an extent that the position of the respiratory folds is recognized only by the more resistant pillar cells Multiple hæmatomas mark the destruction

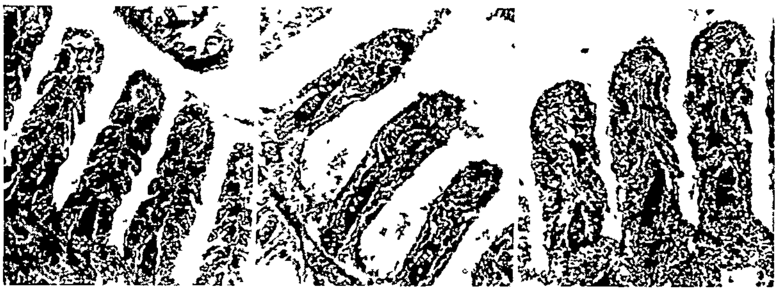

Fig 1 *a*, Untreated control, *b*, concentration of DBS, 5 mgm /l , length of action, 8 hr , *c*, concentration of DBS, 20 mgm /l , length of action, 30 min

The deleterious action of DBS results in a diminished uptake of oxygen by the gills followed by death from suffocation when the concentration of DBS is strong enough or in case of prolonged action even in smaller amounts The deterioration of respiration might be accompanied by a similar impairment of the salt balance and of the metabolism of urea since the gills take part in both mechanisms Details of this work will be published in *Archiv fur Fischereiwissenschaft*, 11 (1961)

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The Species of the Teleostean Genus *Lebetus*

The genus *Lebetus* (Gobiidae) was founded by Winther[1] to include two species, *Gobius orca* and *G scorpioides*, described by Collett[2] in 1874 from examples dredged on the Norwegian coast, and is characterized by the absence of an anterior membrane to the pelvic fins, which are united in the mid-line along their entire length. *Lebetus* occurs in the European Atlantic boreal region, being known from Iceland, Norway, the Skagerrak and Kattegat, the British Isles, and Brittany, but also extends into the Bay of Biscay and perhaps as far south as Gibraltar[3] These fishes are of small size (up to 38 mm total length[3]) and frequent rough grounds , in consequence they are not often taken, and are reputedly rare. However, in the past three years, more than fifty individuals of the genus have been obtained in the course of dredging off the southern end of the Isle of Man This collection, together with earlier specimens from the same region and from several Scandinavian museums and the British Museum (Natural History), has provided considerably more material than has been available to previous workers on this genus

Various characters have been used to identify the two species of *Lebetus* Holt and Byrne[4] attached some importance to the coloration of the second dorsal fin On this basis it has been possible to separate the above individuals into two groups, termed *orca*, where this fin has a thick black superior edge above bright white areas and wide oblique bands of yellow (grey when preserved), and *scorpioides*, with a much thinner dark edge and four narrow lines of black and reddish orange across a predominantly hyaline fin The absence from my collection of female *orca* and mature male *scorpioides* suggested connexion of the two coloration groups with sex and sexual maturity Table 1 shows that mature or developing male *scorpioides* do not occur, and that female *orca* are unknown at any stage of maturation This distribution would seem to indicate that the *orca* coloration is the male livery of a species the females and immature males of which bear the markings of *scorpioides*

Table 1 Relation of Sex and Maturity to Coloration Groups

Group	No of males (mature and maturing)	No of males (immature)	No of females (all stages)
orca	35	1	0
scorpioides	0	10	32

After reaching this conclusion, it was discovered that such a view was put forward by Smitt[5] in 1900, but was not accompanied by any details and appears to have been overlooked by later authors The hypothesis is further supported here by the fact that the known examples of *orca* are 18 0–30 0 mm in standard length whereas male *scorpioides* do not exceed 23 0 mm Tåning[6] has reported juvenile *orca*, but based his identification on meristic characters the validity of which is doubtful and is dealt with later Female *scorpioides* attain the same maximum length as that of *orca* Incipient development of the *orca* pattern in the second dorsal fin can be noted among some of the immature male *scorpioides* The first dorsal fin in the *orca* fishes is a uniform yellowish grey edged with white, but in *scorpioides* is usually banded, with a dark spot on the interradial membrane between the fifth and sixth rays This second pattern is stated to be found in the breeding male of *scorpioides*[4], in fact, it is well represented in adult females, and among male *Lebetus* occurs only in the immature Other aspects of coloration in which the two species have been regarded as differing can be seen in both *orca* and *scorpioides* groups

Tests have also been made on other characters used in the past for distinguishing the two species Diagnostic features attributed to *orca* have been an extra ray in the first dorsal fin, rather more articulated rays in the second dorsal and anal fins, but fewer in the pectoral fin and fewer scales in lateral series[4,6] In body proportions *orca* has been regarded as consistently slimmer[4], and a supposed disparity in the position of the anus with reference to the middle of the body was utilized in a key by Smitt[7] From Table 2 it is apparent that the two groups cannot be regarded as specifically distinct on these criteria The wider range and slightly lower means for fin ray counts in *scorpioides* are due to the occurrence in this group of all the smallest fishes, among which such variation is more pronounced

Table 2 Means and Ranges of Values for Characters used in the Identification of *L orca* and *scorpioides*, determined for the two Coloration Groups A Linear Relation exists between Body Measurements and Standard Length

Characters used in identification	*orca*			*scorpioides*		
	No of obs	Mean	Range	No of obs	Mean	Range
*Meristic						
No of first dorsal rays	25	All 6		32	All 6	
No of second dorsal articulated rays	25	9 2	8–10	31	9 0	7–10
No of anal articulated rays	25	7 2	6–8	31	7 1	5–8
No of pectoral rays	52	19 0	18–20	60	18 7	15–21
No of caudal branched articulated rays	24	9 0	9–10	33	9 0	8–10
No of scales in lateral series	38	26 7	25–29	41	26 8	25–29
Body proportions (in standard length)						
Depth at origin of pelvic fin	21	5 6	5 1–6 7	28	5 7	5 1–6 3
Depth at origin of anal fin	23	6 5	5 9–7 3	26	6 6	5 7–7 5
Depth (least) of caudal peduncle before origin of caudal fin	23	10 0	9 2–11 0	29	10 2	8 5–12 3
Distance from snout to anus	22	1 9	1 7–2 0	18	1 9	1 8–1 9

*British material only

Another distinguishing character of *L orca* is the greater size of the first dorsal fin, which is believed to differ from that of *scorpioides* in being considerably higher than the body[8], and higher than the second dorsal fin[3] In the present work, length of fin ray has been used as an index of height of fin With adults in both the *orca* and *scorpioides* groups, rays II and III of the first dorsal fin are longer than any from the second dorsal fin, and greater than the depth of the body at the origin of the pelvic fins In length of ray II, there is little difference between the groups whereas for ray III and the other rays of the first dorsal fin, as well as in the second dorsal fin, there is a more or less pronounced divergence, *orca* having the longer rays This is very noticeable in the last three rays (IV, V, and VI) of the first dorsal fin and, together with corresponding growth of the interradial and posterior membranes, gives the fin a much greater spread in the *orca* fishes

Comparison of other characters, such as the patterns of sensory papillæ over the head and body, does not reveal any differences between *orca* and *scorpioides* Both 'species' have been recorded throughout the geographical range of the genus, and, in so far as can be judged, display no ecological separation

The two groups are thus different in coloration and in development of the dorsal fins, especially the first As mentioned above, *orca* consists entirely of males, almost all maturing or mature, and *scorpioides* of females and immature males The type specimens of *Gobius orca* and *G. scorpioides* (Zoological Museum, University of Oslo) have been examined, and are a male, maturing if not mature, and two females respectively Sexual dimorphism in colour and length of fin ray is well known among teleosts, and has recently been discussed by Egami[9] for Japanese gobies It seems safe to conclude, therefore, that the two names apply to one and the same species, for which the name *orca* Collett 1874 ranks by page priority as the senior synonym to *scorpioides* Collett 1874

A fuller account of this work, and a re-description of the genus and species, are intended for publication elsewhere

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Manner of expressing Rate of Ribonucleic Acid Biosynthesis

ENRICHMENT of a medium supporting slow, balanced growth of bacteria (*Aerobacter aerogenes*[1] or *Salmonella typhimurium*[2]) is followed by a period of accelerating growth during which the ribonucleic acid (RNA) · protein ratio of the bacterial protoplasm increases considerably It has recently been stated in papers from this laboratory[1] and elsewhere[2] that the rate of RNA synthesis during this adjustment period is even higher than it will be later when the new, high growth-rate is established (The phrase, 'rate of RNA synthesis', refers to the rate of accumulation of total RNA, of which approximately 80 per cent is ribosomal In the conditions of growth employed in the experiments being discussed, measurements of total accumulation are essentially measurements of ribosome synthesis, since turnover is slight) This assertion is based on the fact that when the logarithm of RNA per ml of culture is plotted against time, the slope immediately after enrichment of the culture is usually greater than that which will be maintained afterwards

We wish to point out that the value obtained from such a slope represents the amount of RNA made per hr per unit of RNA ($dR/dt \times R^{-1}$) and is not necessarily a meaningful expression of the rate of the biosynthetic process In fact, unless the synthesis of RNA is truly an autocatalytic process, this manner of expressing its rate can be quite misleading If alternatively, for example, one expresses the rate of RNA formation as the amount made per hr per unit of protein ($dR/dt \times P^{-1}$), the data cited above (as well as new experiments to be reported[3]) give no indication of an overshoot in the rate of RNA synthesis by a culture in accelerating growth Rather, the rate of RNA synthesis (expressed in this manner) simply increases, upon enrichment of the medium, to the value characteristic of the new growth-rate

To illustrate our point we have drawn an idealized curve which describes the accumulation of RNA and protein in such an experiment (Fig 1) The rate of RNA synthesis, calculated per unit of RNA and per unit of protein, is given for three points (*A*) balanced growth before enrichment, (*B*) unbalanced growth immediately after enrichment, (*C*) balanced growth after adjustment of the culture to the new medium For purposes of comparison, each expression has been multiplied by a constant (c_1 or c_2) chosen so as to yield values at point *A* of 1 It is evident that the two ways of expressing the rate of RNA synthesis give qualitatively different impressions of what happens to this process following enrichment of the medium Expressed per unit of protein, the rate of RNA synthesis quickly increases to the new higher value, while expressed per unit of RNA it overshoots (in this example by 100 per cent) The magnitude of the overshoot depends on the absolute increase in the growth-rate (over the range where RNA content of the bacteria is a function of the growth-rate) and on the rapidity with which the rate of RNA synthesis expressed per unit of protein reaches its new value

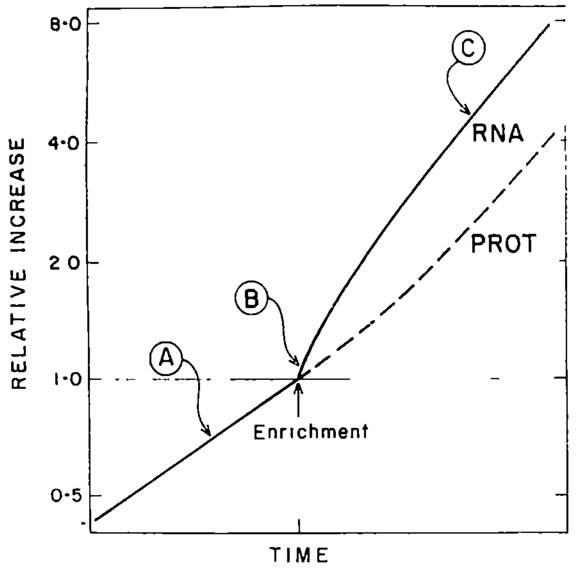

Fig 1 Increase in ribonucleic acid (RNA) and protein (PROT) per ml of culture before and after enrichment

The figure is idealized from data in the papers cited, and fulfils the following specifications (1) The growth-rate finally reached after enrichment is exactly double that before (2) The RNA protein ratio at the higher growth-rate is double that at the lower (3) The rate of RNA synthesis, expressed as the amount of RNA made per hr. per unit protein, increases instantly to the value characteristic of the higher growth-rate Actually, such a curve may be determined simply by the third of these specifications, together with the assumption that the rate of protein synthesis (dP/dt) is proportional to the RNA content of the culture

	$c_1 \frac{dR}{dt} \frac{1}{R}$	$c_2 \frac{dR}{dT} \cdot \frac{1}{P}$
A	1	1
B	4	4
C	2	4

Whether or not one concludes that RNA synthesis is faster during accelerating growth depends on how the kinetic data are expressed, and a choice between methods of expression must await elucidation of how RNA is made

Our work is supported by the National Science Foundation under grant *G*-5887 One of us (D G F) is a National Science Foundation Predoctoral Fellow

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Effect of Temperature and Potassium Gibberellate on Phases of Growth of Algerian Ivy

THE application of gibberellic acid to mature, fruiting shoots of *Hedera* was found[1a,b] to cause a reversion from the shrubby fruiting form to the juvenile vining type of growth Our experiments have confirmed this response but indicate that it depends on the maintenance of a sufficiently high temperature following treatment

Plants of *Hedera canariensis* were treated in midsummer in a 60° F minimum glasshouse with potassium gibberellate in concentrations of 0 0, 0 18, 0 91 and 1 8 mgm a plant, applied in capillary melting point tubes inserted in decapitated stems, using ten plants per treatment In 120 days, all the treated plants were juvenile, but only 30 per cent of the controls exhibited any juvenile characteristics Identical treatments were applied in a lath-house outdoors in January in uncontrolled temperatures in which the minima ranged from 38° to 55° F for most of the experiment Although the plants grew

Table 1 RESPONSE OF ALGERIAN IVY TO TEMPERATURE AND POTASSIUM GIBBERELLATE

Treatment	Temperature	Juvenile or transitional plants	Adult plants	Mean growth of longest shoot (cm)
Potassium gibberellate	50°	0	24	27 5
	60°	19	4	34 8
	70°	21	1	50 1
	74°	18	2	35 1
Control	50°	1	22	12 2
	60°	3	20	16 7
	70°	6	18	19 1
	74°	6	18	15 5

well, none of the treated plants had reverted to a definitely juvenile condition and all the untreated plants remained fully adult after 120 days

Since these observations indicated considerable difference in the ability of gibberellins to promote reversions at different seasons of the year, the effect of temperature was determined experimentally Adult established plants, twenty-four in each treatment, were cut back to one shoot with one or two buds A patch of bark was removed and 1 mgm of potassium gibberellate was applied All plants were placed under 16-hr photoperiods at 1,000 ft -c using constant temperatures of 50°, 60°, 70° and 74° F The reversions produced by the treatments together with shoot growth measurements are shown in Table 1 A few plants died and some of the forms classified as transitional had abnormal growth indicative of excessive concentrations of the chemical

70° F was the best temperature for elongation of shoots Potassium gibberellate doubled the number of bud breaks at all temperatures and was effective in increasing elongation of shoots at all temperatures Reversions were produced in all but the lowest temperatures Several investigators have observed that higher temperatures favoured the production of juvenile growth, including Fisher[2] in *Ranunculus*, Hudson and Williams[3] in *Rubus*, and Njoku[4] in *Ipomoea caerulea* Doorenbos[5] observed reversions on *Hedera* following grafting on juvenile understocks Stoutemyer and Britt[6] verified this effect but showed that it depended on sufficiently high temperatures The observation of Robbins[1b] that reversions were obtained with gibberellin more readily in summer than in winter may be explained as a response to temperature

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Effect of Gibberellic Acid, Kinetin and Other Substances on Seed Dormancy

THE seeds of many plants, especially woody species from temperate regions, are dormant when harvested and require a period of chilling ('stratification') before they become capable of germination As part of an investigation into the physiological basis of seed dormancy, attempts were made to replace the chilling requirement by treatment with gibberellic acid and other substances known to break dormancy Gibberellic acid has been shown to stimulate germination in several members of the Rosaceae[1,2] and in various tree species[3–5].

The effects of gibberellic acid on dormant seeds of hazel, Kent cobs (*Corylus avellana*) and beech (*Fagus sylvatica*) have been examined In germination experiments seeds were sown on filter paper or cotton wool soaked in distilled water or gibberellic acid solution and kept in the dark at room temperature In unchilled nuts of beech and hazel only a few embryos were capable of germination, even after removal of pericarp and testa When treated with 100 mgm /l gibberellic acid, embryos and seeds germinated within 3 weeks Beech embryos responded to a concentration of 1 mgm /l In both beech and hazel, intact nuts failed to germinate, even in the presence of 500 mgm /l gibberellic acid

It was also confirmed that gibberellic acid will stimulate germination of birch seed in the dark[3], but that it has no effect on dormant sycamore seed[6] Delayed epicotyl extension in acorns (*Quercus petraea*) was overcome by treatment with gibberellic acid.

It has been shown[2] that although gibberellic acid will accelerate germination of embryos from dormant Rosaceous seeds, it fails to have any effect when the coat structures are intact This observation was confirmed with seeds of apple (*Malus* sp), varieties 'Stoke Red' and 'Medaille d'Or', and rowan (*Sorbus aucuparia*) (See Table 1) Gibberellic acid stimulated isolated embryos to germinate but had no effect if the endosperm was left intact, even if the testa had been removed Concentrations as low as 1 mgm /l failed to stimulate germination of embryos In all the seeds used, coat structures prevented a response to gibberellic acid, in the case of beech and hazel it was the pericarp, whereas in the case of apple and rowan it was the endosperm plus testa which was effective This lack of response could be due to (i) failure of the acid to penetrate the coat structures, or (ii) failure of embryos treated with gibberellic acid to overcome the retarding effect of the coat structures If alternative (ii) was the case, then gibberellic acid only partially replaced the chilling requirement, since fully stratified seeds germinated despite the presence of coat structures

Table 1 EFFECT OF GIBBERELLIC ACID ON APPLE AND ROWAN SEEDS

	Untreated	Gibberellic acid (100 mgm /l)
Apple var 'Stoke Red' (1956)		
seeds	0	0
minus testa, endosperm intact	0	0
embryos	4	17*
,, chilled 10 weeks		
seeds	1	0
minus testa, endosperm intact	1	0
embryos	20	20
Apple var 'Medaille d'Or' (1959)		
seeds	0	0
embryos	16	20
,, chilled 10 weeks		
seeds	18	—
Rowan (1960)		
seeds	0	0
embryos	1	17*

No germinated out of 20 Apple after 1 week, rowan after 3 weeks
* Significant effect at 99 per cent level of probability (using χ^2 test)

Hazel, beech and rowan seeds were treated with other germination-stimulating substances potassium nitrate, thiourea and kinetin Nitrate had no effect on hazel, beech or rowan Thiourea and kinetin were both effective in stimulating germination in these species, although kinetin, at the concentration used (10 mgm /l), had no effect on intact seeds (see Table 2)

Table 2 EFFECT OF VARIOUS SUBSTANCES ON DORMANT SEEDS

	Untreated		Gibberellic acid (100 mgm /l)		Kinetin (10 mgm /l)		Thiourea (7 5 gm /l)		Pot nitrate (2 gm /l)	
	2*	4	2	4	2	4	2	4	2	4
Beech										
nuts	0	0	0	0	0	0	0	0	0	0
seeds	0	0	5	16*	0	0	11*	13*	0	1
embryos	3	3	20*	20*	15*	16*	14*	15*	3	6
Hazel										
nuts	0	0	0	0	0	0	0	0	0	0
seeds	1	2	10*	20*	0	1	10*	19*	1	1
embryos	2	9	20*	20*	4	18*	8	18*	4	6
Rowan										
seeds	0	0	0	0	0	0	0	0	0	0
embryos	1	1	9*	19*	16*	19*	5	9*	0	1

2, No germinated out of 20 after 2 weeks, 4, No germinated out of 20 after 4 weeks

* Significant effect at 99 per cent level of probability (using χ^2 test)

The effect of gibberellic acid on dormant seeds might be an indication that endogenous gibberellins play an important part in the chilling process Kinetin and thiourea, however, both unrelated chemically to gibberellic acid, can also stimulate germination Interest in the gibberellins lies in the fact that they have been shown to occur naturally in several seeds and that they promote other aspects of plant growth Also gibberellic acid will overcome 'physiological dwarfing' in seedlings grown from unchilled embryos of Rosaceous seeds[2,7]

The work was carried out under the supervision of Prof P F Wareing, part of it while I was supported financially by a grant from the Agricultural Research Council

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Bioassay of Gibberellic Acid using Excised Embryos of *Avena fatua* L.

A NUMBER of bioassays have been described for the detection of the gibberellins A_1 to A_9, particularly for gibberellic acid Almost every assay so far reported for gibberellic acid is of the type which utilizes shoot growth in intact normal, or more often dwarf, species of plants There is thus a need for alternative gibberellin bioassays utilizing other physiological responses This communication demonstrates that the germination response induced in embryos of *A fatua* L treated with gibberellic acid provides an extremely sensitive and rapid micro-test for the acid

The method used by us for assaying chromatograms, or small volumes of solution containing gibberellic acid, is as follows Non-dormant *A fatua* L seeds are allowed to imbibe water for about 15 min to soften the testa Embryos are then excised with a sharp scalpel under a dissecting microscope leaving a thin layer of endosperm attached to the scutellum When dried at room temperature these excised embryos can be stored for several months without affecting their response in the bioassay Twenty-five dry embryos are placed on a standard sized paper (1 × 7/8 in Whatman No 1 chromatography paper) which is then wetted with 0 25 ml of water (for chromatogram segments) or gibberellic acid (for assay of water solutions containing gibberellic acid) Each paper is kept in a small covered dish in a humid atmosphere at 20° C. in darkness. Each treatment is comprised of three such replicates Germination percentages are scored by counting elongated radicles at intervals after 12 hr

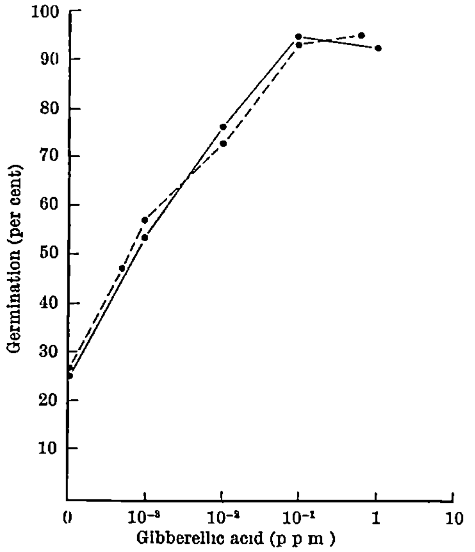

Fig 1 Bioassay of gibberellic acid using the germination response of excised embryos of non-dormant *A fatua* L ---, genetically heterogeneous population of seeds, germination recorded at 14 hr , ——, seeds from an inbred line, germination recorded at 20 hr Each point represents three replicates, each of 33 embryos. *S E* of all points is < ± 3

Two typical dose-response curves of non-dormant embryos to solutions of gibberellic acid are shown in Fig 1 The optimal response to the acid lies between 10^{-1} and 1 p p m It should be noted that concentrations lower than 10^{-3} p p m (0 00025 μgm /replicate of 0 25 ml) can be detected, and that up to 10^{-1} p p m there is a linear germination response to the log concentration of dose of acid There can be some advantage in using an inbred population of seed (solid curve) as opposed to a mixed population of seed (broken curve)

In our material, embryos which have not been after-ripened more than about 18 months show a degree of dormancy which, after maturation of the seed, decreases with period of after-ripening During this period there is a distinct shift in the pattern of reaction, embryos respond to a lower concentration

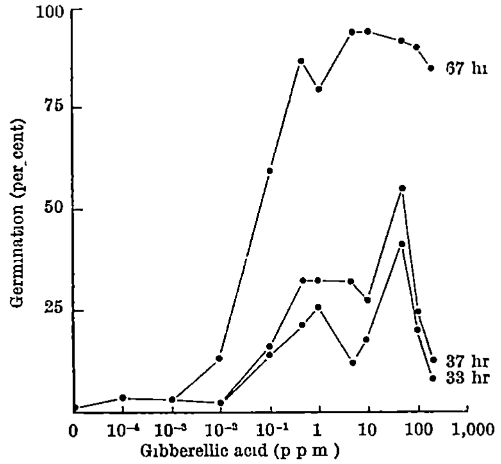

Fig 2 Dose-response curves of semi-dormant embryos of *A fatua* L to gibberellic acid

of gibberellic acid Also the time taken for germination to occur decreases considerably Fig 2 represents the response of seeds after-ripened for 4 months It is apparent that the embryos show two responses to gibberellic acid with distinctly different concentration optima, the higher optimal concentration lying near 50 p p m and the lower between 10^{-1} and 1 p p m Moreover, if seed is mature but not after-ripened the embryos show a response only to the high range of concentrations (10^{-1} to 50 p p m) An explanation of the physiological responses accounting for these differences has been presented in ref 2 The response to high concentrations of gibberellic acid is known to be due to the ability of the acid to eliminate the requirement for exogenous sugar in germination

Accordingly, the assay procedure is simplified by using embryos which have after-ripened to the point where exogenous sugar need not be supplied However, the dose-response curve of dormant embryos to the acid in the presence of sugar is essentially the same as that of non-dormant embryos with the acid alone, with the exception that the time required for the assay is slightly longer for semi-dormant and substantially longer for very dormant seed

Kinetin and indole-3-acetic acid (IAA) show no positive effects in the test IAA induces some inhibition of germination at concentrations of 1–10 p p m , which, however, is reversed by gibberellic acid The specificity of response of the embryos to different substances has not been otherwise investigated It would appear that the cultivated wheat and oat varieties are unsuited for use as bioassay material as their response to low concentrations of the acid disappears fairly soon after the seed is matured

In conclusion, the following points show some advantages of the *A fatua* bioassay (1) The assay can be completed in less than 18 hr (2) Assay material occupies a small space with environmental conditions suited for routine laboratory testing, that is, a simple incubator (3) Because material can be accumulated without loss of activity, large-scale assays are quite feasible (4) Small volumes of solution (0 25 ml /replicate) together with low specific amounts (0 00025 μgm) can be used for the assay This can be important when dealing with extracts of plant material with low specific activity

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ENTOMOLOGY

Occurrence of a Hyperglycæmic Factor in the Corpus Cardiacum of an Insect

ALMOST two decades have elapsed since Abramowitz *et al* [1] first reported the occurrence of a 'diabetogenic' factor in the sinus gland of a crustacean To the best of my knowledge the experiments described here are the first to show that a substance with a similar effect occurs in the corpus cardiacum of an insect The corpus cardiacum was selected for investigation because of its analogy to the sinus glands of Crustacea and its known content of biologically active materials[2-4] In view of the recent finding that the main blood sugar in insects is the non-reducing disaccharide trehalose[5-7] the effect of corpora cardiaca extracts on both reducing and non-reducing sugars in the blood has been investigated

All observations were made on the adult male cockroach, *Periplaneta americana* L , 4–5 weeks after the final moult Blood was removed from the cockroaches by the method in ref 8 and tungstic acid filtrates prepared Part of this filtrate was used for determination of reducing sugar, obtained by determining reducing value before and after fermentation with baker's yeast Because trehalose yields glucose on hydrolysis it may be estimated as reducing sugar Non-reducing fermentable values were obtained by hydrolysing a portion of the original filtrate with *N* hydrochloric acid at 15 lb /in ^{2} for 30 min , neutralizing the hydrolysate with *N* sodium hydroxide and treating in the same manner as the unhydrolysed filtrate The reducing power of the hydrolysed filtrate after correction for reducing power of unhydrolysed filtrate is considered to be a measure of blood trehalose These values are expressed as mgm per cent glucose equivalents Reducing power was determined by the method of Somogyi as modified by Nelson[9]

The corpora cardiaca extracts were prepared by homogenizing a number of pairs of glands, from which the corpora allata had been removed, in a suitable volume of Pringle's insect Ringer The extract was boiled for 5 min and then centrifuged at 12,000*g* for 15 min The supernatant was removed, diluted with Ringer solution to the desired concentration and stored in a deep-freezer until required for injection Table 1 shows the effect of 10 μl of extract, equivalent to one-tenth of a pair of glands, on the level of blood sugar at different times after injection The control animals received Ringer solution only It can be seen that except in two instances the level of reducing sugar was lower in those insects which received extract than in the controls The effect of the extract on the trehalose content of the blood was very striking 30 min after injection it had risen 45 3 per cent and reached a maximum of 150 per cent 5 hr. after injection Thereafter the trehalose content declined, returning to the normal level 48 hr after injection The figures for the control insects compare very well with those obtained for normal uninjected insects, the latter being 74 mgm per cent reducing sugar and 1,174 mgm per cent glucose equivalents of trehalose When older (3 months) cockroaches were used the trehalose concentration was found to be of the order of 1,400 mgm per cent glucose equivalents This figure is in good agreement with that given in ref 10 The extract was found to be effective even at a greater dilution than that used in the experiment described here, an injection equivalent to one-five-hundredth of a pair of glands increasing the blood trehalose by 30 per cent When used in a concentration of one pair of glands/injection the mean increase in blood trehalose was 152·5 per cent, which is comparable to that obtained with one-tenth pair of glands, suggesting that one-tenth of the active material contained in

Table 1 EFFECT OF CORPORA CARDIACA EXTRACTS ON BLOOD SUGAR
Figures are mgm per cent glucose equivalents

Time after injection (hr)	No of insects	Reducing sugar		Trehalose		
		Control	Extract	Control	Extract	Increase (per cent)
0 5	12	25 2	31 4	1,237	1,797	45 3
1	12	70 3	31 8	1,225	2 583	110 8
3	12	29 7	41 7	1,293	2,558	97 8
5	12	37 2	29 3	1,050	2,625	150 0
12	12	66 1	29 1	1,118	2,178	94 8
24	12	43 4	32 3	929	1,941	109 0
48	12	52 4	19 5	1,025	1,160	13 2

the corpora cardiaca is sufficient to elicit the maximum response.

In order to determine whether the hyperglycæmic activity of the corpora cardiaca was specific to this tissue a number of other tissue extracts were prepared and tested for activity. The extracts, prepared from approximately 25 mgm. of tissue, were treated in the same manner as those of the cardiaca. The results of this experiment are shown in Table 2. All values, except one for brain tissue, indicate that none of the extracts had any effect on the level of blood trehalose. One of the values obtained for brain tissue was slightly high and may have been due to traces of cardiaca tissue remaining attached to the brain. The corpora allata were also examined for hyperglycæmic activity, but because of the technical impossibility of completely removing the corpora cardiaca, the results were inconclusive.

Table 2. EFFECT OF VARIOUS TISSUE EXTRACTS ON BLOOD TREHALOSE

Figures are mgm. per cent glucose equivalents

Time (hr.)	No. of insects	Control	Nerve cord	Muscle	Brain
5	6	1,241	1,041	1,147	1,470
5	6	1,119	1,218	1,069	1,263

The results of this work clearly show that the corpora cardiaca of the cockroach contain a powerful hyperglycæmic factor. Perhaps this is the 'insect adrenalin' described in ref. 3.

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Inhibited Oviposition by Females of *Gryllus assimilis* (F.), induced by Radioactive Males, using L-Methionine-Methyl-^{14}C

THE taxonomy of North American field crickets of the genus *Gryllus* L. (until lately all referred to a single species, *Acheta assimilis* F.) has presented considerable problems in the past, and has recently undergone considerable revision[1-4].

In an attempt to help solve certain problems concerning speciation in this group of crickets, work with radioisotopes was initiated, using a laboratory culture of Jamaican *Gryllus assimilis* (F.), *sens. strict.*, and L-methionine-methyl-^{14}C. From these initial experiments an unexpected and interesting result was obtained. Not only did the treatment cause apparent sterility of the males, but also inhibition of egg-laying by females mated with radioactive males.

The experiments reported here were carried out in the Department of Entomology and Plant Pathology, Macdonald College, Ste. Anne de Bellevue, Quebec, in a greenhouse laboratory maintained at a temperature of 80° ± 1° F., and a relative humidity of 75 ± 5 per cent. The radioactive isotope, in distilled water, at dosages ranging from 0.05 to 10 μc./cricket, was injected into the hæmolymph of last-instar nymphs of both sexes of *G. assimilis* by means of an 'Agla' brand microsyringe (Burroughs Wellcome and Co., Ltd.). The needle was inserted laterally through the intersegmental membrane between the sixth and seventh abdominal segments.

At doses of up to 5 μc., the radioisotope had no deleterious effects on the insects and the nymphs metamorphosed to adult crickets and performed all functions of life quite normally, as did the controls which had been injected with equivalent quantities of distilled water alone. Higher concentrations of the radioisotope were lethal within 24 hr.

The nymphs injected with doses of 5 μc., or less, of the radioisotope all retained their radioactivity after moulting. The adult crickets thus obtained were then placed in individual 1-gallon, candy-jar breeding containers, provided, where appropriate, with oviposition cups after the manner described in ref. 5. All virgin females, both radioactive and normal (non-radioactive), began, on maturing, to lay numerous eggs whenever oviposition cups were available. This is usual in *G. assimilis* and certain other *Gryllus* and *Acheta* species, although parthenogenetic eggs have never been observed to develop. Also, when normal (non-radioactive) males were mated with either normal or radioactive virgin females, normal oviposition occurred. The eggs in both cases were viable, those from the radioactive females being also detectably radioactive as were first-instar nymphs which later hatched from them.

However, when radioactive males, derived from nymphs injected with any dose of L-methionine-methyl-^{14}C used, were mated with either radioactive or normal virgin females which had not yet begun laying, no oviposition occurred. Repeated experiments all produced the same results. Table 1 shows the average fecundity of radioactive and normal females, continually associated with normal and radioactive males. Figures for unmated virgin females (all of which oviposited) are also given by way of comparison. All radioactive individuals were derived from last-instar nymphs injected with L-methionine-methyl-^{14}C at a concentration of 1 μc./cricket.

Table 1. RESULTS OF MATINGS BETWEEN RADIOACTIVE (*R*) AND NORMAL OR NON-RADIOACTIVE (*N*) INDIVIDUALS OF *Gryllus assimilis* (F.) FROM DECEMBER 22, 1960, TO FEBRUARY 1, 1961

Matings 5 Males	 5 Females	Average number of eggs per female
R	*N*	0
R	*R*	0
N	*R*	196
N	*N*	126
—	*R*	123
—	*N*	90

It would seem from the results indicated in Table 1 that the radioisotope produced sterility in the males since no eggs were produced by the females with which radioactive males were associated. The urge to mate, however, was not impaired, the radioactive males mated as readily with both normal and radioactive females as did normal males, nor was the production of spermatophores inhibited. Judging from the average number of eggs laid per female, it would seem that the radioisotope may have led to an increase in the fecundity of females whether they had mated with normal males or not. This is indicated more clearly by the complete data than by the condensed table given here. It is also interesting to note that similar results have been obtained by administering the radioisotope orally via the drinking vials. Nymphs and adults were made radioactive by placing treated vials in the rearing jars for 72 hr.

It has, of course, long been recognized that male insects can be sterilized by irradiation with X-rays and/or γ-rays[6-9], but the present experiments appear to give the first indication that sterility may be induced by such a weak β-emitting radioisotope as L-methionine-methyl-^{14}C.

The second point of interest is that, whereas in previous cases of sterility in insects females have laid infertile eggs[7], in the case of *Gryllus assimilis* no eggs are laid by females after mating with radioactive males, even though unmated females normally lay (infertile) eggs. That radioactivity alone does not produce this result is indicated by the fact that radioactive females, either virgin or mated with normal males, oviposit normally; the implication is that some factor inhibiting oviposition is passed to the females from the radioactive males by way of the spermatophores (which were found to be detectably radioactive). It seems unlikely that non-radioactive L-methionine would have this effect, although this possibility is being explored.

Insufficient is known about the mechanism inducing oviposition in insects to indicate what might have occurred in this case, but it could be postulated that some reaction which permits or encourages the production of eggs when normal spermatophores are received by the female is blocked by the presence of a substance or substances originating from the radioactive spermatophores. That something of this kind is possible is indicated by the fact that inhibited females will begin to lay fertile eggs about 2 weeks after being mated with normal males. Similarly, such females isolated afterwards will begin to lay infertile eggs after about the same period. The fundamental question is posed: What causes oviposition to occur? Exactly what process is it that is inhibited by these hypothetical toxins from the radioactive spermatophores?

Whether there is any likelihood of a successful outcome from an attempt to put the type of sterility induced by radiation in *Gryllus assimilis* to practical use is doubtful. Bushland and Hopkins[7] used X-ray-sterilized males of the screw-worm fly, *Callitroga americana* (C. and P.), in the control of that insect in Florida, but this was made feasible because most females of this species mate only once. Females of *Gryllus assimilis*, on the other hand, mate frequently. The exact number of times has not been determined, but Spann[10] states that Kansas females of a related species (not *G. assimilis* although he uses this name) may mate about seven times daily. Thus, even though a radioactive male should inhibit oviposition in many females, it would not be long before the latter recovered and mated with normal males.

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MICROBIOLOGY

Survival of *Rhizobium meliloti* in Soil Culture

THE root nodule bacteria of leguminous plants (*Rhizobium* spp.) have repeatedly been observed to survive as long as 15–16 yr. in various media[1,2]. Bisset[3] has explained this remarkable longevity as due to formation of endospores in a complicated life-cycle.

The culture collection of this Laboratory includes many cultures of *R. meliloti* that have remained viable for much longer periods in a medium of loamy garden soil plus 0.5 per cent mannitol and some calcium carbonate, sterilized by autoclaving; the culture vessels are Freudenreich flasks that have been stored in darkness at room temperature, under which conditions the soil becomes air-dry within about a year. During the autumn of 1960 I examined such cultures of twenty *R. meliloti* strains, seventeen from lucerne and three from *Medicago lupulina*. The age of these cultures ranged from 30 to 45 years, the majority (13) being 38–40 years old.

A pure growth of *R. meliloti* developed from all cultures within a week when small quantities of soil were transferred to slopes of glucose–asparagine agar plus lucerne seedling extract. Plate counts on the same medium were rather erratic but suggested numbers from less than a hundred to about half a million per gm. of soil, with no apparent correlation between age and density of bacteria.

Some pasteurization tests were made on soil suspensions (one part of soil in ten parts of sterile tap water) heated for 5–30 min. in a thermostatically controlled water-bath; duplicate portions were cooled rapidly and ten drops of suspension were transferred to slopes of agar medium that were watched for growth at 25° C. for three weeks (in some cases for six weeks, which made no difference). Eleven strains failed to grow after heating for 5 min. at 50° C. The remaining nine showed somewhat inconsistent growth after heating for 5–20 min., one of them (*L.* 21, aged 39 yr.) even after 30 min. These strains, and five more, were tested at 60° C.; all succumbed after 5 min., except *L.* 21, which, in one instance, survived for 10 min. The rhizobia in the soil are thus about as heat-sensitive as in agar cultures[1], and according to this evidence they do not survive as thermoresistant endospores.

Nutman[4] found *R. trifolii* frequently producing ineffective variants when grown in sterile soil. To see if this had happened in the present cultures, 200 isolated colonies were picked from the plate counts (20 colonies from each of 10 strains) and the isolates were tested for effectiveness on lucerne seedlings in aseptic agar cultures. All isolates formed normal nodules with red pigment; nodule-bearing plants were dark green and healthy-looking and contained 2.7–4.6 per cent nitrogen in dry matter 8 weeks after inoculation, while the uninoculated control plants were dwarfed and yellowish, with 1.2–1.7 per cent nitrogen. One isolate (from strain *L.* 4, aged 44 years) was ineffective immediately after isolation but was normally effective when re-tested a couple of months later. Formation of permanently ineffective variants in *R. meliloti* thus seems to be quite a rare occurrence.

The collection includes no cultures as old as those of *R. meliloti*, but soil cultures of several other organisms proved viable at ages of 10–14 yr. This applies to bacteria (*R. trifolii*, *R. leguminosarum*, *Pseudomonas* *aeruginosa*) as well as actinomycetes (*Nocardia, Streptomyces, Micromonospora*) and fungi (*Trichoderma, Penicillium, Aspergillus*)

Sterilized soil as culture medium thus seems to keep vegetative cells of *R meliloti* alive and effective in a desiccated condition for periods approaching half a century, moreover, it preserves for more than ten years the viability of several other micro-organisms that have never been claimed to form endospores

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Inhibition of Lysozyme by 'Suramin'

IT has been shown that a number of non-chaining bacteria form long chains when grown in the presence of 'Suramin', the chains are rapidly split by filtrates of ordinary 'short form' cultures of the organisms It was assumed as a working hypothesis that separation of bacterial cells is an enzymic process and that 'Suramin' acts by inhibiting the cell-separating enzyme[1]

Detailed experiments showed that while *Streptococcus faecalis, B megaterium, Cl welchii* and *Cl sporogenes* grow well in the presence of 'Suramin' at a concentration of 1 gm in 40 ml (w/v), some chaining still occurs at concentrations up to 1 gm in 5,000 ml (w/v) The hypothesis of enzyme inhibition was confirmed when it was found that the chain-splitting activity of the filtrates was indeed inhibited by 'Suramin' at concentrations ranging from 1 to 15 µgm / ml (w/v)

It is also known that chains of various aerobic and anaerobic rods (obtained by growth in magnesium-deficient medium) and chains of *Streptococcus pyogenes* can be split by lysozyme[2,3] Preliminary experiments confirmed these findings and extended them to *Cl sporogenes* and *Streptococcus faecalis* Relatively high concentrations of lysozyme were required to split the chains in the standard time of 30 min (20–100 µgm / ml w/v), the splitting was inhibited by 'Suramin'

Evidence was afterwards obtained (to be published in detail elsewhere) that despite the similarity of effect between lysozyme and filtrates, splitting of chains is not in fact caused by lysozyme but is due to an enzyme of bacterial origin—apparently released by lysozyme from the cells Since, however, 'Suramin' is a powerful inhibitor of lysozyme it was thought interesting to give an account of it

Crystalline lysozyme (Sigma Co) and 'Suramin' (Imperial Chemical Industries) were sterilized by filtration through sintered glass Lysozyme was assayed by estimating its ability to lyse suspensions of *Micrococcus lysodeikticus* (N C T C 2665) The organism was grown at 28° C on meat-extract broth agar for 48 hr and the cells washed three times with saline Suspensions of 10^9 cells/ml were made in saline-phosphate buffer *p*H 5·5, and falling amounts of lysozyme added Results were read after 30 min at 20° C taking 50 per cent clearing of the suspension on an EEL nephelometer as the end point

The results can be summarized as follows lysozyme caused clearing of the suspensions of *Micrococcus lysodeikticus* up to a concentration of 0·1 µgm /ml (w/v) 'Suramin' gave 50 per cent inhibition of lysozyme when present at equal weight Accepting the mass of lysozyme as approximately ten times that of 'Suramin', inhibition occurred when 'Suramin' was present at a ten-fold molar excess

In some experiments 'Suramin' was first left in contact with the bacterial cells, provided that 'Suramin' was afterwards removed by washing the cells with saline no inhibition was observed In other experiments 'Suramin' was allowed to interact with lysozyme before the cell suspension was added, maximum inhibition was reached rapidly in 5–15 min

We are indebted to the Rankin Research Fund for a grant towards the expense of this work

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Dihydroxyacetone Production in *Acetomonas*

IT has already been shown[1] that a useful primary criterion for differentiating *Acetomonas* strains from those of *Pseudomonas* is the ketogenic action of the former on glycerol which results in the production and accumulation of dihydroxyacetone Thus the 165 strains of *Pseudomonas* studied all failed to show production of dihydroxyacetone, while 49 out of the 50 *Acetomonas* strains produced it abundantly

As regards the single anomalous *Acetomonas* strain (*A suboxydans* N C I B 3734) it was tentatively suggested that this might not be an *Acetomonas* strain at all, but a mutated *Acetobacter* strain which had possibly lost the typical ability of acetobacters further to oxidize the acetic acid produced to carbon dioxide and water, for production of dihydroxyacetone is much less common in *Acetobacter* than in *Acetomonas* There was, however, no way of knowing whether it was originally dihydroxyacetone-negative when first isolated or whether it has lost dihydroxyacetone positivity in the course of long sub-cultivation

However, we now have definite evidence that the ability to produce dihydroxyacetone can be lost by 'mutation' (in the broadest sense) in the course of laboratory sub-cultivation A strain of *Acetomonas*, isolated from cider, was strongly dihydroxyacetone-positive when first isolated Now, after sub-cultivation on apple juice agar for some two years, this strain is completely dihydroxyacetone-negative, although it has retained its original ability to produce deep brown to black water-soluble pigment on yeast extract/glucose/calcium carbonate agar

It thus appears that production of dihydroxyacetone from glycerol, if negative, is not so conclusive as formerly in suggesting that a strain is a pseudomonad, but merely functions as a first presumptive indication to that effect However, even if dihydroxyacetone-production is negative, the other three primary criteria[1] found always to be conclusive for differentiating *Acetomonas* from *Pseudomonas* strains amply suffice to prevent any possibility of confusing these two completely different types of bacteria.

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CYTOLOGY

Chromosome Preparations obtained directly from Peripheral Blood

Abnormalities of both number and morphology of the chromosomes of leukæmic cells in man have been reported[1,2]. All such work reported to date has been made on cells from marrow or from peripheral blood cultured *in vitro*. Such findings are open to the theoretical objection that any observed abnormality might have arisen during the culture process. In order to obviate this possibility, we decided to attempt to take advantage of the situation in leukæmia where the bone marrow is abnormally active, and later developed a method of making preparations without prior culture[3].

This method has now been successfully adapted for application to cells obtained directly from the peripheral blood in cases of leukæmia where again abnormal numbers of cells, many of them in division, may be found.

Venous blood (20 ml.) was collected into the dipotassium salt of (ethylene dinitrilo) tetraacetic acid. The white cells were separated from the red by centrifugation at 400 r.p.m. at 0–4°. The supernatant plasma and white cells were decanted and 0·72 per cent sodium citrate was added for 10 min. After centrifugation at 1,500 r.p.m. and resuspension of the leucocytes in 3 : 1 (ethanol : acetic acid), the material was hydrolysed in *N* hydrochloric acid at 60° C. for 4–6 min (depending on the specimen) and stained with orcein.

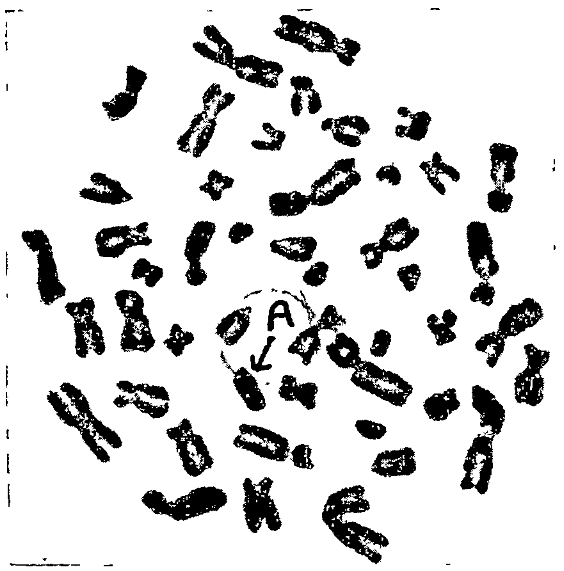

Fig. 1. Direct peripheral blood preparation. Hyperdiploid metaphase ($2n + 1$) in a male with acute leukæmia. The additional chromosome is shown at *A*

It has been found that the dipotassium salt of (ethylene dinitrilo) tetraacetic acid used as an anticoagulant has a colchicine-like action on chromosomes, producing many well-differentiated metaphase plates. There is thus no need for previous administration of colchicine or colcemid. Very satisfactory preparations, suitable for both counting and morphological work, are being obtained by this simple method.

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Surface Granulation in Myeloid Leucocytes

The clinical use of cytotoxic drugs which have a more or less specific action on cells of the lymphoid or myeloid series has made more important the differentiation of one type of acute leukæmia from another. Any technique which points to distinct biochemical or physico-chemical differences between myeloid and lymphoid cells is therefore worthy of further study. This communication is concerned with distinct differences in these two types of cells revealed by certain effects of fixation. These have not been reported before.

In 1957 McNary[1] described the application to blood cells of a histochemical stain specific for zinc. The stain contains diphenylthiocarbazone (dithizone) dissolved in absolute acetone, water, 1 *N* acetic acid, sodium potassium tartrate, and a complex-forming buffer (sodium thiosulphate, sodium acetate, potassium cyanide and glacial acetic acid) at *p*H 5·5.

After staining blood smears for 15 min., neutrophil and eosinophil granulocytes, but not lymphocytes, show a golden-brown discrete 'granulation' in the cytoplasm which is easily seen with high-power magnification, provided a dry objective is used. Viewed under oil immersion, or after mounting, the 'granulation' is no longer visible. The same golden-brown 'granulation' is produced if dithizone is left out of the stain.

Accentuation of this effect is obtained with phase contrast and a dry objective × 45, the neutrophils contain numerous black dots and the lymphocytes contain none (Fig. 1*a*). The black dots disappear when viewed through oil, and where the black 'granules' were previously seen the cytoplasm now shows fine patchy areas which are paler than the rest of the cell (Fig. 1*b*).

This phenomenon can be explained if the 'granules' are regarded as irregular elevations due to folding of the cell surface, resulting from the effects of *p*H and fixation. Light passing through a cell is altered in phase by an amount depending on the product of refractive index by thickness[2]. These elevations would therefore appear black under phase contrast because the cell is thicker in this region. Addition of oil with a greater refractive index than the cell would reverse the picture as there would be now less oil over the elevations than over the rest of the cell. In support of this concept, it is known that precipitant fixatives

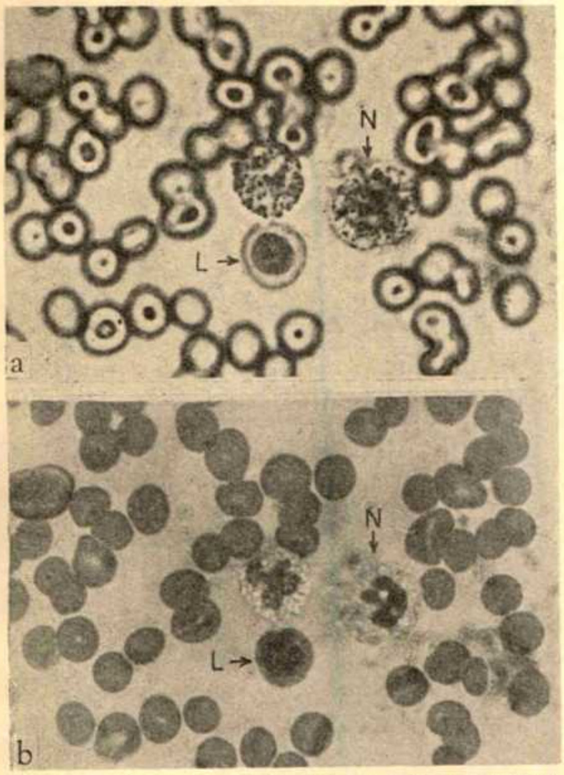

Fig. 1. *a*, Blood smear showing two neutrophils with 'surface granulation' (*N*) and one lymphocyte with clear cytoplasm (*L*). Counterstained with Leishman. Phase contrast × 450. *b*, Same field as in *a* but under oil immersion (× 450). Disappearance of 'surface granulation'

containing acetic acid often produce considerable distortion of the cell[3].

Sodium thiosulphate in the presence of acetone, water and acetic acid is effective in producing neutrophilic 'surface granulation', but the change is dependent on *p*H and is best developed at a *p*H near 5·5. This was determined by testing freshly prepared air-dried blood smears against the various components of McNary's stain. Neither dithizone, sodium potassium tartrate nor potassium cyanide was necessary for the production of 'surface granulation'. Absolute acetone, or acetone plus water, alone does not produce 'surface granulation' although it adequately fixes blood smears.

If blood smears are fixed first in methanol, formalin vapour, or osmium tetroxide vapour, neutrophils no longer show 'surface granulation'. Neutrophils from the dog do not show 'surface granulation' under conditions which produce it in human neutrophils.

The differences described here between human neutrophils and lymphocytes must reflect biochemical differences in cell proteins. These differences are being investigated in lymphocytic and granulocytic cells at various stages of maturation.

This work was supported by a grant from the Anti-Cancer Council of Victoria. I am grateful to Dr. T. E. Lowe for his help.

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ARCHÆOLOGY

Absolute Age of Pleistocene and Holocene Deposits in the Haua Fteah

THE general characteristics of the large coastal cave site known as the Haua Fteah, in north-eastern Libya, have already been described, together with a brief résumé of the most important results obtained up to 1952[1,2]. A further season's work in 1955 provided additional fossil human remains associated with Middle Palæolithic occupation débris[3a,b], enabled a much closer analysis of the stratigraphy, and, by opening up a wider area, made possible the excavation of a deep sounding into earlier cultural horizons than any hitherto examined, down to a total depth of 42·5 ft. In the past six years a detailed programme of study of the cultural, palæontological and other collections has been carried out in this Department, and further samples for carbon-14 analysis have been processed.

From an archæological point of view the site is now seen to offer a nearly continuous cultural succession from a pre-Mousterian stage (provisionally labelled Libyan Pre-Aurignacian by analogy with the "Praeaurignacien" identified by Rust[4] in Syria) up to the present. The stratigraphy containing this succession consists for the most part of remarkably regular horizontal layers, distinguished from one another by minor differences in colour and lithology, and ranging in thickness from about 6 to 18 in. (ref. 2). Mammal bones form a considerable proportion of the occupation debris and are the subject of a separate communication elsewhere[5]. The object of the present note is to make available, in advance, the principal data on which the absolute age of the cultural and biological succession is based, since these would appear to be relevant to other investigations being carried out elsewhere in the Mediterranean and adjacent areas.

Carbon-14 age estimates were obtained by H. E. Suess, at the Washington Laboratory, from samples collected in 1952 (ref. 6), and by the late Hl. de Vries, from samples collected in 1955 (personal communication). In the accompanying diagram (Fig. 1) these ages have been plotted against the cumulative depth obtained by adding together the maximum observed depth of each layer. This device has been adopted in order to minimize the effects of localized denudation, and hence approximate as nearly as possible to the relative time-interval represented by each layer.

It will be noted that as far back as 28,500 before the present the points appear to approximate to a logarithmic curve representing a gradually decreasing ratio of thickness to time unit. This is not unexpected since the effects of compression by increasing overburden, plus possible weathering *in situ* by percolating water, should in general lead towards progressive reduction in thickness.

Below a depth of about 25 ft., however, this tendency seems to be reversed by dates *W*85, *GRO* 2022, 2023, and a preliminary reading for layer XXIX, *GRO* 2050 (de Vries, personal communication). Both Suess and de Vries, however, considered that these dates were best regarded as minimum rather than absolute, owing to the well-known greatly increased disturbance of small contaminations, etc., on readings of this great age. On the whole, therefore, the projection of a line drawn from *GRO* 2586 through the midpoint between the readings for layers XX and XXI would seem to provide as reasonable a first approxi-

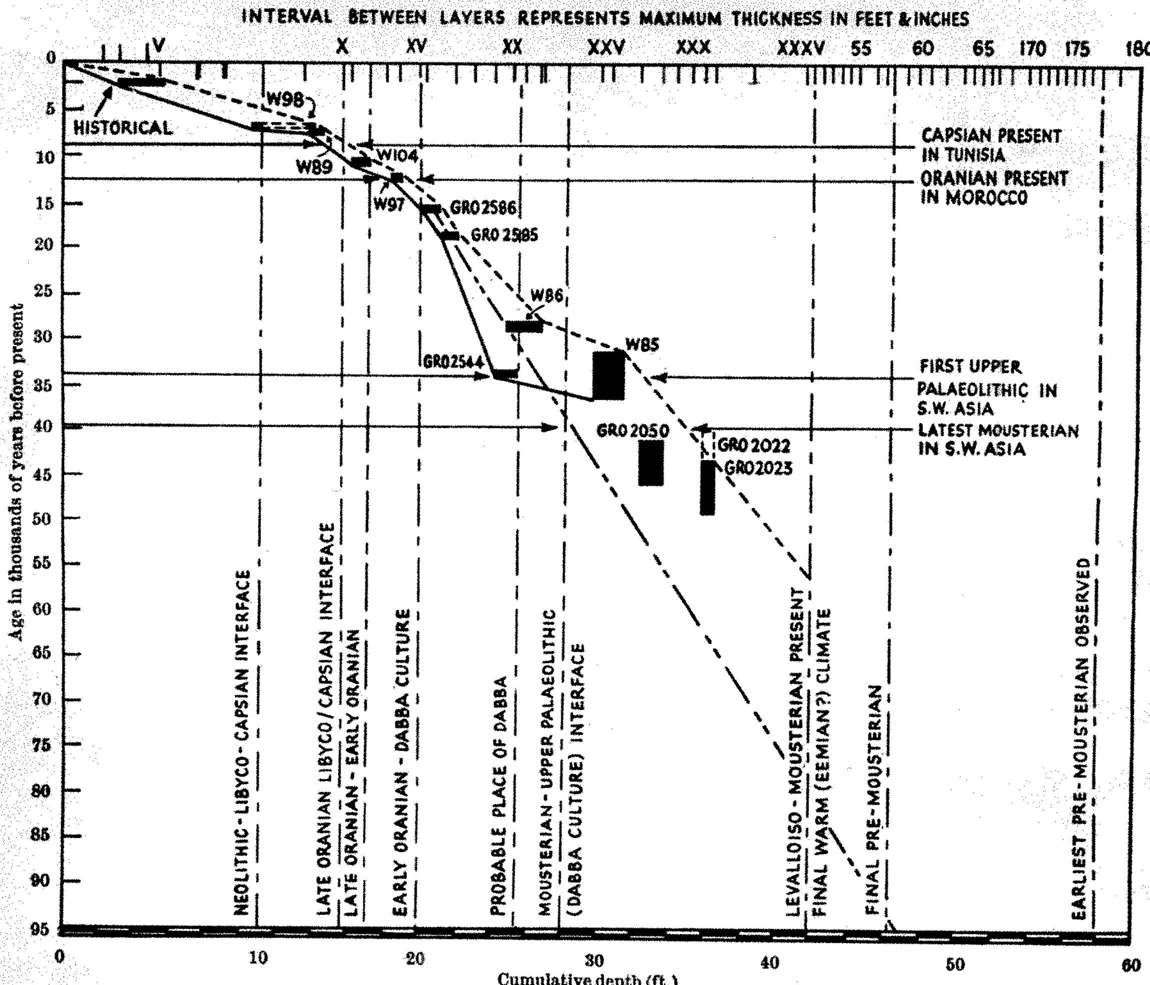

Fig. 1. Graph to show relation between age in thousands of years before the present and cumulative depth (ft.), of carbon samples from the Haua Fteah, Cyrenaica, Libya. The rectangles represent the depth through which the sample was taken and the standard error of the carbon-14 reading expressed in years. The central broken line represents the hypothetical trend beyond the range of the readings regarded as most reliable. The numbers at the top of the diagram are the reference numbers assigned to each layer

mation to the possible ages of the earlier interfaces as the present data allow. Although a wide margin must naturally be left to alternative estimates, an age for the earliest occupation horizon within the limits 100,000–150,000 seems probable.

It is interesting to set this against the geological evidence for an Eemian date recently established for the closest typological homologue, the Pre-Aurignacian of the Levant[7], since the end of the Eemian according to most carbon-14-based estimates would have occurred between 55,000 and 75,000 (refs. 8*a*, *b*, 13).

Using the same gradient, an age of 38,000 would be indicated for the Mousterian–Upper Palæolithic interface at the Haua, which would compare with previous estimates for the corresponding horizon at Shanidar (Kurdistan) of 35,000–40,000 (refs. 9, 10) $\geqslant$ 32,000 at Kara Kamar (Afghanistan) (ref. 10), and $>$ 32,000 (ref. 12) (estimated 36,000, ref. 13) at Istallöskö (Hungary). On the other hand, a date $<$ 39,500 (ref. 14) is indicated by the presence of a Mousterian horizon of this age at Tabun (Palestine).

Again, the age of 14,000 indicated for the earliest Oranian at the Haua may be compared to 12,000 recently established for the middle Oranian horizon at Taforalt (Morocco)[15], whence the earliest Oranian at this site can be estimated as between 11,000 and 15,000 on the stratigraphical context.

On the basis of these figures it may be said that the chronology suggested by the combined carbon-14 readings and stratigraphy at the Haua Fteah is in general agreement with available carbon-14 dates for the corresponding cultural events in not too distant areas.

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[10] Solecki, R. S., *Trans. N.Y. Acad. Sci.*, II, **21**, 712 (1959).

[11] Coon, C. S., *Science*, **123**, 447 (1956).

[12] Vertés, L., and de Vries, Hl., *Arch. Ertésitö*, **86**, 195 (1959).

[13] Movius, H. L., *Current Anthropol.*, **1**, 355 (1960).

[14] de Vries, Hl. (personal communication).

[15] See Balout, L., in *Prehistoire de l'Afrique du Nord* (Paris, 1955).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 20

CIBA FOUNDATION (at the Royal Institute of British Architects, 66 Portland Place, London, W 1), at 5 p m —Prof E Katchalski (Weizmann Institute of Science, Rehovoth) "Synthetic Macromolecules as Protein Models" (Thirteenth Ciba Foundation Lecture)

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Electrical Accidents and their Causes, including the Legal Aspects", opened by Mr S J Emerson, O B E

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S W 1), at 5 30 p m —Dr H Gysin "Triazine Herbicides, their Chemistry, Biological Properties and Mode of Action"

UNIVERSITY OF LONDON (at King's College, Strand, London, W C 2) at 5 30 p m —Dr F A E Pirani and Dr G F Elliott "Ballistic Missiles and Nuclear Weapons—Effects of Nuclear Weapons" * (Sixth of seven lectures in War Studies Further lecture on November 27)

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College Gower Street, London, W C 1), at 5 30 p m —Dr T Sorgenfrei (Copenhagen) "Tertiary of North-West Europe" * (Further lectures on November 21 and 23)

UNIVERSITY OF LONDON (in the Large Lecture Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, London, W C 1), at 5 30 p m —Prof C R Rao (Indian Statistical Institute, Calcutta) "Some New Tests in Large-Sample Theory" * (Further lectures on November 24 and 27)

INSTITUTION OF MECHANICAL ENGINEERS, INDUSTRIAL ADMINISTRATION AND ENGINEERING PRODUCTION GROUP (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "Mechanical Handling of Small Parts with special reference to Automatic Assembling Equipment"

INSTITUTE OF METAL FINISHING (at the Northampton College of Technology, St John Street, London E C 1), at 6 15 p m —Mr L E. Probert and Mr J Rollinson "Detection and Removal of Hydrogen Absorbed during Chemical and Electrochemical Processing"

Tuesday, November 21

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place London W C 2), at 5 30 p m —Discussion on "The Precision Required in the Calibration of Industrial Instruments", opened by Mr P M Clifford and Mr A Felton

PARLIAMENTARY AND SCIENTIFIC COMMITTEE (in Committee Room 12, House of Commons, London, S W 1), at 5 30 p m —Meeting of the General Committee Prof P B Medawar, F R S "The Impact of Biological Research on Advances in Medical Science"

RESEARCH DEFENCE SOCIETY (in the Physiology Lecture Theatre, University College, Gower Street London, W C 1), at 5 30 p m —Sir Hugh Linstead "On Taking Account of Public Opinion" (Thirteenth Stephen Paget Memorial Lecture)

SOCIETY FOR ANALYTICAL CHEMISTRY, PHYSICAL METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W 1) at 6 30 p m —Annual General Meeting followed by the Retiring Chairman s address

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Dr A R Harrison "Primary Hyperparathyroidism" * (Eleventh of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 5 30 p m —Prof K R Popper "Science and the Growth of Knowledge" * (First of four Shearman Memorial Lectures Further lectures on November 23, 28 and 30)

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W C 1) at 6 p m —Prof D R Cox "The Role of Statistical Methods in Science and Technology"* (Inaugural Lecture)

Tuesday, November 21—Wednesday, November 22

INSTITUTION OF GAS ENGINEERS (at Church House, Westminster, London, S W 1), at 9 30 a m on Tuesday and 9 15 a m on Wednesday —27th Autumn Research Meeting

Wednesday, November 22

ZOOLOGICAL SOCIETY OF LONDON (NUFFIELD INSTITUTE OF COMPARATIVE MEDICINE) (at the Zoological Society, Regent's Park, London N W 1) at 10 a m —Conference on "Arterial Diseases"

GEOLOGICAL SOCIETY OF LONDON (at the Royal Institution, Albemarle Street, London, W 1), at 2 p m —Symposium on "Depth and Tectonics as Factors in Regional Metamorphism"

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London Ducane Road London, W 12), at 2 p m —Prof G. H Lathe "Bile-Pigment Metabolism" *

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W C 2), at 2 30 p m —Miss Sylvia Crowe "Landscape and Civilization"

INSTITUTE OF FUEL (at the Royal United Service Institution, Whitehall, London S W 1) at 5 30 p m —Mr L E Reed and Mr S C Wallin "The Effect of Operating Conditions on the Performance of Different Types of Oil Burners"

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W C 2), at 5 30 p m —Dr A S Aldred "Electronic Analogue Computer Simulation of Multi-Machine Power System Networks", Mr J G Miles "Analysis of Overall Stability of Multi-Machine Power Systems"

BRITISH INSTITUTION OF RADIO ENGINEERS, COMPUTER GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Symposium on "Adaptive Optimizing Control"

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster London, S W 1) at 6 p m —Prof E Giffen "Reflections on the Institution's Role in Engineering Education" (James Clayton Lecture)

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square London, S W 1) at 6 15 p m —"Food Technology in Norway". (The film and members' impressions of the 1961 tour)

UNITED STATES INFORMATION SERVICE (in the American Embassy Theatre, American Embassy (Upper Brook Street entrance), London, W 1), at 6 30 p m —Three films from the series "Planet Earth". "Research by Rockets", "Cosmic Rays" and "Radio Waves" *

BRITISH PSYCHOLOGICAL SOCIETY, MEDICAL SECTION (at the Royal Society of Medicine, 1 Wimpole Street London W 1), at 8 30 p m —Mr Murray Jackson, Mr Michael Fordham and Mr Kenneth Lambert "Jung's Later Work" (C G Jung Commemorative Meeting).

Thursday, November 23

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1 15 p m —Dr J H Burns "Jeremy Bentham and University College" *

SOCIETY OF CHEMICAL INDUSTRY MICROBIOLOGY GROUP (at the Royal Society of Medicine, 1 Wimpole Street, London W 1) at 2 p m —Meeting on "Industrial Cell Culture and Vaccine Production".

ROYAL INSTITUTION OF NAVAL ARCHITECTS (at 10 Upper Belgrave Street London, S W.1) at 4 45 p m —Mr M N Parker and Mr James Dawson "Tug Propulsion Investigations"

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W 1), at 5 p m.—Prof Keith Runcom "Palæomagnetism", Prof P Allen "Palæotemperatures"

UNIVERSITY COLLEGE, LONDON (in the Physiology Theatre, Gower Street, London W C 1) at 5 p m —Dr B R Rabin "Some Aspects of Enzyme Catalysis" * (Further lecture on November 30)

UNIVERSITY OF LONDON (at the London School of Economics and Political Science, Houghton Street, London, W C 2) at 5 p m —Dr George Taylor "Britain's Botanical Power House" * (Further lecture on November 30)

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place London, W C 2) at 5 30 p m —Mr F C McLean, Mr H W Baker and Mr C H Colborn "The B B C Television Centre and Its Technical Facilities"

UNIVERSITY OF LONDON (at Queen Elizabeth College, Campden Hill Road, London, W 8) at 5 30 p m —Prof Hermann Bondi "Ideas of Special Relativity" *

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street London W C 1), at 5 30 p m —Prof I E Bush "Hormones of the Adrenal Cortex" * (Twelfth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College Gower Street, London, W C 1), at 5 30 p m —Dr T Sorgenfrei. "Tertiary of North-West Europe" * (Last of three Special University Lectures)

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W C 2), at 6 p m —Discussion on "The 57 Effects of an Electrical Current" opened by Dr G S Brosan

UNIVERSITY OF LONDON (at Wye College, Wye, near Ashford, Kent), at 8 15 p m —Dr. H L Jensen (Denmark) "Recent Advances in Soil Microbiology" *

Friday, November 24

ASSOCIATION OF APPLIED BIOLOGISTS (joint meeting with the HORTICULTURAL EDUCATION ASSOCIATION, at the Royal Horticultural Society, Vincent Square, London, S W 1) at 10 50 a m.—Meeting on "Pests and Diseases of Ornamental Plants"

ROYAL ASTRONOMICAL SOCIETY (joint meeting with the ROYAL METEOROLOGICAL SOCIETY, at Burlington House, Piccadilly London, W 1), at 4 15 p m —Geophysical Discussion on "The Density and Composition of the Atmosphere Above 100 km ". Chairman Mr. J A Ratcliffe, F R S

UNIVERSITY OF LONDON (in the Manson Lecture Theatre, London School of Hygiene and Tropical Medicine Keppel Street London, W C 1) at 5 30 p m —Prof C R Rao (Indian Statistical Institute, Calcutta) "On Problems of Discrimination with Multiple Characters" * (Further lecture on November 27)

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square London S W 1) at 6 30 p m —Dr J R Catch "The Production of Organic Compounds Labelled with C-14 or Tritium"

ROYAL INSTITUTION (at 21 Albemarle Street, London, W 1), at 9 p m —Dr P B. Hirsch. "Seeing Atomic Defects in Metals"

Friday, November 24—Saturday, November 25

Botanical Society of the British Isles (at the Linnean Society, Burlington House, Piccadilly, London, W 1) Conference on Local Floras and Annual Exhibition Meeting

Saturday, November 25

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 3 30 p m —Mr C M Mitchell "The Folk Life of the English People" *

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Assistant Lecturer (preferably with an interest in theoretical or experimental plasma physics) in Physics—The Secretary, The Queen's University, Belfast, Northern Ireland (November 24)

Junior Lecturer (preferably with a registrable medical qualification) in Bacteriology—The Registrar, Trinity College, Dublin 2 (November 27)

Physics Master (graduate with a good honours degree)—The Head Master, City of Oxford High School, Oxford (November 30)

Research Fellow (with a postgraduate degree in geophysics, physics or geology, and preferably, though not necessarily, previous experience in seismological work) in Seismology—The Secretary, The University, 38 North Bailey, Durham (December 1)

Assistant Biochemist (with a degree or equivalent qualification in chemistry, with experience in, or wishing to take up, clinical biochemistry)—The Secretary, Welsh National School of Medicine (University of Wales), 34 Newport Road, Cardiff (December 2)

Lecturers in Pure Mathematics and in Applied Mathematics —The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne 1 (December 4)

Lecturer (with postgraduate experience in research coupled with a keen interest in teaching) in Physics at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Hong Kong and London, December 4)

Technical Officer (with a recognized degree or National Diploma in Agriculture, and preferably experience in seed production) for Seed Multiplication—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (December 4)

Burnett-Fletcher Chair of History and Archæology—The Secretary, The University, Aberdeen (December 9)

Lecturer (with an honours degree in electrical or electronic engineering) in the Department of Electrical Engineering—The Registrar, The University, Leeds (December 14)

Lecturer (with an honours degree in psychology) in Psychology at the University of Canterbury, Christchurch New Zealand—The Secretary Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, December 15)

Lecturer in Psychology at the University of Cape Town—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1, and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (December 20)

Lecturer (preferably with qualifications in one or more of the following fields geomorphology, biogeography, and the geography of soils) in Geography at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, December 30)

Reader in Physics—The Registrar, The University, Hull (December 31)

Mary Glendining Chair of Medicine in the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, January 15)

Chair of Veterinary Science in Massey Agricultural College (University of New Zealand), Palmerston North—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (February 1)

Computer Programmer in the Nuclear Physics Research Laboratories, to work with a research group studying high energy nuclear physics—The Registrar, The University, Liverpool, quoting Ref No CV/N/543

Lecturer (well qualified, and preferably with industrial and teaching experience) in Mathematics—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref 32/G

Lecturer (with a first degree or equivalent qualification in chemical engineering, chemistry, mathematics or physics, and preferably some industrial experience) in Chemical Engineering—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref 31/G

Master to teach Physics throughout the School, with Sixth Form and University Scholarship work—The Headmaster, King's College School, Wimbledon, London, S W 19

Postdoctoral Fellow and a Postgraduate Student in Chemistry for work on the fluorescence of aromatic hydrocarbons under the direction of Dr B Stevens—The Registrar, The University, Sheffield

Professor of Thermodynamics at the University of Malaya, Kuala Lumpur, to develop research in applied thermodynamics and to supervise courses already in existence—Department of Technical Co-operation, 26 King Street, London, S W 1, quoting MAL/861/TCS

Scientific Officer or Senior Scientific Officer (honours graduate in chemistry or biochemistry with interests in the isolation and chemistry of proteins and in studies of enzymes) in the Physiology Department—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, near Reading, quoting Ref 61/16

Senior Lecturer in Mathematics, and a Research Assistant in Mathematics—The Registrar (Department D), Bradford Institute of Technology, Bradford 7

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Department of Scientific and Industrial Research Forest Products Research Bulletin No 46 Identification of Hardwoods a Microscope Key By J D Brazier and G L Franklin Pp viii+96+32 plates (London H M Stationery Office, 1961) 10*s* net [258

Fitzwilliam Museum, Cambridge Annual Report for the year ending 31 December 1960 Pp 8+4 plates Friends of the Fitzwilliam Museum Fifty-second Annual Report for the year 1960 Pp 8 (Cambridge The Fitzwilliam Museum, 1961) [258

Ministry of Housing and Local Government Essex Rivers and Stour Hydrological Survey, Hydrometric Areas 36 and 37 Pp iv+123 (London H M Stationery Office, 1961) 20*s* net [258

Iron and Steel Institute Bibliographical Series, No 15b The Rolling of Iron and Steel (1955–1960) Pp 164 (London The Iron and Steel Institute, 1961) 40*s* [258

Philosophical Transactions of the Royal Society of London Series A Mathematical and Physical Sciences No 1036, Vol 254 (24 August 1961) Axially Symmetric Plastic Deformations in Soils. By A D Cox, G Eason and H G Hopkins Pp 1–45 (London Royal Society, 1961) 14*s* [258

Gateways Guide to Courses of Further Education in Middlesex, 1961–1962 Part 2 General Interest and Recreational Courses Pp 52+4 plates (London Middlesex County Council Education Committee, 1961) [258

N L L News, No 1 (August, 1961) (A News-Sheet of the National Lending Library for Science and Technology) Pp 4 (Boston Spa National Lending Library for Science and Technology, D S I R , 1961) [258

Report of the Hydrographer of the Navy for the year 1960 Pp 33 (4 plates). (London Admiralty, 1961) [258

Other Countries

Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin Klasse fur Medizin Jahrgang 1960, Nr 1 Chemische Mutagenese-Erwin-Bauer-Gedächtnisvorlesungen I, 1959 Veranstaltet vom Institut fur Kulturpflanzenforschung Gatersleben der Deutschen Akademie der Wissenschaften zu Berlin, 26–28, Juli, 1959 Herausgegeben von H Stubbe Redaktion H Bohme Pp vi+136+3 tafeln (Berlin Akademie-Verlag, 1960) 18 50 D M [258

Deutscher Wetterdienst Deutsches Meteorologisches Jahrbuch, Saarland, 1945–1955 Pp 38 (Offenbach a M Selbstveralg des Deutschen Wetterdienstes, 1961) [258

Dental Caries from the Nutritional Point of View By Alfred Åslander Pp 7 (Stockholm Alfred Åslander, Royal Institute of Technology, 1961) [258

Canada Department of Mines and Technical Surveys Geological Survey of Canada Bulletin 76 Spores with Proximal Radial Pattern from the Devonian of Canada By D C McGregor Pp ix+11+1 plate (Ottawa Queen's Printer, 1961) 75 cents [258

Geological Maps of Canada Map 1092A Sevogle, Northumberland and Gloucester Counties, New Brunswick (Sheet 21 P/4 West Half) Map 2–1961 Rabbit River, British Columbia (Sheet 94M) Map 5–1961 Dean Lake, Ontario (Sheet 41 J/3) Map 6–1961 Wakwekobi Lake, Ontario (Sheet 41 J/6) (Ottawa Geological Survey of Canada, 1961) [258

Forty-fourth Annual Report of the National Research Council of Canada, 1960–61, including the Annual Reports of the Medical Research Council and Canadian Patents and Development Limited Pp 48 (N R C 6322) (Ottawa Queen's Printer, 1961) [258

Canada Division of Building Research National Research Council Canadian Building Digest No 20 Corrosion in Buildings By P J Sereda Pp 4 (Ottawa Division of Building Research, National Research Council, 1961) [258

VI^{e} Symposium International de Nematologie, Gand, Belgique, 24–28 juillet, 1961—Résumés des Communications Pp 122 (Bruxelles Ministère de l'Education Nationale et de la Culture et du Ministère de l'Agriculture, 1961) [258

Canada Department of Northern Affairs and National Resources National Museum of Canada Bulletin No 174 (Anthropological Series, No 51) Tsimsyan Myths By Marius Barbeau Pp v+97 1 dollar Natural History Papers No 11 (July 24, 1961) Placement of the Prowfishes, *Zaproridae*, in the Subfamily Stichaeoidae By D E McAllister and R J Krejsa Pp 4 No 12 (July 3, 1961) New Records of Fresh-Water Amphipod Crustaceans from Oregon By E L Bousfield Pp 7 (Ottawa Queen's Printer, 1961) [258

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PROGRESS IN INDUSTRY

A PAPER which was read by Mr. M Zvegintzov before Section F (Economics) at the Norwich meeting of the British Association on September 1 examined some of the accusations which have been levelled against British firms—and even whole industries—as unprogressive or backward or obsolete, in contrast to those of other countries The paper also advanced some constructive suggestions for remedying this position in so far as it can be substantiated A recent book by Michael Shanks, for example, roundly condemns the British economy as a stagnant society, and, although he may have picked out industries which are scarcely representative in regard to the proportion of employees with technical qualifications—in the example quoted it was 0 6 per cent—some of his more general charges have considerable substance Mr Shanks is by no means the first to point out that Britain should, by and large, be relying on brain-power for exports, and exporting the products which require scientific and technical skills, or even exporting her own trained man-power, rather than exporting the products of bulk production Reliance on the amateur and distrust of the specialist are admitted weaknesses, and that in too many British firms promotion comes by nepotism rather than merit is unlikely to be challenged within the reservations which Mr Shanks rightly makes, even by those indisposed to accept his view that our class system is mainly responsible for all these weaknesses

Mr Zvegintzov begins by pointing out that the man-power outlook in Britain is extremely tight, but he anticipates a useful increase in the productive age-groups by 1985, when the population is expected to reach some 56 million What he stresses more particularly, however, is the need to take steps to re-deploy continuously for new demands the limited available man-power This he regards as a *sine qua non* to avoid a halt in the rise of the standard of living The expansion of world trade, the industrialization of undeveloped countries and rising standards there and in the developed communities, all put the emphasis on a change in the character of world trade, and this assumption is more or less implicit in the recent report which attempted to forecast the long-term demand for scientific man-power

With this capacity for change must go flexibility and mobility Mr Zvegintzov is ruthless about real backwardness and unadaptability and looks unequivocably to the elimination of such firms by the process of economic competition To attempt to preserve them by protective measures or restrictive practices would be disastrous and place an unjustified strain on the limited resources of man-power in Britain, the critical problem is really how to translate the valuable craft tradition and accumulated experience which characterize a substantial proportion of the medium size or smaller firms, which constitute a significant proportion of British industry, into a modern scientific idiom The aim, as he sees it, should be to devise and supply to the craftsman the most modern and delicate tools which will enhance his craft, and this in turn involves further capital investment with saving in labour, and, even more important, educating the craftsman to demand these additions to his craft

Acceptance of this challenge by scientist and technologist as well as technician or craftsman and industrialist could well falsify some of the estimates made by the Committee on Scientific Manpower, although the question of technicians is still to be examined by it Mr Zvegintzov rightly points out that success here would go far to enable us to meet overseas competition, and that with a high craft tradition, using delicate instruments and high-precision tools for detecting, initiating and maintaining adjustments, very high standards as well as flexibility could be achieved without recourse to the further research and high expense which would be necessary for automatic production with low-grade labour Moreover, he visualizes the possibility of easy transfer of the habit of mind and inherited skill, which characterize craftsmanship, to other and perhaps new requirements as in the design and construction of scientific instruments

Perhaps the most important part of Mr Zvegintzov's paper, however, is that in which he discusses the difficulties that attend innovation in long-established and well-developed industries, and particularly in deciding where further investigation or development is likely to be most worth while It is essentially a question of how much research and where, and Mr Zvegintzov lays particular stress on the importance of effective communication between science and technology on one side, and finance and commerce on the other He visualizes, for example, a detailed cost breakdown analysis, possibly with comparisons from other countries, to bring out the features where the incidence of cost is considerable, followed by comparisons of this breakdown in a group of firms, and finally a joint study by technologists and cost-accountants to assess whether any scientific or technical innovation, or even a complete re-orientation of operations, could remove or ease this incidence of cost

In all this, close co-operation is imperative at working-level, alike between firms and between management and accountancy and the technologist, particularly if budgetary control techniques are not to confuse ends and means and impose rigid procedures which ignore circumstances and stifle initiative This kind of analysis, Mr. Zvegintzov points out, has not been tried, though departments of economics or industrial management at universities in certain industrial areas would appear to be well placed to undertake it This is possibly due to the absence of finance for this type of experimental economics, and, if so, it should be easily remedied if the Chancellor of the Exchequer's new structure is to stimulate expansion and development in industry in Britain

It is worth noting that something of this is foreshadowed in a paper which Mr G S J. White contributed in January 1960 to the *Journal of the Society of Dyers and Colourists* Limiting himself specifically to the development of dyestuffs for textiles and looking especially at the needs of the consumer, Mr White sought to assess the magnitude of the research effort which it is worth while expending on the discovery and development of new dyestuffs It is exactly this type of critical and realistic appraisal that is all-important if Britain's limited resources of trained man-power are to be deployed where they offer the greatest prospect of benefit to the national economy, and it is at this point that Mr Zvegintzov's analysis links up with the importance of effective management

It is indeed to the limitations of management, particularly in smaller firms, that Mr Zvegintzov attributes the lack of receptivity to new ideas, and he does not seem optimistic as to the possibility of overcoming such resistance otherwise than by the use of consultants This, in turn, he admits, depends on the initiative of managements if effective use is to be made of such consultants, but he sees ground for hope in the expansion of the colleges of technology and of the scientific and technological faculties at the universities Apart from its direct educational influence, this expansion offers a potential source of consultants and contacts between industry and the frontiers of science and technology, which should create a much more favourable climate of opinion in industry, and for this reason alone every effort should be made to encourage the movement of staff between industry and the universities and colleges of technology

How far the regional offices of the Department of Scientific and Industrial Research could assist in this process may be open to debate, but, from the point of view of information service alone, there seems sound sense in Mr Zvegintzov's plea that these regional offices should be strengthened and staffed with able men Moreover, there can be no doubt that the Research Associations could function very effectively here, and that their educational function, both from the point of view of information and as a source of trained staff, has been under-estimated or overlooked in the past The Robbins Committee could well give more than a passing glance at the position and functions of the Research Associations in the organization for higher education in Britain, but meanwhile the Council for Scientific and Industrial Research should examine the contribution which the Associations could make in such ways to industrial efficiency and development

Mr Zvegintzov is indeed rather more optimistic as regards the future of the smaller firms, because he believes a new generation of management is coming into office which is much more alive to scientific and technological opportunities This is the more important for, as he also contends, the rapid progress of technological co-operation and even integration in Europe may present a more important feature of the Common Market than ordinary commercial competition Here the influence of the Research Associations may be beneficial, though it is likely to be weakest in the traditional industries where it is needed most Incidentally he comments here on the need for more research in the human sciences where industry still relies far too much on guess-work, but he suggests that for the smaller and more specialized firms a powerful stimulus to co-operation could come from their suppliers of materials or equipment, and from the users of some of their products Most of these firms are bigger and with wider contacts both at home and abroad, and thus may be better placed to know what is new and promising

Whether in fact such firms may be willing to help with expert advice is a matter of opinion, and Mr Zvegintzov could be too optimistic on this point, though he suggests the possibility of joint studies under some co-operative trade association Here the Common Market could help in providing a large pool of experience, and Mr Zvegintzov in fact suggests that entry into the Common Market could provide the biggest single step for stimulating the unprogressive, though if, in fact, the operative factors are financial, the policy initiated by the Chancellor of the Exchequer at the end of July should be directed to that end Nevertheless, where the process acquires also much qualitative judgment, involving identification of needs, assessment—qualitatively and quantitatively—of advantages, and the establishment of priorities, it is more than a little doubtful how far the Exchequer is likely to be of direct assistance

The changes recently recommended by the Plowden Committee should go some way, however, to facilitate the kind of outlook that is required, and some of Mr Zvegintzov's concluding words are strikingly in line with that report Continuous progress, he believes, can only come through enterprise and boldness, which are only likely when there is confidence in the future and assurance of continuity The general climate of opinion is thus psychologically very important, and for this reason alone he condemns the current 'stop–start' policy as extremely dangerous Furthermore, the inexorable consequences of entering into scientific commitments cannot be ignored, and to insist that some co-ordination is needed between official scientific and economic organization is not to advocate rigid planning on the Russian model

Mr Zvegintzov himself leans to something on the lines of the French system of the Commissariat aux Plans, attached to the Prime Minister's Office, but he insists that above all development must come from within industry itself There is no substitute for efficient self-management, and probably the wisest national policy for Britain is one that provides definite encouragement to the enterprising and efficient, removing obstacles and providing positive advice and even help, rather than one that attempts to stimulate the backward or unprogressive

Much of Mr Zvegintzov's argument, it is true, can be traced in Sir Harry Pilkington's Fawley Lecture of three years ago, "Facing the Future in Industry" Sir Harry made the vital points about effective leadership, the choice of priorities, particularly in regard to the direction of research, and the real co-ordination of research, technical development and production, but it is hard to point to substantial progress in many sectors of industry since that Lecture was given Nor does Mr Zvegintzov himself really face some of the issues that can arise in such an industry as shipbuilding or the machine tool industry, if the industry itself really fails for internal reasons to meet the challenge Is the Government to stand idly by if employers fail to seize their opportunities and make the necessary adjustments, or if trade unions cling to obsolete and restrictive practices that prevent full advantage being taken of technical advance ?

It would be foolish to pretend that Sir Harry Pilkington, Mr Zvegintzov or Mr Shanks has provided the answer to these problems, nor is it probable that they would claim to have done so They have indicated certain aspects of the problem which call imperatively for further and urgent examination and for some clear and constructive thinking There are matters which the Minister for Science as well as the Chancellor of the Exchequer and their advisers should be examining, and there are others to which the Minister of Education should be giving attention But the major challenge is to industry itself, and to its co-operative organizations, both of employers and trade unions The situation is far too serious for either management or labour to rest content with putting the blame on others even for admitted mistakes The need for efficient management is once again overwhelmingly demonstrated, with all that that implies regarding training and selection for management No less clearly, however, is demonstrated the need for thinking about production in terms of the techniques and possibilities of to-day, and not in terms of practices of a previous century, and in that the trade unions have at least as large a responsibility as that of management

The primary emphasis, moreover, must be on flexibility, imagination and information On information, because it is only the free dissemination of information that prevents the duplication of effort which is so wasteful of resources—material and man-power—and provides the basis for sound judgment as to the objectives most worth while in research or in development That reason justifies the Committee of Public Accounts in pressing for more informative accounts from the Atomic Energy Authority, and the relevant passage in the Committee's Third Report for the Session 1960–61 * should not pass unnoticed The Committee fully appreciated that it is necessary on security grounds to restrict the information provided by the Authority's published accounts, but although the Public Accounts Committee was concerned in this instance more particularly with the control of recoverable expenditure relating to reactor research and development, and the manufacture and sale of fuel elements, it is clear that it is not satisfied that sufficient information is disclosed as to the Authority's expenditure on research and its distribution

Imagination is necessary, because on this factor depends very considerably the ability of Great Britain to exploit some of the possibilities of new techniques in automation and the electronic computer Some of these possibilities were indicated by Dr B V Bowden in his evening discourse, "The Impact of Automation", before the British Association at Norwich, when he hinted at possibilities of their use by the Exchequer in economic controls The importance of imagination both in the use of such techniques and in training a generation of scientists competent to use them was particularly well stressed by Prof R Courant in an address last year at the closing session of an international symposium arranged by the International Computation Centre in Rome and which has now been published in *Impact*

Flexibility is also essential, because of the demands for adjustment and a new outlook which it makes at all levels There is the demand on education to provide men and women with minds alert to the new possibilities and equipped to use the new opportunities There is the demand on management with which Mr Zvegintzov dealt, which involves not merely facing present-day problems of development but also foreseeing the human and technical problems involved in expansion, in automation and in redundancy, and in the recruitment of managers competent to deal with these problems in the future There is the demand made on manager and trade unionist alike to face these problems as they arise, and to adjust practices to new needs, developing habits of co-operation rather than restriction, and preparing for change rather than shrinking from it or ignoring it Solutions to these interlocked problems will not be found in isolation, nor can the necessary policies be implemented without mutual understanding and co-operation, and the task of creating in industry and in Britain generally the appropriate climate of opinion is one to which all possible efforts should already be directed

COSMIC RADIO WAVES

Cosmic Radio Waves

By I S Shklovsky. Translated by Richard B. Rodman and Carlos M. Varsavsky Pp xvi+444. (Cambridge, Mass Harvard University Press; London Oxford University Press, 1960) 65*s* net.

THOSE of us who were fortunate enough to receive a copy of Shklovsky's book when it was first published in Moscow in 1956 realized that a significant contribution had been made to radio astronomy by this Russian scientist Now the value of the book, and the understanding of Shklovsky's personal contribution, has been greatly increased by the appearance of this English translation The author has himself revised the work to bring it up to date so far as the contributions to radio astronomy which were presented at the Paris Symposium in 1958 Even so, in view of the tremendous growth of the subject and the productive research of the world's major radio telescopes since that date, it might be expected that the book would already be too old to be useful In fact, the book makes vivid reading and will be an inspiration to all concerned with the problem of radio waves from the cosmos.

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* Third Report from the Committee of Public Accounts, Session 1960–61 Pp 40 (London H M S O , 1961) 2*s* net

The main reason for this timeless quality of the work is to be found in the great theoretical contribution which Shklovsky has himself made to radio astronomy About ten years ago radio astronomers in the West were under the influence of the radio star idea as an explanation of the origin of the radio waves from the local galaxy, by which a distribution of radio sources somewhat similar to that of the common stars was believed to be the probable origin of the galactic radio emissions. Shklovsky held that this view was untenable He believed that the main generally distributed radio waves from the galaxy had two separate origins, first, a 'flat' component closely concentrated in the plane of the Milky Way arising as an emission with thermal characteristics from the free-free transitions in the ionized hydrogen gas in the plane of the galaxy, second a 'corona' or 'halo' component, surrounding and enclosing the galactic system of stars and arising as synchrotron radiation from relativistic electrons moving in the interstellar magnetic field He believed that a few of the intense discrete radio sources of emission were supernovæ remnants lying in the plane of the galaxy (as exemplified by the already well-known case of the Crab nebula in Taurus), and that the majority were extragalactic.

At first Shklovsky's views received little attention, but during the past few years there has been clear observational proof that they provide a reasonable explanation of the emission both from the local galaxy and from the individual extragalactic nebulæ Shklovsky's explanation in this book of the analytical procedure by which he arrived at these conclusions from a study of the spectra and isophotes of the radio emission made by radio telescopes which confused the issue by their poor resolving power is a masterly example of scientific procedure which should be studied by all young research workers

Another long section of the book which deals with the emission of light and radio waves from the supernovæ remnants still further exemplifies the fruitfulness of the conceptions of Shklovsky and his Russian colleagues The discovery of the polarization of the light from the Crab nebula, and (since the book was revised) of the polarization of centimetre radio waves from the nebula, provides firm observational support for these ideas that the emission arises as synchrotron radiation from the motion of high-speed electrons in the magnetic field of the nebula In due course these electrons move away from the nebula to form the electrons in the corona of the galaxy and to become, with other energetic particles resulting from the explosion, the primary particles of cosmic rays.

The scope of the book is specifically limited to the problem of the cosmic radio waves of galactic or extragalactic origin. The radio or radar astronomical aspects of the subject concerned with the solar system are excluded For all who are interested in the cosmos this book makes exciting reading, while for astronomers of all kinds and for those physicists who are concerned with the problem of the origin of cosmic rays the book will be an invaluable acquisition

BERNARD LOVELL

THÉODORE SCHWANN

Lettres de Théodore Schwann (1810–1882)

Éditées et annotées par Prof Marcel Florkin. Pp 274 (Liège Société Royal des Sciences de Liège, Université de Liège, 1961) 250 francs

THE 'cell-theory' is the story of the recognition of the cell as the essential unit in the structure and function of the body. The first suggestion of this coming change occurred in the work, *Die Zeugung*, which Lorenz Oken produced in 1805, but the important dates are 1838 and 1839, when Matthias Jacob Schleiden (1804–81) and Theodore Schwann (1810–82) enunciated in clear form the new ideas

In the volume reviewed here a distinguished Liège professor and medical historian shows how the second of these two men, who was born at Neuss-am-Rhein, apparently failed to justify the high hopes of his earlier years He had shown great promise when he was working in the laboratory of Prof Johannes Muller in Berlin but in 1839 he became a teacher in Louvain and afterwards in Liège, and in Belgium he published nothing but a few unimportant treatises and a brochure accompanying the apparatus which enabled one to exist in an irrespirable medium, it was shown in the Universal Exhibition in Paris in 1878

F G J Henle (1809–85) said that Schwann, during his five productive years, did enough for science to justify the entire lifetime of a great man He also noted that Schwann had been forced to lose much time studying systematic human anatomy when his real forte was physiology, and, moreover, when the disputes about general anatomy were repugnant to his sensitive nature.

At Louvain and at Liège the Roman Catholic Church was not concerned with the matter, for only at the end of his life did Schwann become a devotee to the faith, and he was unhampered in both his ideology and his religious experience

In 1842 at Louvain he began an intimate diary or *Tagebuch innerer Ereignisse*, part of which was published in 1936 An unpublished part, of exceptional autobiographical interest, was produced by Florkin in 1951, and there is also a series of treatises by Schwann relating to his inner life

Three periods can be distinguished in his story, namely, an ethical one, during which he strove for knowledge and the perfecting of self, a scientific one, which was rational and Promethean, and a mystical or metaphysical one, all three being characterized by intellectual eagerness Schwann's contemporaries and the generations separating us from him knew only his scientific output, considering it an intellectual oasis in the general drabness of a life devoid of eminence They were unaware that behind the decorous existence of the famous and conscientious but unproductive faculty professor was being carried on an unceasing intellectual struggle

Florkin enlarges on these three periods In the first he has much to recall about one Wilhelm Smets, in the second he recaptures the liberal Berlin atmosphere dominated by the powerful personality of Johannes Muller, and Schwann's attempts to make biology a province of physics In an appendix to his *exposé* of his cell-theory, Schwann disclosed a philosophy which rejected vitalism and sought the final cause of the universe in the actual properties of atoms. He affirmed the physico-chemical nature of life, this affirmation being his crowning achievement as a scientist, taking him away from scientific inquiry and orienting him towards metaphysics It was at this period in 1838, in the course of an attack of conscience, that he renounced rationalism and the scientific method in an attempt to know his Creator and thereby truth.

Florkin forecasts that Schwann's personality will certainly stimulate various works, not only by historians of science but also by psychologists given to the 'characterology' of the scientific investigator and to that of the meanderings of the spirit of man

Besides renewing biology by his discoveries and scientific theories, Schwann's philosophic and religious pre-occupations, and the transfiguration of science into religious emotion which he sought in his theories, the intensity of his inner life and his long resistance to orthodoxy outline a life at once sad and serene, untiringly devoted to the most exalted and moving aspirations of the human soul. As Edmond Scherer said of another great and lonely figure, Frédéric Amiel, Schwann was "de ceux qu'a touché de son aile l'ange des visions ineffables et des divines tristesses"

KENNETH J FRANKLIN

WEALTH OF THE FOREST

Les Plantes Utiles du Gabon

Essai d'Inventaire et de Concordance des noms Vernaculaires et Scientifiques des Plantes Spontanées et Introduites Description des Espèces, Propriétés, Utilisations Économiques, Ethnographiques et Artistiques Par André Raponda-Walker et Roger Sillans (Encyclopédie Biologique, Tome 56) Pp x + 614 + 53 planches (Paris Éditions Paul Lechevalier, 1961) 250 NF

TO us who depend increasingly on 'supermarket' and slot-machine, one of the most interesting aspects of life in less sophisticated lands is the wide use made of local plants to supply daily needs Nearly every plant seems to have its use; this one as a cure for fever, the next providing fibre for fish-nets, a third giving wood for musical instruments Of interest, therefore, is a new book describing useful plants of that western part of equatorial Africa known as Gabon Dr Albert Schweitzer's reference to this coast as the edge of the primeval forest comes to mind, and, although the vegetation of the coastal and riverine areas is now well known and is much altered by man's activities, the vast area of the interior, mainly forest, remains inadequately recorded

In a pleasant foreword by Prof Trochain, the book is described as the joint product of a priest-botanist-ethnologist and a botanist-draughtsman-artist During thirty years of missionary work in that country, the senior author, the Abbé Raponda-Walker, amassed the information on plants and their local names and uses which forms the bulk of the text The notes being placed in the hands of R Sillans, he attempted, as is explained, to bring the material to life, to depict the vegetation of the region as known to the inhabitants and as it enters into their daily life

The result is a book which is full of information for both botanist and ethnologist It opens with short introductory chapters on the history of botanical exploration in Gabon, on the climate and types of vegetation, and the vegetable products entering trade In the main part of the book, the plants which are put to use are described in turn, the genera being arranged alphabetically in their families. For each species is given a short description, a note of its use in native custom, and a list of vernacular names by which the plant is known among various of the 27 tribes in the country The artist provides 52 full-page botanical drawings of important species In addition, the text is enlivened by figures and illustrative sketches If the writer tells us that wood of *Alstonia* is used for ceremonial masks, the artist employs his pen to show the masks worn by some tribal society in their fetish rituals The book is well indexed, and nearly 8,000 entries in the index of vernacular names are a measure of the Abbé Raponda-Walker's diligent observation

Many plants described here are common also to forest country of West Africa to the north-west, and it is possible to compare the uses of them in Gabon with those so well described earlier by Dalziel, or in Irvine's *Woody Plants of Ghana* (see p 695 of this issue) Others again are peculiar to the region of Gabon, ranging in economic importance from the great okoumé tree, *Aucoumea klaineana*, the timber of which constitutes the greatest wealth of Gabon to so small and nasty a plant as *Ataenidia* of the arrowroot family, the leaves of which, thrown into the cooking-pot, cause discord in the household or village Ferns are among the plants of which use is made, and an unusual record is the weaving of the black shining rhizomorphs of a fungus into ornamental cords to wear on the body An appendix gives the vernacular names of recognized cultivars of some staple crops, including banana, cassava, and sugar-cane

The useful plants of Gabon, as here presented, include many introduced plants Some, like banana and maize, have long been adopted by the Gabonese and have become part of their life. Others have not penetrated farther than the trial-ground or mission-garden, and, although mention of this is made, their appearance at all in the main text as useful plants of Gabon may well be questioned To find included the Judas tree, *Cercis siliquatrum*, a Mediterranean plant ill-suited to the humid tropics, is as unexpected as it may be misleading But this, and the mis-spelling of some botanical names, are minor blemishes in a work which contains much of value and of charm

T A. RUSSELL

FORMAL LOGIC IN RETROSPECT

A History of Formal Logic

By Dr I M Bochénski Translated and edited by Ivo Thomas Pp xxii + 567 (Notre Dame, Indiana University of Notre Dame Press, 1961) 20 dollars

IN this monumental work, Prof Bochénski presents us with the results of some forty years of research Scholars are already aware of the merits of the German edition, published in 1956, especially the accuracy of the transcripts from Aristotelian texts Now we are enabled to have the outcome of this 'labour of love' in English, thanks to the University of Notre Dame Press, and a competent translator In the event, these pages are very much more than a source-book They will be consulted by logicians and historians for many a generation maybe, before anything comparable appears again The decimal system of entries—both extracts and commentaries—of the original is maintained with trifling exceptions, which makes reference easy within the volume itself, and to the German version

An introduction to the present edition has been added, which has as its main object the slightly unusual one of augmenting the very brief (and perhaps inadequate) treatment of Abelard in the original. It is good to have this, though one would have thought that a more appropriate place could have been found for it. But we are left in no doubt that formal logic was well away in its scholastic form by the twelfth century.

In his own introduction, the author says plainly what he conceives his task to be, and how he intends to carry it out. Logic did not develop continuously, rather it started at various places and at various times almost independently. But, characteristically, men soon found that they were grappling with much the same essential problems, approaching them by different paths. This concept sets the pattern for the whole book. Thus we have ancient logic, scholastic logic, mathematical logic, and Indian logic. The somewhat arid period of 'classical' logic is briefly reviewed. Before discussing these sections in greater detail, it is clear that two figures stand out in bold relief, Aristotle and Frege, the rest grouped around them with the symmetry of an artist. It is almost as if Prof. Bochénski had painted a new version of Raphael's great picture, adapting the stance of the figures to subsequent history as the grand drama of the pursuit of reason unfolds.

Ancient logic (Part 2) shows the author at his best, and text is superb. Here is the essence of the esoteric syllogism, displayed both in its intellectual power and its æsthetic appeal. Aristotle scarcely achieved corresponding success with modal syllogistics, and that probably explains why Prof. Bochénski gives this subject relatively little attention. The criterion throughout is that of quality, and the extent to which original minds have been able to approach perfection of presentation and methodological rigour. Included in this section are, of course, the Stoics and the Megarians, together with a host of commentators. It is good to read such a vivid extract from the tree of Porphyry, with its focus on extension and intension, and the principles of classification. This was not an epoch of discovery, but the literature is valuable and its synthetic character important. Theophrastus, pupil and successor of Aristotle, receives more than honourable mention. In addition to his logic, we salute him as a pioneer in meteorology.

Scholastic logic (Part 3) will be welcomed not only by logicians but also by medievalists. Naturally, there is a good deal here that stems from the Stoics and the Megarians. But imperfect as our knowledge of this period may be, it is one of supreme consequence since in fact such giants as Albertus Magnus and St. Thomas Aquinas brought to Aristotelianism not only a new life but also gave mankind something to live for, and thus realized, for the time being in any event, the concept of philosophy as the serving-maid of theology. This is not what is said in so many words, but the author's mastery of his material is such as to commend his scholarship to students of subjects sharing boundaries with those of formal logic.

Part 4 (classical logic) has already been mentioned. With Part 5 (mathematical logic) we are brought right up to 1931, with Godel's famous theorem, announced in that year. The highlights here, as one would expect, are the works of Boole, Schröder, Frege, Whitehead and Russell.

Indian logic (Part 6) forms a survey of a branch of learning all too little understood in the West. Prof. Bochénski makes a point to the effect that had India produced a Plato or an Aristotle at the appropriate point in history, a long stretch of time would have been saved in the working out of principles. But this was not to be. Seen against the background of centuries, creative genius acts somewhat like a modern computer, solving almost in an instant of time some problem previously wholly intractable.

The bibliography is extensive, running to several thousand entries. It has, for reasons of economy, been photocopied from the German, a sensible plan which will hurt nobody. In addition, are a series of indexes, dealing with names, subjects and signs.

There has been nothing like this book before. It is far from easy reading, but the planning is itself so logical, and the commentaries themselves so apt, that one is left with the impression of a veritable masterpiece, set off by a frame and background at once wholly sympathetic and comely.

F. I. G. Rawlins

FUNCTION THEORY AND ITS APPLICATIONS

Lectures on the Theory of Functions of a Complex Variable

By Prof. G. Sansone and Dr. Johan Gerretsen. Vol. 1: Holomorphic Functions. Pp. xvi+488. (Groningen: P. Noordhoff, Ltd., 1960.) 45 D. fl., 12 dollars, paper bound; 48.75 D. fl., 13 dollars, cloth bound.

Modern mathematics is in the process of becoming more and more abstract. We are in general less interested in numbers than in spaces of objects having certain properties. However, this process of abstraction necessarily moves more slowly in disciplines with physical applications.

It is for this reason that Sansone and Gerretsen's *Lectures on the Theory of Functions of a Complex Variable*, although a little old-fashioned, is bound to make a lot of friends, particularly among those who wish to learn function theory in order to use it. The authors claim to address those interested in advanced methods of analysis. They rather quickly cover the foundations, including Cauchy's theorem, and concentrate on what one can do once the basic theorems are proved. There is, however, a fair amount of theoretical stuff such as, for example, the Vitali convergence theorem, the Borel–Caratheodory inequality and the theory of integral functions of finite order. Picard's theorem for functions of infinite order and subharmonic functions are not covered. Conformal mapping, both theoretical and practical, is left for a second volume.

Among the practical topics which are covered are the inversion of power series, theta, elliptic, gamma, Legendre and the Riemann zeta functions, Laplace integral, Dirichlet series, as well as some other special functions but not Bessel functions.

The many applications include the Euler–Maclaurin and Poisson summation formulæ, and the analytic proof of the prime number theorem, asymptotic expansions of various kinds, use of the Bernoulli numbers, summability of power series, by the methods of Borel and Mittag-Leffler, and special series of many kinds, including one for Euler's constant.

The book should be very valuable to anybody who is interested in function theory, mainly in order to use it. The English is nearly always good. The term "monotonously increasing functions" should appeal to many students.

W. K. Hayman

Woody Plants of Ghana with special reference to Their Uses

By R F Irvine Pp xcv + 868 + 34 plates (London Oxford University Press, 1961) 105*s* net.

THIS beautifully produced book, illustrated by 142 full-page line drawings and 34 plates, is primarily concerned with the useful properties of Ghana's trees and shrubs It incorporates much of the material in Dalziel's *Useful Plants of West Tropical Africa*, and supplements it with Dr Irvine's own extensive knowledge of this subject There is a wealth of fascinating information on economic products and medicinal properties, both factual and fancied, for the 'doctrine of signatures' is implicit in much of African medicine This is ably supported by a comprehensive series of indexes and glossaries, and by an extensive collection of vernacular names

The purely botanical part of the book is less happily conceived Each species is accompanied by a short botanical description, but this is quite inadequate to enable the species to be identified and there are no keys, while the errors in nomenclature are numerous The citation of individual specimens seems unnecessary in a book which is not primarily a taxonomic work, and it is surely pointless to cite unnamed species known only from incomplete specimens when they have no known uses

It is understood that this volume is to be followed by another on the herbs of Ghana It is suggested that much of the botanical detail might profitably be omitted, leaving the author free to concentrate on the useful properties where he is clearly an expert, and enabling the publishers to offer the book at a cheaper price W D CLAYTON

Re-educative Treatment of Suppression Amblyopia

Being an abridged English version of Jean Sédan's "Post Cure de l'Amblyope Rééduqué" By T Keith Lyle, Cynthia Douthwaite and Jill Wilkinson Pp xii + 135 (Edinburgh and London E and S Livingstone, Ltd , 1960) 25*s* net

THIS is an English version of Dr Jean Sédan's well-known compilation of test types and pictorial exercises designed for use in the treatment of amblyopia associated with uniocular strabismus. Dr. Keith Lyle, who was responsible for the preparation of this version, states in his preface that he has found Sédan's methods of the greatest value in the work of the Orthoptic Department at Moorfields Eye Hospital, London.

Three types of exercise are represented In the first, the patient is required to detect misprints in test passages in a variety of sizes of print In the second, he has to solve simple problems or detect errors in exercises presented in numerical, diagrammatic or pictorial form (Some of the pictures are delightfully French) In the third, there is a number of special exercises intended for patients with eccentric fixation or related disturbance of spatial localization.

It is unfortunate, perhaps, that no evidence as to the efficacy of these procedures is presented None the less, Sédan's methods would seem very well worthy of trial in the treatment of squint and related conditions in which systematic eye-exercises are prescribed Some of his material too, might find useful application in the study of other types of visual disorder, in particular the defects of perception which may result from injury or disease of the brain.

O. L ZANGWILL

Inductive Probability

By John Patrick Day. (International Library of Philosophy and Scientific Method) Pp xvi + 336 (London Routledge and Kegan Paul, Ltd., New York The Humanities Press, 1961) 40*s* net.

THE author of this book has performed a most valuable service This consists essentially in treating his subject philosophically rather than in the more 'restricted' sense as an essay in logic. That little mathematical knowledge is required of the reader is neither here nor there the approach is not esoteric Probability as a concept is used by many professional people, but even ordinary mortals use it quite widely Much the same can be said of evidence The writer comments on the advantages and disadvantages of the jury system on balance, it comes out on the right side, in spite of the fact that sheer quantity of evidential material is a strain on the layman This is only another example of our national inclination to keep the expert in his place

The scheme of the present volume comprises a full discussion of inductive probability, subject–predicate formulæ, functional formulæ and determinants, theory of elimination, inductive reasoning and inductive logic In spite of a commendable determination to write clearly, the author seems occasionally to share with no less a person than John Ruskin a tendency to make meanings obscure by over-emphasis on detail Also, his use of the word 'generally' (for example, on pp 51, 58, 72) is not altogether happy, supposedly he means 'usually'

All in all, scientists would do well to read this book carefully, both for its meticulous scholarship and for its discipline Their weighing up of what is true and what is likely to be true will be greatly helped, with corresponding confidence in their conclusions F I G RAWLINS

Crystallization

By Dr. J W Mullin Pp ix + 268 (London · Butterworth and Co (Publishers), Ltd , 1961) 60*s*

THE first half of this book surveys ably, with much detail. the physical chemistry, properties and phase equilibria of crystals and solutions with some reference also to melts The final chapter summarizes in useful fashion the techniques and problems of sizing and size analyses In between we have a chapter summarizing briefly the better-known theories of the mechanisms of nucleation and crystal growth It would be a superhuman task to render the literature on these topics into a single, readable chapter and the author might well have condensed his material yet more without loss As it stands, however, it is slightly incongruous to find 103 papers, presented at three symposia, dismissed in a single reference as "able summaries", while an almost irrelevant reference to gas absorption merits a whole paragraph and the beard of the late W H. Perkin a whole sentence

It is particularly significant that the remaining chapters, treating re-crystallization, industrial crystallization and equipment, are wholly qualitative. This characterizes the status of crystallization as a unit operation. Due to the difficulties involved and perhaps to the guarding of technical secrets, almost no quantitative investigations of the process have been published The real utility of this book depends to a large extent, therefore, on engineering design data being made available for the process chemists and chemical engineers for whom it is intended

S H. BRANSOM

THE INSPIRATION OF SCIENCE*

By Sir GEORGE THOMSON, F.R.S.

Master of Corpus Christi College, Cambridge

WE are all well accustomed to science as the basis of technology, the source of the fundamental knowledge which it is our duty to apply to the service of man, but there is a quite different side to it and one not less in importance, that is science as truth, or at least one of the great aspects of truth, science as a human value—something which exists for itself and is self-justified as is music or painting, or indeed the human relations. This side should be an inspiration to all who are concerned with science in whatever form. Surely we have reason to be proud that such an insignificant creature as man should even be aware of his own insignificance? That he should be able to reckon up the countless billions of stars in the Galaxy and of galaxies in the universe and understand something of how they are made, that he should be able to measure the atoms and to determine their structure, that he should be able to understand even a little of the astonishing meaningful complexity that goes to make life? But besides its emotional appeal science is the philosophy of our age, and we who practise it should spend a little time in thinking of it from this point of view and seeing in what ways it differs from some of the older philosophies.

First of all physics relates to the outside world; it requires that a consensus of opinion be possible between different observers, or in other words that the observations made by different people can be adjusted so as to be consistent with a rational theory. One need not go into the complications of relativity, it is enough merely to consider the different judgments of relative position which two people sitting in an ordinary room will make and which are, normally at least, consistent with the furniture and the room being rigid objects and with light going in straight lines. It is in fact precisely this which is the justification for believing in an outside world: the fact that one can describe it in a way that can be accepted by all observers. Compare this outside world with the private world which each conscious person carries about with him. In this private world are dreams, emotions, pain and so on, things which he may be able to explain by symbols to his fellow men but for which, in the last resort, they must take his word and which they cannot directly share.

Physics, like other sciences, is based on concepts which have names, things such as mass, temperature, energy, rays of light, electrons and so on. It is these concepts which determine the stage to which physics has arrived because they determine what statements can in fact be made, and major advances in physics are, to a great extent, discoveries of the right concepts.

Concepts are usually suggested by experiments, but they seldom remain directly tied to them, at least not to simple experiments. Temperature is a good example. Starting from sensations of warm and cold, which (in so far as they measure anything) measure a rather complicated mixture of the temperature and conductivity of the substance in contact with the skin, temperature was early measured by the expansion of solids and liquids. These expansions are not uniform, and the scale of temperature derived from one body is near to but not identical with that derived from another. If temperature is to be a fundamental concept it must be removed from this dependence on the particular substance. It is well known how this has been done, by making it dependent on the ideal efficiency of certain heat engines, but this is not, of course, the way that anybody ever actually measures it.

Much the same kind of thing happens with space, spacial measurements, and most of geometry, depend on the assumption of rigid bodies to act as measuring rods or theodolites. There are no rigid bodies, but it is possible to make satisfactory corrections for the lack of rigidity of the nearly rigid ones. Some concepts are even less closely connected to experiments than these: for example, that of molecular chaos. It was a long time before this could be directly observed, yet the idea was most valuable to physics. The same is true to an even greater extent of the concept of atoms, and one of the lessons of the history of science is that one should beware of the purism which demands very close connexion between a concept and what is at the moment experimentally verifiable. One of the most interesting things in theoretical physics is the way in which certain concepts develop a usefulness far beyond that for which they were originally intended. Atoms are a case in point—so are quanta which, starting from a theory of black-body radiation, are now fundamental to all physics.

But not all concepts conform exactly to Nature, they are approximations, but they are not to be despised on this account. Take, for example, rays of light; they are still more important in practical applications of optics than are waves or quanta, although even in the time of Newton it was known that they were not the whole story. Another example is the use in physics of the concept of a surface as a mathematical discontinuity between two bodies, for example, solid and gaseous. There is no such thing as a surface in this sense, speaking strictly, and it is certainly not mathematically smooth. Atoms do not have sharp edges, there are vague regions round them in which they exert force, yet the concept of a surface is of immense value not merely to engineers but also to most of classical physics. Some concepts are absorbed by others, or perhaps one should say become tributary to them, for they still continue to be used, as the concept of mass has to that of energy.

There has been much discussion as to the 'reality' of certain concepts, for example of the electron. I think sometimes this fails to realize how artificial are the concepts of ordinary life, of common-sense things. Few people think it necessary to justify the idea of a chair or to ask whether it is real, yet it is in fact a most complicated idea. It is derived from a variety of different impressions, visual and factual, and is defined partly by appearance and partly use. A chair seen by the engineer is a collection of stressed material; seen by the botanist, if it is wooden, a collection of plant cells; seen by the housewife, something on which people can sit. While electrons are countable so that in any given circumstances they are a whole number, in a group of chairs there may be some broken ones of which it is doubtful whether they rank as chairs or not. The chair is really a very vague idea, and if one must argue about reality (which perhaps should be considered a bad word) the electron's claims are, I would say, better than the chair's.

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* Substance of the first Thomson Lecture of the Society of Instrument Technology, delivered on October 19.

Concepts are used to form hypotheses, theories, and laws which are statements of varying certainty about concepts. It seems to me that the differences between these words lie more in the emotional attitude of the user than in anything else. A law is some statement about concepts in which you really believe, a hypothesis is one about which you choose to remain uncertain. I will just use the middle word 'theories', the emotional content of which is somewhere between the two. All physics is based on experiment ultimately, and on the idea that experiments can in some way be repeated. By experiment I do not necessarily mean something in a laboratory, but merely something that can be observed; for this purpose the explosion of a supernova is an experiment. The first point that I want to make is that one cannot repeat an experiment unless one has some sort of theory, because repeating an experiment implies that the conditions are the same on the two occasions. Now all the conditions are never the same: life moves on, the planets are in different positions, the apparatus is either made of the same bits of material (in which case it has been worn) or is made of different material, in which case how do you know it is effectively the same? The answer is, of course, to say that the relevant conditions must be the same if you wish to repeat an experiment. But who is to say which conditions are relevant and which are not? This can only be done by a theory. The theory need not be a detailed one, it may merely say 'I believe that all that matters for the experiment I am now going to perform is the state of the objects on this table' and this may well be true if you make some allowances for the gravitational direction of Earth. But your experiment may go badly wrong if someone is making electrical disturbances in the next room. The theory must be sufficiently wide to give you an idea as to what matters.

Dr. K. Popper has made the very good point that theories in science exist to be disproved; they can never be proved. Since they are general statements that something will always occur, one can never be certain that a case of exception will not occur; if it does occur the theory is disproved and is either abandoned or, if it has shown previous merits, is somehow altered to fit the new evidence. The great strength of science as compared with philosophy is that theories can be disproved or at least shown as inadequate and that this can be done in a manner which compels the assent of all, or almost all, reasonable people; in short, that a consensus of opinion is possible and one which appears to be lasting. This consensus, philosophy never seems to have achieved, or at least only for short periods.

Personally, I think that the existence of technology is an enormous support to theoretical physics in this way. It is impossible not to believe in the validity of theories which enable complicated machines like television sets to be made and operated by people who are wholly ignorant of the scientific principles which we scientists say they depend on. It seems to me psychologically impossible not to believe that such principles have, in fact, a validity external to our minds. It is not necessary for this purpose that the principles are absolutely true or extend over all conditions: it is possible that Maxwell's equations do not apply in the interior of an atomic nucleus, but this does not detract from their validity for television.

Of course, the prestige of a theory goes up enormously if it can predict the unexpected, and rightly so. The mere fact that a theory covers known experiments counts for very little; after all, it is possible to draw curves through any finite number of points in an infinite number of ways, no one of which is in principle better than another. Experiments can always be represented by empirical formulæ which need have no further validity whatever. A theory must be æsthetically satisfying as well as fit the facts. This is equivalent to saying that Nature in some way respects our æsthetic judgment. In a sense I think it does, but this is very largely because our judgments of what is an æsthetically acceptable theory are based on our experiments on theories of the past which have been successful. In other words, we are assuming that Nature is somehow of a piece, and that we can hope to recognize theories which conform to the pattern just as one might hope to recognize somebody's handwriting in a few words though one has never seen those actual words written by him before. I am not sure whether this implies a personification of Nature or whether it is a consequence of the way our brains work.

A good analogy of the relation between physical theories and external realities is given by an ordinary map of Earth's surface. Like the map, the theory is either something on paper or in the mind of the physicist, just as the map may be in the mind of the traveller. The concepts of physics correspond to the signs on the maps denoting rivers, roads and the like. The making of such a map consists in translating Nature on to a piece of paper, and the use of the map involves reversing this process, reading the map, deciding, that is to say, that one particular point on the map is the place where you are standing, that the hill on your right is represented by a certain group of concentric contour lines on the map and so on. All theories are approximate in practical application, ignoring small effects; just so, all maps leave out details. They may show hills and slopes but they will not show molehills. This, however, does not prevent them from being useful. Again, every map of Earth on a flat piece of paper is necessarily distorted since you cannot bend a plane to fit a sphere. Several kinds of maps are possible corresponding to the different methods of projection; some are more suitable for one purpose, some for another, but there is none which is universally the best. Yet a map can be right or wrong; a map which shows Piccadilly Circus north of Oxford Street or Birmingham in the middle of Spain is definitely erroneous.

This applies to scientific theories. They can be so badly wrong as to be useless. They can never hope to be completely right, with the rarest and doubtful exceptions. Two quite different theories may be equally valid: compare for example, the thermodynamics and the kinetic theory approach to the properties of gases; they start from wholly different ideas yet arrive at practically the same conclusions, but for some purposes one is better than the other.

The great authority of Lord Kelvin is often quoted to extol the importance of the quantitative, which implies measurement, in physics. He was, of course, right in urging its importance; but it should, I think, always be remembered that in the last resort we are trying to do something qualitative. The pure scientist is aiming at producing a theory which will be convincing and satisfactory, which will make people feel that they understand, and which will predict something new and exciting. The object of the applied physicist or engineer is also in essence qualitative. For example, if he is designing aeroplanes the question of top speed is very important and it can be measured. But speed, predicted or measured, is important because of its qualitative implications. You desire an aeroplane which will get you to your destination before your friends are tired of waiting for you, or if it is for war, one which will return safely from its sortie having shot down the enemy. The quantitative predictions, important as they are, are really the means to the end, not the end itself.

I have spoken of a good theory being æsthetically satisfying because as we have seen a mere empirical formula is hopeless. Comparatively little has been written on the æsthetic side of science apart from mathematics. Some of the same criteria apply here as do there: for example, the theory must be general, simple and striking. It should not involve arbitrary constants or functions, unless indeed it can be shown that the constants which are introduced are related to something quite outside the theory itself: for example, Maxwell's equations and the velocity of light. But I am inclined to think that the most important feature in making one accept a new theory is what I have already said, its general resemblance to the successful theories of the past.

The quantum theory has made, as we are all aware, an alteration of immense importance in our ideas of physics. It has replaced determinism by probability. The uncertainty principle of Heisenberg is, I think, the first occasion on which science has laid down limits to knowledge as opposed to the familiar limits to what man can perform given by such principles as the laws of thermodynamics. It means an alteration in the sense in which Nature is rational. One cannot predict certainties, one can only predict probabilities and it becomes a matter of some difficulty to know what is meant by a probability when you deal with a unique event, an event which cannot be repeated—for example because the apparatus is changed or broken and is too complicated to re-make. I am thinking in particular of experiments on brains, animal or human. In more ordinary laboratory experiments one can use an apparatus or series of apparatus, each of which conforms to the same theory. This theory asserts that results *a*, *b*, and *c* are probable in calculable proportions, and by prolonging the experiment it is possible to verify the theory, if true, to any degree of accuracy required. Each experiment is really a quite separate one and the only feature in common is that you believe them to be representable by the same theory.

Recently Born has pointed out that quantum indeterminacy has wider implications than one might suppose. Consider, for example, the Brownian movement. In what possible circumstances can the motion of the tiny drops of butterfat seen in a microscope moving about in all directions in the liquid be regarded as determinate? Their motion in the view of nineteenth-century physics is controlled by the collisions they make with the molecules of the liquid, but who is to calculate the initial positions of the molecules of the liquid and their collisions with one another? To make the matter a little more definite let us imagine a more precise experiment. Suppose that we have a Geiger counter divided into two halves by a grid and filled with gas at suitable pressure. Let us introduce a single radioactive atom into this counter, at a known place. The atom is supposed to stick to a wall of the counter as soon as it touches, as it eventually will. Now try to calculate in which half it will end up. It will explode sooner or later and that half will be discharged giving an observable effect. To attempt to predict this, one would have to trace the zig-zag path of the atom diffusing through the molecules of the gas. If you try to consider the accuracy with which you would have to measure initially the positions and velocities of the millions of molecules concerned you will, I think, agree that to regard such an experiment as determinate is a misuse of language, even on the simplest assumptions such as assuming the molecules to act like billiard balls. The motion in each collision is sensitive to the position of the moving balls at the moment; a very small error in determining this will result in a big change in the direction and velocity of the balls after the collision, and so effect to a still greater extent all subsequent collisions. The errors in fact are magnified enormously, and long before the process comes to an end with the active atom reaching the wall, the initial accuracy required for prediction becomes fantastic. In the nineteenth century the physicist could say "yes, but in principle the motion is determinate". Since Heisenberg we can no longer do this; the motion is indeterminate. But I want to point out that this conclusion depends very little on the precise magnitude of *h* provided it is not absolutely and mathematically zero. I think that there are probably far more examples in practice of these truly indeterminate events than one is apt to suppose. One may be too impressed by the smallness of *h*. Take, for example, aerodynamics, where one is concerned with turbulent motion set up as an instability, as when, owing to changed speed, the laminar boundary layer of an aerofoil changes to a turbulent one. This may depend, of course, on roughnesses on the surface and therefore be determined by them, but it is quite likely if the surface is smooth that the instability is triggered off by a fluctuation, in the kinetic theory sense of the term, and it is within limits indeterminate when and where this happens. Most cases of instability are like this; they may become determinate because of some deviation from the ideal in the geometrical shapes, but if these are accurate enough there will come a time in which wholly unpredictable micro-effects will set the instability off. It seems quite likely that details of meteorology may be unpredictable in this fashion, and even quite major features may depend on unpredictable events in the Sun. All physics can do is to predict probabilities, not merely for atomic events but also for many other classes even where large effects are concerned. There are a great many trigger actions in Nature, especially where biology comes in.

It is perhaps scarcely necessary to urge that the discovery of new effects is fully as important a test of the merits of a theory as the explanation of old ones; but some writers on the philosophy of science seem to regard the main duty of a theory as the proper ordering and correlating of known facts. This seems to me an unimaginative view. In the past, theories have gone much beyond the known facts and profoundly helped physics by doing so Maxwell's equations connected the velocity of light with the ratio of electrostatic and electromagnetic units, but they also suggested waves of very different lengths, some of which Hertz discovered, and the theory also inspired von Laue to try X-ray diffraction The idea that many crystals are formed by the close-packing of nearly spherical atoms was put forward by Pope and Barlow , their theory was wrong in detail, but suggested to Bragg ideas now fundamental in the study of crystals

Imagination indeed is one of the qualities which mark the great scientist Even a hint of it may lift a man out of the rut. There is only one quality which is more important and that is enthusiasm, the feeling that science is the one thing that really matters and that to be allowed to spend one's life in its pursuit is the greatest possible privilege

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION, AUSTRALIA

DIVISION OF FOOD PRESERVATION · NEW LABORATORIES

THE Division of Food Preservation of the Commonwealth Scientific and Industrial Research Organization, Australia, has moved into new laboratories at North Ryde, New South Wales. This Division began its work in 1932 in laboratories situated in the Brisbane Abattoir, Queensland, where a branch laboratory is still maintained In 1938 the Division transferred its headquarters to laboratories in the Abattoir at Homebush, New South Wales, but in twenty years outgrew the accommodation there

The new laboratories are located on an elevated site, about eight miles from Sydney, in an area that is being preserved as a green belt to the city. Eleven buildings with a total floor area of 71,000 sq. ft are set in landscaped grounds about 10 acres in extent The disposition of the buildings on the site was determined largely by a desire to provide for future extensions to individual buildings without interference with other buildings and services

In the centre of the site (Fig 1) are the service facilities—workshops, stores, boiler house, engine room, and canteen, and also a Taste-Testing Laboratory, which has been carefully planned to provide a standard environment for sensory testing Flanking

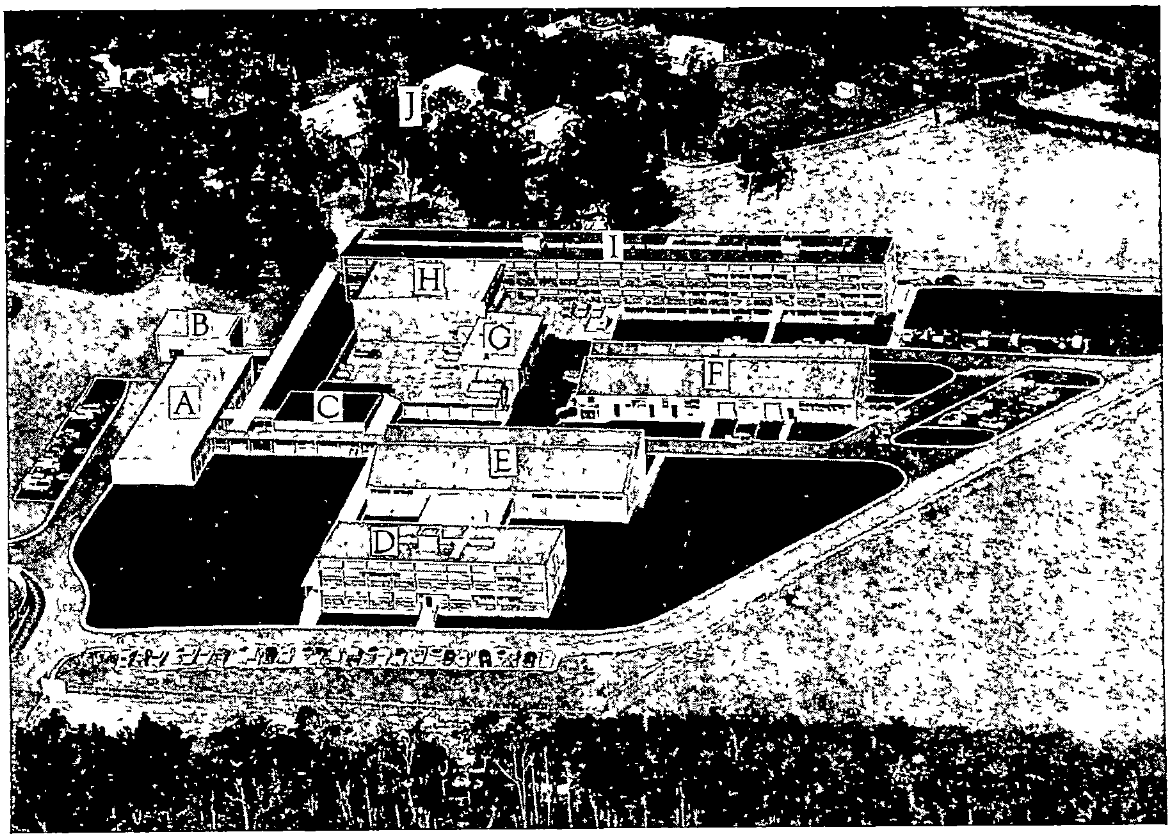

Fig 1 Aerial photograph of the new laboratories of the C S I R O Division of Food Preservation *A*, administration, *B*, Hicks meeting room, *C*, canteen and Taste-Testing Laboratory, *D*, food technology, *E*, food processing, *F*, workshops, *G*, boiler house and engine room, *H*, cold room block, *I*, food science, *J*, Bread Research Institute

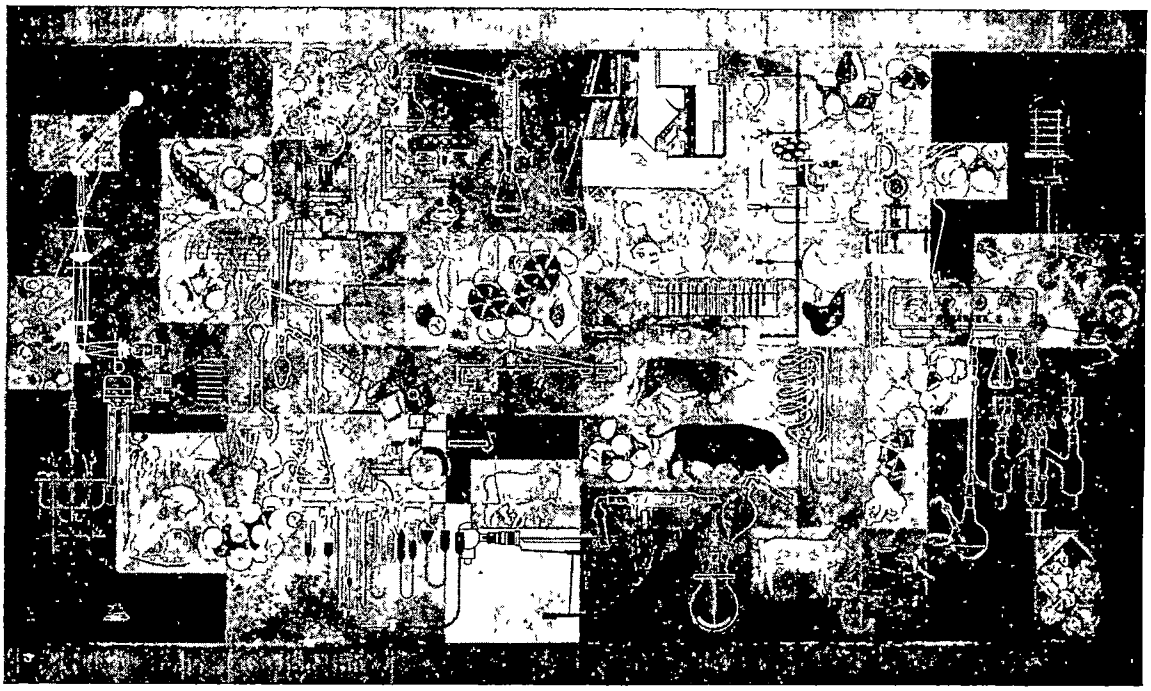

Fig. 2. Mural by Miss Eileen Mayo which decorates the foyer of the administrative building in the new laboratories

these buildings to the east are a single-storey building housing administration and the library, and the E. W. Hicks Meeting Room, named in honour of a pioneer member of the Division. On the south side of the site is the Food Science Building, which contains forty laboratories and accommodates the Physics, Chemistry, and Microbiology Sections, and also the Fresh Fruit and Vegetable Storage Section, and an Animal Products Section dealing with meat, fish, poultry and eggs. The other technological Sections of the Division—Canning, Packaging, Dried Foods and Frozen Foods—occupy the twenty-two laboratories in the Food Technology Building, and also use the adjoining Food Processing Building, where there is an open processing area 130 ft. × 50 ft., containing pilot-scale equipment for the production of experimental packs of processed foods. In a separate building is a sulphuring room for work on the treatment of dried fruits with sulphur dioxide and a chemical processing laboratory for operations involving volatile solvents.

The laboratory buildings have a central corridor with load-bearing walls in brick, and reinforced concrete supporting piers at 12-ft. centres on the external walls. Aluminium has been used for roofs and window frames, and also for the coloured enamelled spandrels, which are removable for access to the services running in perimeter ducts along the north and south walls. Penetration of summer sunshine on the long north and south sides of these buildings is controlled by continuous concrete hoods and vertical pre-cast concrete fins. The hoods also act as working platforms when access is required to the perimeter ducts.

The services available in the laboratory blocks are hot and cold water, distilled water, gas and alternating-current power, with compressed air and steam in some laboratories. Fume cupboards are provided with individual exhaust fans, and in some sections separate fume rooms containing two fume cupboards have been installed. Water and gas are run in copper pipes, distilled water in stainless steel, and sink wastes in polythene and glass piping. The library, the taste-testing booths, and several specialized laboratories are air-conditioned, while the remaining buildings are heated by hot-water radiators.

The buildings are linked by an internal road system and by covered footways which also provide overhead carriage for some services.

Two general types of layout have been used within the laboratories, according to whether the function is predominantly chemical or biological, but officers were given considerable freedom in the detailed planning. A variety of service rooms has been provided, ranging from balance and instrument rooms to a suite of preparation and sterilizing rooms for microbiological work. Many of the internal partitions are removable to permit changes in layout, and bench units and service lines can be readily dismounted and re-used. Laboratory bench tops are solid timber finished with a clear epoxy resin, while fume cupboards are finished in a white pigmented epoxy resin. Natural wood finishes have been used extensively on laboratory furniture, and in conjunction with the light-coloured walls the general effect is very pleasing.

In a wing of the Food Science Building are 31 controlled-temperature rooms with a range of constant temperatures from −30° C. to 50° C. Four of these rooms are gas-tight for the storage of food in controlled atmospheres.

Immediately adjoining the Division of Food Preservation at North Ryde is the Bread Research Institute of Australia, a research association financed by the Bread Baking industry and the C.S.I.R.O. jointly, and this Institute houses a C.S.I.R.O. Wheat Research Unit. There is thus a useful centralization of food research establishments at North Ryde.

The Division of Food Preservation has a total staff of 150, of whom 78 are graduate research officers and experimental officers, and 49 are technical assistants The field of research in which these workers are engaged is the handling, preservation, processing, storage and transport of meats, fish, eggs, fruits and vegetables More than half the resources of the Division are devoted to fundamental research seeking a deeper understanding of the properties of foods and the changes which they undergo during processing and preservation In addition, therefore, to technological projects on the preservation of foods by canning, dehydration, and refrigeration, the research programme includes many projects in the basic sciences

Present chemical research is concerned with applications of gas chromatography to volatile constituents of foods, with anthocyanin and other flavonoid pigments in foods, and with reaction mechanisms in the non-enzymatic browning of foods and its control by sulphur dioxide Workers in muscle biochemistry are elucidating the effects of rigor mortis and freezing on the protein and other constituents of muscle Theoretical work on physics, on heat- and mass-transfer across laminar boundary layers, are related to many problems encountered by food technologists Fundamental microbiological studies on the effects of water activity on microbial growth and on the germination and growth of bacterial spores are designed to give precise information on factors governing the control of micro-organisms which cause food spoilage and food poisoning Studies in plant physiology on the metabolism of carbohydrates and organic acids and on the properties of plant cell membranes are related to outstanding problems in the preservation of fruits and vegetables

The new laboratories were officially opened on September 18 by Dr. D A Cameron, Federal Minister for Health and for the C S I R O , in the presence of a large gathering of representatives of Government and university departments and the Australian food industry On the four days following, a Food Science Conference was held and was attended by 250 Australian food scientists and technologists and a few overseas visitors. The programme included symposia on "Chemical Aspects of Food Processing" led by Prof C O Chichester of the University of California, Davis, on "Meat Quality" led by Dr E. C Bate-Smith, Low Temperature Research Station, Cambridge, and on "Food Preservation and the Organization of Plant and Animal Tissues" led by Dr J B Biale, University of California, Los Angeles, and also panel discussions on public health aspects of handling and processing foods and on trends in food research

UNCONVENTIONAL METHODS OF ENERGY CONVERSION

A JOINT session of Sections A (Mathematics and Physics) and G (Engineering) of the British Association was held in Norwich to discuss the present situation with conventional methods of converting heat into electricity, the need for new methods, the principles of the new methods on which research is in progress, and the prospects for the future

In the opening paper, Prof. T G Cowling (University of Leeds) pointed out first that with the established methods of using solar energy indirectly, by fossil fuel or by water-power, the proportion of the incident energy which is utilized for energy conversion is extremely small This proportion is nevertheless, up to the present, readily available The nuclear fission process is now well established, and can be controlled and made available satisfactorily The direct employment of solar energy is, however, difficult because, although 1 5 kW /m ^{2} arrives from the Sun outside the Earth's atmosphere, the problems of concentration mean that it is not readily available Even greater difficulties with availability of the energy apply, of course, to thunderstorms, and so far to nuclear fusion processes Thus, there are no new sources of energy likely to compete for some time with the existing sources For new methods of utilizing existing sources of energy, those of interest appear to be thermoelectricity, the thermionic diode, magnetohydrodynamics and the fuel cell The last of these was omitted from this session, and dealt with in a meeting of Section G on the following day

Mr F H S Brown (Central Electricity Generating Board), in the second paper, considered the incentives for establishing unconventional methods of energy conversion The conversion of mechanical energy to electrical is now very efficient indeed, and the problem remaining with conventional methods is the conversion of heat to mechanical energy This is, of course, limited ultimately by the efficiency of the Carnot cycle, though the best efficiency obtained is only about 70 per cent of this To raise the efficiency of the Carnot cycle requires an increase in the temperature at which heat is fed into the cycle With present materials, no engine with highly stressed moving parts can be operated continuously at a temperature much above 600° C Thus, although a fuel may be burned at 1,700° C , no such temperature can be fed into an engine continuously. In the internal combustion engine, the explosion produces a high temperature which falls rapidly during adiabatic expansion, thus lowering the mean temperature of the engine parts However, there is a limit in practice to the size of the internal combustion engine, it is expensive, and moreover, since it is of the reciprocating type, it suffers from friction, wear and noise to an unacceptable degree

The steam cycle is thus more satisfactory, although it has a limiting temperature near 600° C The efficiency obtainable is about 40 per cent, and in this case, unlike the internal combustion engine, scaling up improves the performance obtainable The gas turbine involves again continuous exposure to the maximum temperature, the limit of which at present is near 600° C ; but a better performance can be obtained compared with the steam cycle because of the lower pressure and the larger heat rejection at the minimum temperature of the cycle

It is clear that it would be very desirable to increase overall efficiency above 40 per cent, and the methods of achieving this could in principle be: (1) finding an intermittent process cheaper and more reliable than the internal combustion engine, (2) evolving better materials for use at high temperatures, for example, up to 1,700° C , (3) evolving a continuous method which does not stress working parts at high temperatures

In the third paper, Dr R G Siddall (University of Sheffield) described the work of Prof M W Thring and himself on producing electricity directly from fast-moving plasmas Here the ionized gas of the plasma replaces the conductor in a dynamo, and moves through a magnetic field, converting its kinetic energy directly into electricity To obtain useful power, the plasma must have an electrical conductivity near 1 ohm^{-1} cm.$^{-1}$, and it must move at several thousand miles per hour through a magnetic field of 10,000–20,000 oersteds Moreover, the path-length through this field to extract the energy must be many feet Ordinary gases would have too low a conductivity at any feasible temperature, but by 'seeding' with a small proportion (say 1 per cent) of the vapour of an alkali metal, temperatures near 2,500° C would be sufficient The simplest scheme would be to preheat air, compress it and mix with a fuel, burn it at about 2,500° C , and expand through a nozzle to obtain the velocity Unfortunately, this would cool the gas almost to 1,000° C , to maintain it near 2,500° C after expansion would need a combustion temperature near 6,000° C Thus, more sophisticated schemes are necessary

Three ideas due to Prof Thring are under investigation First, continuous combustion at 2,500° C followed by expansion through a nozzle is used to produce the high velocity Secondly, short bursts of preheated oxygen and fuel seeded with potassium are injected periodically into the combustion chamber immediately before the expansion nozzle This burning gas is swept along in 'slabs' by the main gas stream These slabs are expected to retain their initial temperature during their passage through the magnetic field The second scheme involves obtaining the high velocity without pre-compressing and expanding The combustion occurs at constant volume, in a chamber with inlet and exit valves which achieve pulsating combustion, so that again the hot gas passes in slabs through the magnetic field

The third idea utilizes shock waves produced successively at the opposite ends of a tube, so driving a plasma to and fro through the magnetic field This will generate power at each passage, and will produce alternating current It is thought that these methods, if successful, will produce efficiencies of 50–60 per cent

Mr P Dunn (Atomic Energy Research Establishment) described, first, the basic principles of the thermionic diode as an energy converter, using the analogue of the steam boiler to illustrate the electron evaporation from the cathode and collection at the anode He showed that for a given cathode operating temperature, the cathode work function should be below a maximum allowed value which is a function of the operating temperature, but that there is no optimum value He indicated the importance of low anode work function, and of minimizing the effects of space charge either by using very close-spaced vacuum diodes or by positive ion neutralization of the space charge The former method is impracticable for generation of large amounts of power, though it is valuable for space vehicles With cathodes operated at 1,400° K , present diodes give an output of 0 7 V , 1 amp /cm ^{2}, with an efficiency near 15 per cent Higher outputs and efficiencies will be obtained with higher cathode temperatures For use in a reactor, supposing that the fuel elements could be run at temperatures up to 2,000° C , but not higher, a cathode with a work function less than 3 eV would be required This cannot be a metal, and none of the known oxides used as cathode materials is satisfactory at 2,000° C Uranium carbide has, however, a work function near 3 eV , and may be useful both as cathode material and, suitably enriched, as the fuel element Using cæsium vapour to give the positive ions, this should maintain a cæsium coverage on the anode which would give a reasonably low anode work function It is not certain yet whether the cæsium would be adequately ionized in such a diode, and it is known that there are considerable difficulties in using cæsium due to attack of other metals, glass and ceramics Thus other forms of ion injection are under investigation

The thermionic diode is conveniently used with fission nuclear power, as it is easy to obtain the required heat flux to the cathodes To produce a plasma jet would not be easy with a reactor Conversely, with fossil fuel, it is not difficult to produce such a jet, but the heat flux to a cathode is difficult to obtain The thermionic diode is suitable for 'topping', in that the heat injected at the cathode at 2,000° C is conveniently rejected at the anode at temperatures of 400–600° C , so that it could then be used in a conventional steam cycle The thermionic diode therefore promises a useful step forward under the third heading above Incidentally, the high-velocity plasma cannot be used after the hottest part of the gas has cooled below about 2,000° C , so that this method could also be used for topping

In the final paper, Prof D A Wright (University of Durham) discussed thermoelectricity as a means of generating power directly from heat He first outlined the basic principles, showed why the performance of metallic thermojunctions was poor, and contrasted the possibilities with semiconductors. With the latter there are many complications, but the main requirements with the 'broad-band' type are that they should have a sufficiently high energy gap, depending on the required temperature of operation, that it should be possible to prepare them n-type or p-type, with a Seebeck coefficient α near $\pm$ 200 μV /deg C , and that they should then have a high electrical conductivity σ and a low thermal conductivity K The usefulness of a material is, in fact, determined by the quantity zT, where $z = \alpha^2\sigma/K$ and T is the absolute temperature It would be necessary to prepare the material reliably and repeatably, reasonably cheaply, with a high degree of uniformity of properties over every sample, from sample to sample, and from batch to batch It must be possible to make low-resistance reliable contacts, and the material must not melt or decompose in operation, the deliberately added impurities must not diffuse out, and impurities from the contacts or from the environment must not diffuse in

One of the best materials so far is bismuth–antimony–telluride, which has a value of zT slightly greater than unity, but cannot be operated above 150° C , because of its low-energy gap With the hot junction at 150° C , cold junction at 0° C , a thermojunction between p- and n-type material would give a junction efficiency near 7 per cent Lead telluride can be operated at 500° C , but with zT less than unity, so that the junction efficiency between 500° C and 0° C is about 12 per cent Unfortunately, the figures realizable in practice are lower than these because of resistance at contacts, temperature drop at contacts, and, with fossil fuels, problems of heat transfer to the hot junctions following combustion

Progress in finding materials which can be operated at higher temperatures is slow because there are incompatibilities in the requirements To obtain higher energy gaps and so permit higher operating temperatures one needs compounds of lower atomic weight which, compared with bismuth and lead tellurides, tend to have higher thermal conductivities and lower values of the electrical conductivity for a given Seebeck coefficient Thus the trend is for materials which can be operated at higher temperatures T to have lower values of z, and in fact zT, than materials which can be operated only at low temperatures Indium arsenide containing gallium or phosphorus can be operated to about 700° C , but zT is only about 0 5 More promising are some compounds such as $AgSbTe_2$ and its analogues formed by cross-substitution from the same columns of the Periodic Table These have lower thermal conductivity than their mean atomic weight would predict Values of zT as high as 1 5 up to 700° or 800° C seem likely to be attained with these compounds Materials which could be operated to 1,100° or 1,200° C have so far lower values, for example, cerium sulphide, zT near 0 5, and nickel oxide, zT still lower Thus the highest junction efficiency which is theoretically obtainable at present between 1,200° C and 0° C is about 17 per cent, while the best actually obtained so far for this range is only about 6 per cent This is in a 'cascaded' system in which three or four stages are used, the heat from the first stage passing to the second and so on, and different materials being used in the different stages, to match the temperature involved

To obtain useful performance for 'topping' in power generation, values of zT near unity would be necessary at temperatures up to 1,600° C , while for direct replacement of present generators operating up to 600° C , values of zT would have to exceed 4 There seems little prospect at present of obtaining values as high as this in either temperature-range Meanwhile, research is continuing, and this type of generator is useful in spite of low efficiency for the production of low power in portable equipment and in remote situations where such a generator has to run unattended for long periods

D A WRIGHT

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The International Council of Scientific Unions was established in 1931 Its precursor, the International Research Council, had been set up in 1919 but proved unsatisfactory because it was restrictive, in no sense fully international, and because it allowed feelings engendered by the First World War to cloud its proper function These mistakes were avoided after the Second World War, and the recent assembly reaffirmed its previously declared non-political philosophy by admitting to membership under the terms of its rules Hungary (Academy of Sciences), North Korea (Academy of Science, Phyongyang), South Korea (Academy of Science, Seoul), Ghana (Academy of Learning), Ceylon (Association for the Advancement of Science), and East Germany (Berlin Academy of Sciences)

The Council has several objects It aims to coordinate the activities of the international scientific unions, and to be a co-ordinating centre also for the national organizations which are in membership It seeks to encourage scientific activity in fields which do not fall directly within the scope of any existing organization, to enter through the national organizations into relations with Governments and to maintain relations with the United Nations and its Specialized Agencies The Council consists of national members and scientific members There are now fifty-one national members, each representing the authoritative scientific organization in the country concerned—an academy, national research council or similar institution or, in a very few cases where no appropriate body exists, the Government itself Fourteen international unions are now members Of these, the general unions include astronomy (IAU), biology (IUBS), pure and applied chemistry (IUPAC), geodesy and geophysics (IUGG), geography (IGU), pure and applied physics (IUPAP), mathematics (IMU), and geology (IUGS), which was admitted at the recent Assembly The specialized unions include crystallography (IUCr), history and philosophy of science (IUHPS), theoretical and applied mechanics (IUTAM), scientific radio (URSI), physiology (IUPS), and biochemistry (IUB)

ICSU has neither the wish nor authority to interfere with the autonomy of these unions, nor to disturb the work which they are specifically designed to undertake The range of their work is enormous It includes the arranging of international symposia on matters of universal importance, the preparation of reports by specially appointed commissions, and the instigation and organization of research which requires international collaboration For example, IAU has dealt with atomic time standards, and has helped astronomy and space research Through its activity, also, many astronomers have been enabled to obtain the use of instruments in countries other than their own IUGG has concerned itself with problems of Earth and its atmosphere, such as earthquakes and volcanoes, the atmospheric absorption of solar radiation, and the levels of oceans and the penetration of radiation into them The data which it has collected are published and available to anyone In the future it will participate in projects of the International Committee on Geophysics (CIG) discussed later IUPAC has recently published reports on nomenclature and terminology, on internationally agreed analytical standards, on problems of foods and fats, and in collaboration with IUPAP has laid down a revised scale of atomic masses based on carbon-12 IUBS has dealt with quantitative methods in pharmacology and toxicology and with problems of cellular biology, embryology and genetics, and IUB has made a complete survey of known enzymes URSI has been considering the results of the International Geophysical Year (IGY) in relation to the ionosphere, problems of radio communications in space, and has given advice about the allocation of frequency channels. IUTAM has studied the problems of atmospheric pollution, and IGU has set up groups to deal with new world maps, erosion and coastal geomorphology, and the problems of humid tropics or arid zones

The function of ICSU is to co-ordinate and integrate the work of the different disciplines, and to catalyse research in fields not covered by the separate organizations For the latter purpose, special committees have been set up, supported in the early stages by funds from ICSU, although each must later find support through the participating national organizations A strikingly successful example was the International Geophysical Year, in which 67 countries took part, some 2,000 observing stations being established, 57 of them in the Antarctic The results accumulated during this project are still being analysed, but publication will soon be complete In order to exploit this success, the International Committee of Geophysics (CIG) was set up, with more than 60 national committees, the appropriate unions and the World Meteorological Organization taking part, to maintain the operation of the World Data Centres, to ensure further inter-disciplinary co-operation and to arrange universal publication of the relevant scientific data Its future plans include a World Magnetic Survey, the study (1962–65) of Earth's Upper Mantle—a 1,000-km crust, and in 1964–65 the International Year of the Quiet Sun (IQSY) IQSY will obtain data on geomagnetism, aurora, airglow, the ionosphere, solar physics, cosmic rays and space research during a period of sun-spot minimum, complementary to those obtained in the IGY period of sun-spot maximum

Another special committee is SCOR, dealing with oceanographic research of all kinds There is the effect of circulation on the welfare and migration of the plankton, the spread of radioactive wastes, the study of surface waves in relation to the design of ships or to erosion, and new methods of studying the sea floor More specifically SCOR has planned for 1963–64 an Indian Ocean expedition, in which at least thirteen countries and more than forty ships are expected to co-operate, and for which a good deal of reconnaissance has already been completed The expedition will make measurements on marine geology, geophysics and bathymetry, physical, chemical, and biological oceanography, and meteorology from the air-sea interface to the upper atmosphere The results may lead to a better understanding of the natural factors which affect the large population living in countries bordering on the Indian Ocean

The Special Committee on Antarctic Research (SCAR), has continued work along the lines of the IGY, involving a long-term programme of measurements at many stations on a variety of geophysical matters Twelve nations are at present taking part and the agreement by treaty to freeze all territorial claims for some thirty years shows a high degree of co-operation Advice sought from SCAR by several Governments has been freely given

Another descendant of the IGY is the Special Committee on Space Research (COSPAR) which aims to use rockets or rocket-propelled vehicles for carrying out scientific research in the widest sense In the public eye, space science is loaded with military and commercial interests and also provides some satisfaction to sensation-hunters It is most important, however, to preserve in proper perspective unbiased space research on a world-wide basis and to ensure that the top scientists concerned are maintained in personal contact COSPAR, first set up by ICSU in 1958, had many problems in its first year, but under an agreed charter (1959) can now hope to perform a valuable service It is not concerned with technical problems of rockets or with manned space flights Ten of the international unions and 16 national members are affiliated to it, and relations are maintained with the United Nations, the World Meteorological Organization, and the International Telecommunications Union, all Governmental bodies COSPAR is only just beginning to achieve its object, but two examples may serve to illustrate its potential value First, if the science of radio astronomy and of space research itself is to be continued at all, some frequency bands must be kept free from interference The 21-cm band has at present been exclusively preserved, but as Earth-to-Earth communication by satellites for commercial, military or other purposes is developed, larger parts of the spectrum will be claimed COSPAR, with IAU and USRI, must explain and emphasize the need Secondly, space experiments are now possible, or even in progress, which may for ever prevent some aspects of proper scientific research quite apart from their damaging effects on mankind The creation of a belt of needles, in the project 'West Ford' was considered as possibly hampering or even stopping future work on both optical and radio astronomy For these sound reasons, the ICSU Assembly agreed unanimously to invite COSPAR "to examine any proposed experiments or other space activities that may have potentially undesirable effects on scientific activities and observations, to arrange for careful objective quantitative studies and to make available to unions, academies, and at contacts, and, with fossil fuels, problems of heat transfer to the hot junctions following combustion

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Governments the facts and analyses needed by them for making wise and proper decisions concerning the proposed experiments" It further appealed to all Governments planning to launch space experiments which could possibly affect other scientific research adversely to make available to ICSU the information and data about the proposed experiments in sufficient time to ensure their proper consideration

ICSU has other commitments The Federation of Astronomical and Geophysical Services (FAGS) provides an international time bureau, a gravimetric bureau, and Earth tide service and a central ursigram service for the transmission of coded ionospheric, solar and geomagnetic data The Joint Committee on Radioactivity (JCAR) provides standardization of radioactive isotopes, and is linked with the International Atomic Energy Agency The ICSU Abstracting Board has done much to co-ordinate abstracting in both physics and chemistry Funds are also to be sought so that a revised set of bathymetric charts of the oceans can be published An international committee has recently been set up to examine the teaching of science at university level

The London assembly took an important decision to initiate a world-wide programme for studying the biological basis of human welfare The physical and biological condition of the surface of Earth is undergoing a more rapid and extensive change than might ever have been expected Much of this change is due in one way or another to the development and application of physical and medical science. Desirable as much of this may be, some of the influences may lead to serious changes in the frequency of certain genes Higher levels of radioactivity, too, although at an apparently insignificant dose-level, may in the long term be harmful to the progeny of man or animals or plants We need not only to investigate these problems, but also to educate the public on how to preserve satisfactory world conditions ICSU has therefore resolved to study, through the Unions of Biology, Biochemistry and Physiology, the effect on living communities of changes in natural environment, and the augmentation through basic research of natural resources and the reduction of losses and waste It is hoped to examine the somatic and genetic variation induced by environmental factors on man and other organisms, at the individual and population levels, and to find the best means for the preservation of biotic communities which are in danger of destruction or transformation In planning this programme it is intended in the first place to restrict it to clearly defined small projects of limited scope which can only be done by international co-operation The work will be basic in character and although it will be co-ordinated with related activities of a more applied kind being carried out by such bodies as UNESCO, FAO, WHO, or IUCN, it will involve no duplication

It is not surprising that after a period of such ferocious development the question has been raised whether the structure and mechanism of ICSU as laid down thirty years ago needs some revision The great activity of the separate international unions may have concealed the importance of the national academies Yet these latter have a vital part to play, especially to prevent a too extensive fragmentation of science, and through interdisciplinary integration to preserve science as a whole They, too, by contact with Governments, are primarily involved in the search for funds The whole structure must be designed to allow for this, and to ensure a satisfactory geographical distribution of members while still preserving both efficiency and flexibility Also, one cannot escape the fact that this enormous growth of activity requires a larger central secretariat in the headquarters which are shortly to move from The Hague to Rome Yet policy must always be decided by scientists themselves, and bureaucracy must be limited to the essential minimum More money is needed. The Assembly agreed to double the subscriptions of National Members and to ask for additional voluntary contributions UNESCO, an inter-Governmental organization, has certain overlapping interests with ICSU, but a mutually beneficial arrangement has been maintained, and UNESCO has continued to transfer funds to ICSU for use by the unions and special committees which are carrying out work in which it has a direct interest and on which it frequently seeks advice

The ICSU Assembly has appointed a special committee to examine all these problems of structure and operation in the near future Dr E W R Steacie, who succeeds Sir Rudolph Peters as president, will have charge of this committee. The next general assembly is planned for 1963 or 1964 in Zurich

The spirit of ICSU to-day can still be expressed by the resolution of the 1946 assembly, also held in London, acknowledging the duty of scientific workers (*a*) to maintain a spirit of frankness, honesty, integrity and co-operation and to work for international understanding, (*b*) to promote the development of science in the way most beneficial to mankind and to exert their influence as far as possible to prevent its misuse, (*c*) to serve the community not only by their specialized work but also by assisting so far as they are able in the education of the public in the purposes and achievements of science

H W THOMPSON

OBITUARIES

Mr. B. B. Osmaston, C.I E

B B OSMASTON, known to many in India as "B B", was born at Yeldersley Hall, Derbyshire, in 1867, the ninth of a family of fifteen, two of whom survive him He was educated at Cheltenham and at the Royal India Engineering College at Coopers Hill, where those entering the India Forest Service were then trained He passed in and out first of his year at Coopers Hill, and was made a Fellow of the College He joined the Forest Service in India in 1888, and served in the United Provinces (including Dehra Dun Forest College), Bengal, the Andaman Islands, a short time in Burma, and finally retired as chief conservator of the Central Provinces, being appointed C I E

After retirement he lived with his family for some time at Srinagar in Kashmir, and for the rest of his life he lived at Oxford

In 1892 he married Catherine Mary, daughter of General and Mrs Hutchinson, and they had sixty-eight years of married life together His wife died in 1960 but two sons and three daughters survive him, one son having been killed in the First World War

He was keen on any natural science subject which he took up chemistry, botany, spiders and, above all, ornithology He loved birds from boyhood, and he was very familiar with the habits and songs of Indian birds

Mr Osmaston's life in the jungle inevitably brought a large share of excitement and adventure in the form of encounters with dangerous wild animals, but one of the most exciting occurred within three months of his arrival in India, and is well worth recording again He was posted to the district of Jaunsar in the hills, where there had been a man-eating tigress taking a toll of human life for ten years Mr Osmaston, then aged twenty-one, and a forest student named Mr Hansard, decided to try to stalk the tigress in the heat of the day, when, they felt sure, she would be lying up in some secluded place They went cautiously down a steep ravine which was covered with thick scrub one of them on each side of the steep banks bordering the small stream They had not been going for more than five minutes when Mr Osmaston heard a scuffle and a terrible noise of growling, mixed with cries and groans above him The tigress had stalked the stalkers, and had pounced on Mr Hansard from behind Mr Osmaston, who had never before seen a tiger outside a zoo, had to risk a shot at the tigress while she was mauling Mr Hansard The shot made the tigress let go her hold, and the second barrel discharged at her as she came headlong down the bank towards him finished her off Mr Hansard's life was saved, though he had been badly mauled, and the scourge of the countryside was dead Mr Osmaston had achieved what many a famous hunter had tried to do during the previous ten years

His adventures included an encounter on foot with the rogue elephant which had caused the death of Mrs Anson, an exciting escape from drowning when shooting some rapids in a canoe, navigating a small launch by compass and map without previous experience in order to find a certain uninhabited island in the Andamans which was reputed to be the only habitat of some very rare hornbills This adventure included the onset of a storm resulting in the loss of the landing boat while the party was ashore, which necessitated a dangerous swim in rough water infested with sharks, back to the launch, which was anchored in deeper water

These few incidents serve to show Mr Osmaston's adventurous spirit He was abstemious and thrifty all his life, and his disregard for personal comfort and safety took him to many strange and interesting places, and he had very wide knowledge of all things to do with life in the jungle

After retirement he participated in two strenuous expeditions in pursuit of further knowledge about birds, the first to Ladakh at very high altitudes, and the second in Africa Both these expeditions were undertaken in the company of his good friend Admiral Lynes, another keen ornithologist

By his death, at the age of ninety-three, the world has lost a very courteous, charming and adventurous personality

Dr T P Colclough, C.B.E.

TOM COLCLOUGH, as he was known to ferrous metallurgists, who died on September 22, aged seventy-six, was one of those rare personalities who commanded respect in both industry and research He was educated at the Universities of Manchester and Sheffield, and awarded the D Sc of Manchester and the D Met at Sheffield He entered the Brown–Firth Research Laboratories in 1916 under W H Hatfield and in 1920 became chief chemist at Park Gate Iron and Steel Co , Ltd He was appointed in turn chief metallurgist, open-hearth manager and technical officer In 1929 he became technical director of H A Brassert and Co and was directly concerned with subsequent developments at Corby, Ebbw Vale and other steel works His capacity for sensing the direction of developments in iron and steel contributed to an international reputation covering Germany, Austria Turkey, China and New Zealand

During the Second World War he was technical adviser to the Iron and Steel Control of the Ministry of Supply, and was appointed C B E for his services in 1947 In 1945 he continued in this field as technical adviser to the British Iron and Steel Federation, which post he held at the time of his death His post-war travel ranged from work with the Control Commission in Germany at the end of the War, to advise the Durgapur works in India and the R I S C O works in Southern Rhodesia He was the leader of British Iron and Steel Federation missions to the U S S R , Poland and Yugoslavia

Research was not forgotten amid this industrial activity, for he participated in the work of the British Iron and Steel Research Association, and from 1950 served as a member of its Council He was awarded the Bessemer Gold Medal of the Iron and Steel Institute in 1954, and elected an honorary vice-president of that Institute in May 1960

Until recently he delivered an annual lecture in the Department of Metallurgy at Manchester, which was always received with eager concentration by the students He had the power to hold the attention and stimulate his audience which scarcely changed with advancing years In these days of specialization, his combination of forward thinking with the necessary restrictions of practice was as welcome as it was unusual C R TOTTLE

Dr. E. McKenzie-Taylor, C.I.E , M.B.E.

DR E MCKENZIE-TAYLOR died at his home at Basildon on October 17 He was a North-country man and received his early training at Armstrong College, Newcastle upon Tyne, before the First World War, for a short time he was a demonstrator in agricultural chemistry there His first appointment elsewhere was as lecturer in agricultural science at the Harper Adams Agricultural College From there he moved to the East Anglian Institute of Agriculture at Chelmsford, where he became head of the Department of Agricultural Chemistry, and as such was concerned primarily with analytical work connected with agricultural problems

After service in the Royal Artillery during the First World War, he returned to Chelmsford until 1921, when he was appointed senior chemist to the Cotton Research Board of the Egyptian Ministry of Agriculture Here, with A C Burns, he carried out important work on the daily and seasonal changes in soil temperature and on other factors influencing the yield of cotton In particular, they investigated the steady decrease which had been experienced in the yield of cotton in Egypt during the previous quarter of a century, under intensive irrigation by free flow from the Nile

McKenzie-Taylor returned to Great Britain in 1926 and was appointed University lecturer in agricultural chemistry in the School of Agriculture at Cambridge, a post which he held until 1930 In that year he became director of irrigation research and land reclamation in the Panjab Here again he applied his active mind to the soil problems connected with irrigation With his colleagues he was concerned to elucidate the mechanism of increasing salinity of the surface soil in irrigation areas, the possibility of the influence of a rising water-table on it, the difficulties of removing the accumulating salts, and the significance, in connexion with 'water duties', of using extra water to leach them out

During the Second World War, McKenzie-Taylor became director of military engineering research, G H Q India, where he held the rank of brigadier After returning to England on retirement, he had, since 1952, acted as agronomist to Rotary Hoes Ltd

H H NICHOLSON

NEWS and VIEWS

Royal Society Awards Royal Medals

H M THE QUEEN has approved recommendations made by the Council of the Royal Society for the award of the two Royal Medals for 1961 as follows to Sir Wilfrid Le Gros Clark, Dr Lee's professor of anatomy in the University of Oxford, in recognition of his outstanding contributions to neuroanatomy and primate morphology, which he has combined to provide new knowledge of human evolution, to Prof C F Powell, Melville Wills professor of physics in the University of Bristol, in recognition of his pioneering work on the development of photographic emulsion technique in the investigation of cosmic rays and the outstanding results derived therefrom on the elementary particles in cosmic radiation

Experimental Physics in the University of Sussex

Prof K F Smith

DR K F SMITH, at present lecturer in the Cavendish Laboratory, Physics Department, University of Cambridge, has been appointed to the chair of experimental physics in the new University of Sussex at Brighton Prof Smith was born in 1923 and went to Colfe's Grammar School at Lewisham (London) In 1942 he went to Cambridge with a minor scholarship to Sidney Sussex College His studies were interrupted in 1945 by work on atomic energy, first in Canada and then at the Atomic Energy Research Establishment at Harwell In 1948 he returned to Cambridge and began to build an apparatus for measuring nuclear spins, using the molecular-beam method, developed by O Stern and further by I I Rabi, but not previously used in Great Britain He aimed in particular at the study of unstable, that is, radioactive nuclei, of which only very minute quantities can be obtained In subsequent years he and his students developed various ingenious ways of recording the faint trickle of atoms that pass through the severe scrutiny of narrow slits, two inhomogeneous magnetic fields and a radio frequency field which causes the spin of the atom to change direction Research Fellow of Sidney Sussex College since 1950, Senior Exhibitioner in 1952, Dr Smith became a demonstrator in the University of Cambridge in 1953, and lecturer in 1958 He spent a year's sabbatical leave in 1958 as an associate professor at Berkeley, California He will be followed to Brighton by the good wishes of the molecular-beam fraternity everywhere

Iodine-131 in Milk in Britain

RESULTS are now available for measurements, made by the Agricultural Research Council Radiobiological Laboratory, of iodine-131 in milk during the week ending November 11 The method of sampling was the same as that already described (see *Nature*, November 11, p 504) Milk is collected daily from 23 sites in different parts of England, Wales, Scotland and Northern Ireland In addition, on one day in each week country-wide samples are provided by more than 200 depots which together handle more than 40 per cent of the total milk produced throughout the United Kingdom The mean results of the daily samples are shown in Table 1

Table 1

Week	$\mu\mu c$ Iodine-131/litre
October 15–21	114
October 22–28	158
October 29–November 4	127
November 4–11	117

The survey of iodine-131 in milk throughout the United Kingdom has been in progress for 8 weeks and an analysis of the combined results of the daily and country-wide sampling now enables the overall situation to be assessed in terms of the recommendations made by the Medical Research Council It was stated that the acceptable radiation dose would not be exceeded for infants under one year of age, the most susceptible age group of the population, unless the average concentration of iodine-131 in milk rose above 130 $\mu\mu c$/litre over a period of one year, or higher concentrations were maintained for correspondingly shorter periods This recommendation took into account the likely extent of variations in the contamination of local milk supplies The significance of the quantity of iodine-131 which has so far entered milk can thus be assessed by comparing it with that which would give rise to the annual average specified by the Medical Research Council Table 2 shows that in different regions of the country, between 9 and 20 per cent of the specified annual total had been reached on November 11, the country-wide average being 14 per cent

Table 2 SUMMARY OF RESULTS OF DAILY AND COUNTRY-WIDE SAMPLING OF MILK FOR IODINE-131 DURING EIGHT WEEKS, SEPTEMBER 16–NOVEMBER 11

	Zone	Average contamination ($\mu\mu c$/litre)	Percentage of quantity which would give rise to annual average specified by the Medical Research Council
1	Northern England	103	12
2	North-West England	98	12
3	East and East Midland	72	9
4	West Midland	106	13
5	Wales	165	20
6	South and South-East England	94	11
7	Mid-Western England	100	12
8	Far-Western England	133	16
9	Scotland	157	19
10	Northern Ireland	164	19
	Country-wide mean	115	14

The highest values have been found in Wales, Northern Ireland, Scotland and far-western England, but even these would not lead to the annual average specified by the Medical Research Council unless they continued for at least a further seven months or more. Regional differences can result not only from climatic and geographical factors but also from differences in the diet of cattle. The quantity of iodine-131 which they consume depends primarily on the quantity of fresh grass which they eat; thus, recently deposited fission products enter milk less readily when, owing to the onset of winter, cattle are provided with increasing quantities of other foods. In 1958, after the previous series of weapons trials in the Arctic, this effect was marked from the middle of November onwards, and results from Scotland suggest that it is occurring again. It cannot, however, be assumed that the contamination of milk will decrease steadily in the future, because for some weeks after the detonation of nuclear weapons the rate of deposition may vary widely depending on meteorological factors. A careful watch will therefore be maintained so long as appreciable levels of contamination remain.

Paul Instrument Fund Grants

AWARDS of grants by the Paul Instrument Fund Committee have been made as follows: £44,500, with the possibility of an increase to £52,000, to Dr. V. E. Cosslett, University lecturer in physics, University of Cambridge, to design and construct a very high voltage electron microscope and to explore its applications in biological and metallurgical research; £1,000, in supplement of a previous grant, to Prof. D. Gabor, professor of applied electron physics in the Imperial College, University of London, and Dr. D. Jones, for work on the development of an electron interference microscope; £3,500 to Mr. D. W. Hill, research department of anæsthetics, Royal College of Surgeons of England, for the construction of an infrared gas or vapour analyser using interference filters; £2,500 to Dr. N. Kurti, reader in physics and senior research Fellow, Brasenose College, Oxford, for the construction of a nuclear thermometer for use in the micro-degree Kelvin temperature-range; £1,500, in supplement of a previous grant, to Dr. J. H. Sanders, University lecturer and demonstrator in physics, Clarendon Laboratory, Oxford, for the construction of an optical maser. The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much improved types of physical instruments and apparatus for investigations in pure or applied physical science".

Department of Technical Co-operation

IN a written answer in the House of Commons on November 14, the Secretary for Technical Co-operation stated that of the expenditure on overseas information services, 32 per cent was attributable to the British Council, 35 per cent to the British Broadcasting Corporation, 13 per cent to the Central Office of Information and 20 per cent to overseas departments. The Department had taken over 87 administrative and advisory staff from other Departments, including 31 from one of the Overseas Departments, 155 executive staff, and 306 ancillary staff. With four exceptions, the staff had been previously employed on work which had been transferred with them. He was now considering how the Department's ability to help with advice on the economic aspects of development could be strengthened.

Technical Colleges and Schools in Britain

IN reply to a question in the House of Commons on November 16 regarding the number of technical colleges and schools in Great Britain, the Minister of Education, Sir David Eccles, said that in England and Wales there were 761 grant-aided major establishments, 232 maintained secondary technical schools and 5 direct-grant secondary technical schools. Students in the two latter numbered 97,039 and 838, respectively, in January 1961. In the 1959–60 sessions, 112,768 students attended full-time courses in the major establishments, 10,038 sandwich courses, 501,003 part-time and 784,584 evening courses. In the 92 major establishments in Scotland, there were 12,549 full-time students, 35,609 part-time and 52,696 evening students. Construction is in progress on 126 major projects in England and Wales and 18 in Scotland, and 217 further projects in England and Wales and 25 in Scotland have been approved.

High-flux Reactor at Petten

THE high-flux materials testing reactor at the Petten research centre of the Reactor Centrum Nederland became critical for the first time on November 9. This is the second reactor to become critical at Petten since September 28, 1960, when a low-flux reactor was put into operation. The nuclear part of the high-flux reactor was designed and delivered by the U.S. firm of Allis-Chalmers Manufacturing Company, the remaining part having been built in co-operation with several Dutch companies. Most components, also those delivered by Allis-Chalmers, were manufactured by Dutch industries. The reactor, which is essentially similar to the U.S. Oak Ridge research reactor, is intended for the testing of materials. The neutron beams emitted by the reactor can also be used for carrying out neutron physics research and for the production of radioisotopes. During the forthcoming months the reactor will be operated at low power. Afterwards the power will be gradually increased up to the maximum level of 20,000 kW. It is expected that this level will be reached in the spring of 1962. In the meantime, the reactor vessel, in which the actual core of the reactor is situated, will be replaced by a new one, which has been manufactured in the United Kingdom.

Digital Computation at King's College, Newcastle upon Tyne

A NEW automatic digital computer has been ordered by King's College, Newcastle upon Tyne, to replace the *Pegasus* computer in the Computing Laboratory. The new computing system is based on the *KDF* 9 machine manufactured by the English Electric Company. A grant towards the cost of the machine has been promised by the University Grants Committee, while the balance remains to be found by the College. The new system is capable of very high speeds of calculation and has facilities for storing large quantities of information on magnetic tape. Results in both numerical and alphabetical form can be printed at speeds up to 600 lines per min. On some problems the new machine will operate at about one hundred times as fast as the existing machine, and will greatly assist many research projects in both pure and applied science not only for the University but also for industry It will permit also the training of many students in the techniques and applications of automatic computing At the present time, computing systems are being ordered in increasing quantities for both scientific and commercial applications, and the machines are being built faster than people are being taught how to use them There is thus great need for enlarged training facilities of the kind the Computing Laboratory can provide

National Research Council of Canada

The recently published forty-fourth annual report of the National Research Council of Canada outlines the scope of work carried out during 1960–61, including the provision of 7 1 million dollars to support pure research in the universities (including 775 grants, 550 scholarships and fellowships) and an additional 2 3 million dollars through the Medical Research Council (Pp 48 N R C 6322 Ottawa Queen's Printer, 1961) The National Research Council employs 670 scientific research staff (including 130 postdoctorate fellows) and 953 personnel, and operates five laboratory divisions covering the fields of applied biology, applied chemistry, pure chemistry, applied physics and pure physics, and also controls the four engineering divisions, including building research, mechanical engineering, the National Aeronautical Establishment, and radio and electrical engineering as well as two regional laboratories, one at Halifax and the other in Saskatoon During 1960–61 it sponsored thirty-six associate committees, operating in such diverse fields as the control of hospital infections, experimental psychology, plant breeding, radio science, and soil and snow mechanics, and dealt with 14,500 technical inquiries from Canadian industries The outstanding event of the year was the establishment of the Medical Research Council as an autonomous body associated with the National Research Council The report also includes the annual reports of the Medical Research Council and Canadian Patents and Development, Ltd

The *Review of the National Research Council*, Canada, for 1961 (Pp 377 NRC No. 6421. Ottawa National Research Council, 1961. 75 cents), describes the work of the Council to March 31, 1961, and besides reports of the directors of divisions and heads of sections includes accounts of the work of the Council's committees and the first review of the work of the Medical Research Council, 1960–61 Lists of publications during the period are appended to the several reports of divisions, etc, and with the directory of staff and list of staff serving on outside organizations the *Review* is a comprehensive reference work on the two Research Councils, supplementing the information given in the annual report for the period and in the report on university support

Safety in Mines Research

The annual report of the Safety in Mines Research Establishment of the Ministry of Power for 1960 gives a concise yet easily readable account of the wide range of work undertaken by the Establishment (London H M S O Pp 72 + 4 plates. 5s net) Within the main body of the report sections are devoted to explosives and blasting devices, explosion hazard, fire hazard, rescue apparatus, engineering and metallurgical research, dust measurement and the pneumoconiosis hazard, investigation of mining incidents, examination of equipment after mine service and testing services. Special attention is being paid in the field of coal dust explosions to the use of stone-dust barriers and a full-scale explosion gallery is to be built at the Buxton laboratories Most coal dust explosions underground are initiated by the ignition of firedamp, accordingly, the work on layering of firedamp and its accumulation has been extended together with an examination of preventive and remedial measures At the same time research on the frictional ignition of gas, particularly between metals and rock, is in progress Ignitions of gas due to shot-firing are very few compared with the tens of millions fired each year; but such ignitions are always serious, present work in this field is concerned with the safety of millisecond-delay firing and with improved methods of testing explosives Much of the work described in the report will be of interest to many fields of industry other than the coal industry not only in connexion with explosion and fire hazards but also in connexion with breathing apparatus, engineering and metallurgical research, dust measurement and pneumoconiosis

Wool Research Colloquia in Australia

A new series of one-day meetings has been inaugurated in Australia to discuss particular aspects of wool research. They are being organized by the Commonwealth Scientific and Industrial Research Organization Wool Research Laboratories, and invitations are extended to those research workers in university and C S I R O laboratories who are working on the subject under consideration Wool Research Colloquium No 1, which was held at the Division of Protein Chemistry, C S I R O, Parkville, Victoria, on September 20, was convened by Dr R D B Fraser Wool structure was considered at this meeting under the headings of molecular structure, chemical structure, biosynthesis and physical properties and structure. Wool research scientists from several States of the Commonwealth attended the meeting Prof H Fraenkel-Conrat, of the Virus Laboratory, University of California, who had been in Melbourne as guest lecturer during a series of lectures in protein chemistry, and Mr I K Walker, director of the Dominion Chemical Laboratory, Wellington, New Zealand, were guests at the Colloquium These meetings will be a permanent feature and they are intended to provide specialist discussions on major aspects of the C S I R O wool research programme

Research and Education in Physics

An international seminar for research and education in physics, co-sponsored by the International Atomic Energy Agency, the United Nations Educational, Scientific and Cultural Organization and the Central Committee for Swedish Technical Assistance, is to be held as a one-year course at the Institute of Physics, Uppsala, during September 1, 1962–July 1, 1963. The aim of the seminar is essentially to provide possibilities for individual participation in qualified experimental research work in one of various fields of physics (for example, solid-state physics, X-ray physics, nuclear physics, atomic physics, etc) under the guidance of experienced scientists An introductory course in the operation of, and the coding for, a modern computer, IBM 1620, will be given The seminar will be open to non-European students and scientists, mainly from developing countries, who are working in the above-mentioned subjects and who are connected with the teaching and/or research of a university or national laboratory, and who are not less than twenty years and not more than forty years of age Application forms and further information can be obtained from the International Seminar, Institute of Physics, University of Uppsala, Uppsala, Sweden

University News

Belfast

THE following appointments to lectureships have been announced Dr R H J Sellin (civil engineering), F J Smith (applied digital mathematics computing), Dr Uno Opic (applied mathematics)

Bristol

THE following appointments to lectureships have been announced C Andrew (mechanical engineering), Dr R Binks (organic chemistry), J S E David (veterinary surgery—diseases of reproduction), Dr R P Mercier (theoretical physics), Dr J G C Milne (physics), D B Peacock (bacteriology)

Cambridge

THE following elections at Churchill College have been made *Fellowships*, J A Pateman, University lecturer in genetics, and C K Phillips, deputy treasurer of the University, *Junior Research Fellowship*, J S Gibson, of the University of Sheffield, in genetics, *Extraordinary Fellowship*, Prof Jacques Barzun, professor of history, Faculty of Political Science, provost of Columbia University for 1962–63, and Prof Kenneth J Arrow, professor of economics and statistics, Stanford University, for 1963–64, *Overseas Fellowship*, Dr P W Anderson, of the Bell Telephone Laboratories

Ibadan

THE Calendar 1961–62 of the University College, Ibadan, Nigeria, lists the officers, standing committees, principal standing boards and committees of the Senate, honorary associates and the staff of the College (Pp 110 Ibadan The University College, 1961) Besides particulars of entrance regulations, courses of study, scholarships and prizes, there are notes on the Department of Extra-Mural Studies, Institute of Education, Institute of Child Health, and the Nigerian Institute of Social and Economic Research A list of graduates for 1961 is also included

London

PROF K G DENBIGH, professor of chemical engineering at the Imperial College of Science and Technology, has been appointed to the Courtaulds chair of chemical engineering tenable at that College The following titles have been conferred professor of geography, on Dr W R Mead in respect of his post at University College, professor of organic chemistry, on Dr L N Owen in respect of his post at the Imperial College of Science and Technology, reader in anatomy, on Mr D W James in respect of his post at University College, reader in chemistry, on Dr C A Vernon in respect of his post at University College

Miami

DR CARL H OPPENHEIMER, formerly of the University of Texas Marine Institute at Port Aransas, has been appointed research associate professor at the Institute of Marine Science, University of Miami, where he is at present head of the microbiology group

Announcements

PROF J B S HALDANE, formerly professor of biometry in the University of London and later research professor in the Indian Statistical Institute, from which post he has recently resigned, was awarded this year the Kimber Gold Medal for genetics of the U S National Academy of Sciences He has also received a Premio Feltrinelli for biological sciences of the Accademia dei Lincei, Rome Prof Haldane attended a special meeting during the summer of the Physiological Society held in Oxford to commemorate the centenary of the birth of his father, J S Haldane, and received an honorary doctorate of science from the University He expects to take charge of a small laboratory for research in genetics to be set up by the Council for Scientific and Industrial Research, India

DR LEO L BERANEK, president of Bolt Beranek and Newman, Inc, of Cambridge, Mass, has been presented with the Wallace Clement Sabine Award of the Acoustical Society of America for his "internationally recognized achievements in all phases of architectural acoustics" The Pioneers of Underwater Sound Award has been presented to Dr Albert B Wood, of the Admiralty Research Laboratory in Teddington, Middlesex, "for nearly half a century of original work in physics and for decades of pioneering leadership in underwater sound"

DR F RALPH KOTTER has been appointed chief of the newly established High Voltage Section of the Electricity Division at the National Bureau of Standards, U S Department of Commerce Since 1955, Dr Kotter has been working on standards of inductance and capacitance at the Bureau

DR THOMAS R LAWRENCE has been appointed to the National Bureau of Standards, U S Department of Commerce As a member of the Engineering Metrology Section, Dr Lawrence will investigate the applicability of the helium–neon gaseous optical maser to precision measurements of length

THE Systematics Association is organizing a lecture on "The Systematics of Man", to be delivered by Dr B G Campbell at the British Museum (Natural History) on February 8 Further information can be obtained from Mr Norman Tebble, Systematics Association, British Museum (Natural History), Cromwell Road, London, S W 7

AN international symposium on the growth and development of the lower archegoniate plants will be held in the rooms of the Linnean Society of London, Burlington House, Piccadilly, London, W 1, during April 5–6, 1962 Further information can be obtained from the General Secretary of the Society

ERRATA In the article entitled "The Mechanism of Action of Liver Alcohol Dehydrogenase" in *Nature* of October 7, p 47, the following corrections should be made Table 1 $\varphi_{12} = 0\ 9$ sec μM^2, $\varphi_{12}' = 10{,}500$ sec μM^2, $K_{E,\mathrm{alc}} = 6060\ \mu M$, in Fig 1 2 2 μN LADH in (3), (6) and (7), col 1, line 5 from bottom of p 49, ($pK = 8\ 6$)

DR D B E STROUD, writing in connexion with the article "Mannosyl-D-Glucosamine Hydrochloride Isomers" (*Nature*, 191, 142, 1961), states that the optical rotation $[\alpha]_D^{20} + 55°$ quoted on p 143, three lines from foot of col 2, refers to the original mixture of fractions $A + B$, and not to fraction B

WATER POLLUTION RESEARCH IN GREAT BRITAIN

A FURTHER stage in the development of the Water Pollution Research Laboratory of the Department of Scientific and Industrial Research was marked by two open days at Stevenage on October 11–12, when interested members of the public could see a wide range of work in progress and also inspect the new wing which came into use during the summer

The new west wing, designed by the Ministry of Works, provides an additional floor space of nearly 20,000 sq ft and comprises four pilot-scale laboratories, with several smaller laboratories and offices (Fig 1) One of the pilot-scale laboratories rises through three storeys and the others are two storeys in height, giving adequate headroom for treatment equipment of all kinds The construction is in load-bearing brickwork, which facilitates the fixing and support of apparatus A full range of services is provided, and the keynote is flexibility

The fact that this new wing has become necessary —it is already almost fully occupied—only seven years after the Laboratory moved to Stevenage from its temporary headquarters at Watford is a sign of the increasing need for research into all aspects of the pollution of water Three recent Acts, the Clean Rivers (Estuaries and Tidal Waters) Act, 1960, the Rivers (Prevention of Pollution) Act, 1961, and the Public Health Act, 1961, have extended control over the discharge of wastes into rivers and estuaries, and the field of pollution prevention and control is now a very active one, in which local authorities, river boards and industry are all closely concerned

In order that the work of the Laboratory can be more closely applied in Scotland, an out-station has recently been established at East Kilbride, near Glasgow This station carries out its own local research and can also call on the resources of the main laboratory at Stevenage when necessary

The functions of the Water Pollution Research Laboratory are, broadly, to study and improve methods of treating polluting liquids, including both domestic sewage and trade effluents, and to study the effects of pollution on surface waters The scope and variety of the work involved are so great that only a few examples can be described Two subjects of wide general interest may be mentioned first

The survey of the Thames Estuary, which was started in 1948, is now nearing completion Its primary object was to provide information for the guidance of the Pippard Committee, the report of which is now with the Minister of Housing and Local Government and is expected to be published shortly Analytical records collected by the London County Council during some sixty years, supplemented by detailed surveys made by the Laboratory staff, have been used to study the complex relationships between dissolved oxygen, temperature, pollutional load, freshwater flow and other factors A theory of mixing developed at the Laboratory has enabled predictions to be made of the effect of changes in the pollutional load on the condition of the estuary water It has given good results when applied to changes known to have taken place in the past Fresh problems have presented themselves as the survey has proceeded For example, the role of nitrates as a reserve of oxygen, the rate of nitrification of polluting matter, and the effect of synthetic detergents on the rate of settling of suspended solids have all required special study before the complete picture of conditions in the estuary can be built up

The second subject of general interest is the search for synthetic detergents which can be broken down biologically in sewage treatment works and will not produce the familiar and objectionable foam on rivers During recent years the Laboratory has tested 74 new materials for manufacturers on a repayment basis It has also collaborated in the 'Luton experiment', in which a 'soft', straight-chain detergent, sodium 'Dobane *JN*' sulphonate, has, since 1958, been distributed by the manufacturers in Luton and the surrounding district instead of the normal 'hard' detergent An extensive programme of sampling at Luton sewage treatment works and in the River Lea has shown a reduction of about 40 per cent in the surface-active material entering the river This is rather less than expected on the basis of laboratory experiments, a result which may be due to the unusual method of treatment used at Luton, consisting of high-rate activated sludge treatment followed by high-rate biological filtration. A greater reduction of the new material has been observed at some other sewage works in the area, and this disparity between different methods of treatment is being further examined in the Laboratory

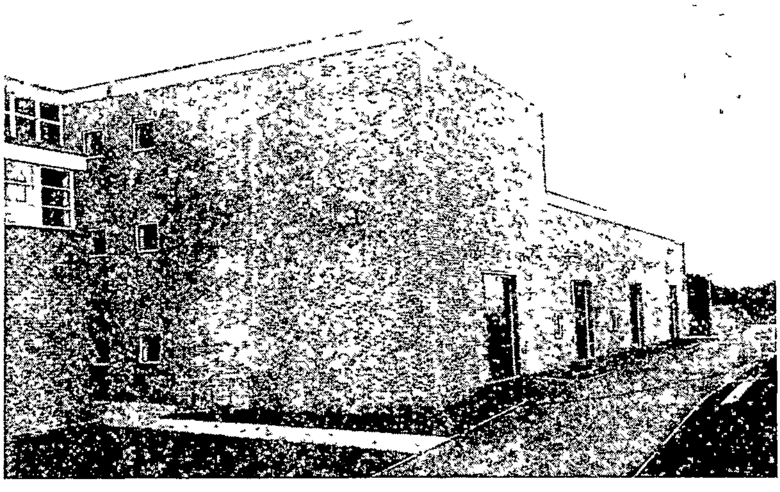
Fig 1 The Water Pollution Research Laboratory Pilot-scale laboratories in the new west wing

Treatment of Sewage and Industrial Wastes

Little information has hitherto been available on the relative merits of the various filter media used in the biological filtration of sewage Since April 1959 a portion of the Stevenage domestic sewage has been treated on a battery of eight small filters filled with two different sizes (nominally about 1 in and 2½ in) of four different media (clinker, blast-furnace slag, broken rock and rounded gravel) With the exception of the gravel which, in the smaller size, gave some ponding trouble during last winter, the smaller media have consistently produced better effluents than the larger media The media with the larger surface area per unit bulk volume, namely, the clinker and slag, gave better results than those of more regular shape, namely, the broken rock and rounded gravel These relative efficiencies of treatment applied not only to the removal of biochemical oxygen demand but also to the reduction in bacterial counts

Investigations into the activated sludge process have been principally concerned with the efficiencies of various methods of aeration The work has suggested ways in which the efficiencies (on a basis of oxygen put into solution per unit of power supplied) accepted in normal sewage treatment practice could be considerably improved by such means as altering the geometry of the aeration tanks Whether the gain in efficiency would be outweighed by other disadvantages such as increased capital cost would depend on local conditions

Interesting fundamental studies have been started on the kinetics of the activated sludge process, to determine the relative importance of the method and intensity of aeration, the level of dissolved oxygen and the concentration of sludge maintained in the tanks, the degree of turbulence and longitudinal mixing and other variables

There is an increasing demand for investigations into methods of pre-treating specific industrial wastes, and this work is done by the Laboratory on repayment Recent and current work of this nature includes the removal of ferrocyanide from engineering wastes, the electrolytic oxidation of cyanide, the bacterial oxidation of thiocyanates, the use of beds of calcined magnesite to neutralize acid wastes, and the treatment of washings from cattle sheds and other farm premises—this last being a growing problem which sometimes causes difficulty at small sewage works

River Pollution

A large part of the work on rivers is devoted to assessing the relative effects of surface aeration, photosynthesis and plant respiration on the oxygen balance As a positive step towards increasing the oxygen content of a polluted river, experiments are being performed on air entrainment in a Venturi tube, which could be inserted in an effluent pipe near the point of discharge to a river The method is simple and, on the laboratory scale, has shown promising results

Work on the toxicity to fish of ammonia and other chemicals has continued, and the effects of mixtures of toxic substances are now being examined

Measurement Techniques

It is inevitable that a laboratory such as this should from time to time find that existing standard methods of analysis and measurement are unsuited to its needs in many specialized lines A valuable part of its work is therefore the improvement of old methods and the development of new Among its contributions in this way are the improvement of established methods for determining nitrites and nitrates, the introduction of new techniques for assessing organic carbon and for the separation of radioactive strontium and barium, and the design of automatic sampling equipment for sewage, and recorders for dissolved oxygen and suspended solids.

The work of the Water Pollution Research Laboratory is thus seen to be a judicious combination of fundamental investigation and practical application for the benefit of the man on the job On the impressive evidence put forward during the open days, it seems to be doing this work very well indeed

F E Bruce

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION, AUSTRALIA

THE thirteenth annual report of the Commonwealth of Australia Scientific and Industrial Research Organization for the year ended June 30, 1961, besides being a general review and a brief survey of some of the more important developments arising during the year out of the research being carried out by the Organization, includes a list of papers published during the year and details of the membership of the Advisory Council, the State Committees and staff[*] Total expenditure amounted to £9,603,963 and the Treasury appropriation to £7,571,104, of which £1,058,246 was in the animal research laboratories, £886,518 on plant research, £334,613 on food preservation, £396,600 on forest products, £856,192 on the chemical research laboratories, £246,683 on fisheries and oceanography, £767,705 on the National Standards Laboratory, £451,278 on radio physics, £574,252 on wool research, £280,904 on fuel research, £333,237 on land research and regional survey, £51,700 on research association grants and £95,069 on overseas research studentships While since 1950 the total budget increased from £2 7 million to £9 6 million, the research staff only increased from 750 to 880, and the Executive believes that this increase in staff is quite insufficient

The giant radio telescope being erected at Parkes for the Division of Radiophysics is structurally completed and is in fact now available full time for research The Controlled Environment Research Laboratory is scheduled for completion early in 1962

Among the research developments noted in the review are a new method of stimulating the germination of legume seeds by exposing water-soaked seeds to low concentrations of carbon dioxide, a practical and economic means of controlling posthitis in wethers by implanting under the skin small pellets of the male hormone, testosterone; progress in work on worm parasites of sheep and cattle, and evidence that selection of cattle for their resistance to ticks may be a highly satisfactory means of minimizing losses due to tick infestation Codling moth infestations have been found to depend largely on the larvæ which over-winter in an orchard, and these, in turn, on the amount of cocoon shelter available in the orchard This can be reduced by adequate management, and, if trap bands are provided to which the overwintering individuals are attracted in large numbers and destroyed by special toxic deposits, relatively few insecticidal treatments are needed in the following season Work at Katherine has shown that nitrogen in a form available to plants concentrates just under the soil surface during the dry season and the greatest loss occurs during the wet season, owing to leaching by rain

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[*] Commonwealth of Australia Thirteenth Annual Report of the Commonwealth Scientific and Industrial Research Organization for the year ending June 30, 1961 Pp iii + 150 (Melbourne Commonwealth Scientific and Industrial Research Organization, 1961)

It has now been demonstrated that when the appropriate fertilizer dressings to overcome deficiences in nitrogen, phosphorus, potassium, sulphur, calcium, zinc and molybdenum are applied to the infertile coastal lowlands of southern Queensland, pastures can be grown on these soils which grow and fatten cattle at stocking-rates of a beast to 1 5 acres, with annual live-weight increases of 300 lb per beast Investigations on the role of elements in plants have shown that manganese deficiency inhibits a reaction involved in the evolution of oxygen during photosynthesis and also that manganese is a constituent of normal chloroplasts Studies on the life-habits of the freshwater snail *Lymnea tomentosa*, which infests grazing lands, have revealed great difficulties in applying effective control measures, as if the population is drastically reduced each surviving snail will harbour so many liver flukes that the end result is much the same Spoilage of dried prunes of high-moisture content has been prevented by hot-filling the prunes into laminate bags

A direct-reading tester for increasing the regain of wool has been developed, consisting of a hot-air blower to dry the wool and a balance which indicates regain directly Evidence has been obtained that substantial numbers of freezing nuclei occur at altitudes of 50,000–60,000 ft, and work has been completed on a recorder, designed to operate for periods up to 12 months, to provide continuous records of water-level in streams, dams or bores for gauging purposes, or of rainfall, temperature, humidity, wind speed or evaporation A process has been devised for production of pure thorium by converting thorium carbide to volatile thorium iodide and depositing thorium metal from this on to a heated surface. A new cyclone elutriator, the 'Cyclosizer', developed for precise separation of solid particles in the subsieve ranges, is based on the stable flow patterns and high centrifugal forces obtainable in hydraulic cyclones of suitable dimensions

A thorough examination has been made of resistance measurements to establish clearly the possible limits of accuracy using both direct and low-frequency alternating current, and fundamental investigations of cell wall organization of the tissue elements of wood and of the pits between them has demonstrated that in hardwoods the initial penetration path is through the vessels and then via the pits to the rays and adjacent fibres or vertical parenchyma In softwoods the path is from tracheid to tracheid, or tracheid to parenchyma, through the pits A notable advance in the metal-staining technique for the electronmicroscopy of wool has revealed further structural details within the microfibrils and a resolution of 1 in 4,000 has been achieved with a mass spectrometer constructed at the Chemical Research Laboratory, while new techniques, involving an optical filter of narrow band-width, have provided photographs showing the velocity distribution in selected areas of the solar chromosphere

SCIENTIFIC RESEARCH IN NEW ZEALAND

THE report of the Department of Scientific and Industrial Research, New Zealand, for the year ended March 31, 1961*, includes the Minister's Statement, the report of the Council of Scientific and Industrial Research and that of the Secretary, while the third annual report of the Ross Dependency Research Committee is appended Lists of publications during the year are given under the respective branches of the Department together with lists of projects in hand.

Expenditure during the year amounted to £1,963,486 net, of which £201,421 was on the Dominion Laboratory, £225,114 on the Dominion Physical Laboratory, £131,777 on the Grasslands Division, £124,885 on the Geophysics Division, £114,532 on the Geological Survey and £111,986 on the Soil Bureau The Council again points out that New Zealand is still lagging behind in the application of science and the scale of research, and her expenditure in 1959–60 of about £3 98 million on scientific work or 0 32 per cent of the gross national produc compares with 2 3 per cent in Britain and 2 7 pe cent in the United States in 1957–58 Professional staff at April 1 was 401 compared with 390 in 1959–60, total staff being 1,121 and 1,067, respectively, and the Secretary refers to a loss of 30 professional staff during the year, overseas, nearly all experienced scientists, although the overall calibre of the Department's staff has substantially improved over the past decade. During the past five years M Sc graduates in physics in New Zealand universities have averaged 10, in chemistry 21 and in mathematics 13, but about half those obtaining first-class honours accept posts overseas and do not return The Secretary stresses the importance of being able to attract scientists of high quality and indicates some of the factors, besides adequate salary, which are important In 1960 the establishment of two senior research fellowships tenable in the Department's laboratories for up to two years was approved and two fellowships have now been awarded The Council is gravely concerned, however, that no action has been taken towards securing the 40–50 increase in staff per annum

* New Zealand Report of the Department of Scientific and Industrial Research for the year ended 31 March, 1961 (H 34) Pp 87 (Wellington Government Printer, 1961)

over the next five years which is required to implement fully the Department's present programme of work

After discussions with the University Research Committee and the chairman of the new University Grants Committee the Council proposes to terminate the present type of research grant made to the universities by the Department From the end of the present financial year the Department will sponsor and provide funds for research units in universities in fields of study deemed essential in the national interest, and in which an outstanding man is stationed in the university, and by entering into research contracts with universities Grants to research and allied institutions in 1960–61 amounted to £176,862, of which £33,810 was to universities and agricultural colleges and £91,376 to research associations

The Council's recommendation for the establishment of a new Wool Research Organization as an incorporated research association with an annual income of £100,000, half from the New Zealand Wool Board and half from the Government has been approved, and the new buildings for the Meat Research Institute were completed during the year The Council has decided to continue New Zealand's scientific programme in Antarctica at about its present level (£86,606 in 1960–61), and topographical and geological survey work will be continued until the reconnaissance of the Cape Adare–Beardmore section is complete Some concern is expressed as to the effect of delay in developing the Institute of Nuclear Sciences on the research programme in this field

Points in the research work completed during the year noted in the Secretary's report include the completion of the first volume of the new *Flora of New Zealand*; the high yields now being obtained with Aotca wheat, bred at the Crop Research Division and released in 1957, the marked difference in the distribution of radioactive selenium in plants, the variation of the nitrate-levels and of sodium, potassium, calcium, manganese, aluminium, copper, titanium, iron, zinc, sulphate and phosphate in rye-grass plants, and the study of the systematics and taxonomy of bacterial plant pathogens

The Fruit Research Division's apple-variety collection has led to the selection of the Canadian variety Spartan and a local seedling, Red Fresca for extensive commercial trial, and satisfactory drying of tobacco leaf has been obtained by two methods which enable a kiln to be loaded with much less labour and permit the use of smaller kilns A study has been made of the Adelie penguins breeding at Cape Royds, Ross Island, and a set of 54 soils selected representing a range of New Zealand soils for agricultural, forestry and highway purposes is being examined comprehensively by chemical physical and biological tests

An intensive mineralogical study of volcanic ash showers has been commenced, and the four-mile geological map project commenced in 1956 to be completed in 1964 has already indicated areas meriting more detailed examination. A rugged tester, in which the sample is dried for 5 min in hot air and weighed on scales calibrated directly in water content (corrected for buoyancy) has been developed by the Dominion Laboratory, and 20 of these machines are now in use in scouring works and freezing works throughout New Zealand for controlling the drying of wool A fœtal heart-rate recorder has been constructed using a heart-sound pick-up which delivers this conglomeration of sounds to a system specially designed to deal with a fœtal pulse which may be mostly even below the level of background noise Investigations of the variation in the essential oil derived from samples of *Pinus radiata* have been initiated, and an instrument developed for rapidly and accurately determining the density of a wood sample taken from a living tree, utilizing a very narrow beam of beta-radiation from a strontium-90 source A novel type of navigation light for marking narrow channels has been developed which can be used when the configuration of the land in line with the channel will not permit the use of more than one light. An emergency wheat-testing service provided by the Wheat Research Institute avoided substantial loss during the 1961 wheat harvest A survey of currents in McCurdo Sound established that at least in summer there is a definite movement of water towards the Antarctic Ice Shelf

FOOT-AND-MOUTH DISEASE

In a report issued by the Research Institute (Animal Virus Diseases), Pirbright, Surrey*, the director, Dr I A Galloway, describes the investigations made into foot-and-mouth and related virus diseases over the past five years

The research programme of the Institute is considered in relation to foot-and-mouth disease in Britain, and, in turn, as a world problem In Britain, periods of relative freedom from the disease (or at least of low incidence) are interrupted by times when as many as 200 farms may become infected in one month These periods of higher incidence do not generally last more than three or four months, and the number of animals involved in relation to the animal population of the country remains less than half of 1 per cent It is generally agreed that a vaccination programme against the disease in a country where it is endemic is unlikely to reduce the incidence much below this figure If, therefore, the disease were allowed to become endemic in Britain, and vaccination were decided on, the ultimate goal which could reasonably be achieved would be an incidence equivalent to that which now obtains At that point, a slaughter policy would undoubtedly be invoked to attempt to achieve eradication of the disease If the situation in the European countries is considered, it is seen that, in spite of an increasing extension of vaccination since the 1951–52 epidemic, the incidence of the disease, though greatly reduced, is still somewhat higher than in Britain Some countries are applying, and others considering, limited slaughter of infected animals as a further step towards eradicating the disease Outside Europe, in endemic areas, effective control of the disease is still some way off, and it is here perhaps that the new improved vaccines have the greatest part to play, if the cost of the vaccination campaign can be reduced and the efficiency of the vaccine increased Assistance in reduction of the incidence of the disease elsewhere than in Britain is at present the greatest contribution which can be made towards the control of the disease within the country

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* Research Institute (Animal Virus Diseases), Pirbright Report for the years 1956–1960 Pp 52 (Pirbright, Surrey Research Institute (Animal Virus Diseases), 1961) 2s. 6d

A considerable proportion of the research effort at Pirbright has always been directed at improved methods of immunization against the disease. The period under review has seen a new bias on work on immunization The development of live modified strain vaccines received a new impetus by the demonstration by Skinner in 1951 of the possibility of infecting unweaned mice and, in 1954, of passaging virus in day-old chicks and chick embryos Serial transfers, repeated sometimes many hundreds of times, have the effect of reducing the capacity of the virus to produce visible signs of the disease in the cattle inoculated By multiplying in the animal, however, the virus would stimulate the defence mechanism of the host and so produce a solid long-lasting immunity Such vaccines are used in many other diseases, for example, in rinderpest, rabies, African horse sickness, blue tongue, and in yellow fever and also in smallpox in man Workers developing such strains aim at the point at which the virus will 'infect' the animal but produce no clinical signs The yellow fever vaccine inoculated in man is an example of a live virus vaccine which has reached this point With most live vaccines, the ideal is not attained, and a compromise between slight infectivity in the sense of producing the clinical disease on one hand, and a capability which falls short of the maximum in producing immunity on the other, has to be accepted This has been true in developing the strains in foot-and-mouth disease and the problem has been made more difficult by the demonstration that not all strains become attenuated or lose virulence at the same rate On the other hand, there has been little indication of difficulty in some other ways, for example, there has been no restoration of virulence and no spread to normal animals in contact with those vaccinated Limited experiments on pregnant animals have indicated no unfortunate sequelæ, and young animals have been successfully vaccinated.

The variation in behaviour of viruses has made the development of suitable strains for each of the seven types of virus now known a complicated operation, especially when several different strains of one type have had to be developed to explore the possibilities of different methods of attenuation Nevertheless, strains of six of the seven types have reached the point at which tests for non-infectivity in cattle have been sufficiently encouraging to warrant investigations on the immunity produced These tests at Pirbright have now involved approximately 2,000 animals

The qualified successes obtained under experimental conditions at Pirbright led to the next phase in development of these strains Since the step from the laboratory to field conditions might not be without risk, it was considered that first trials outside the laboratory should be conducted in areas where the disease was endemic The Institute has had the willing and enthusiastic co-operation of the Governments of countries in South and East Africa in a series of trials These experiments have indicated the potential usefulness of suitable strains in such animal populations while, at the same time, bringing to light many of the suspected difficulties inseparable from immunization of a mixed population with possible previous experience of the disease Some of these experiments, notably those in South Africa, have involved tests of live attenuated vaccine on a scale which has not been undertaken with any other agent of this type, in respect of severe challenge with fully virulent material Even under these conditions, 70 per cent protection was obtained and subsequent use of the vaccine in an infected area resulted in an incidence of less than 1 per cent on vaccinated farms, while, on unvaccinated farms, the incidence was between 60 and 70 per cent These observations are essentially preliminary, many problems require investigation

The problem of the use of inactivated vaccines has not been neglected Frenkel-type vaccine in which virus is grown in culture of cattle tongue epithelium, adsorbed on aluminium hydroxide and inactivated with formalin and mild heat, has been a useful agent and will continue to play a most important part in the control of the disease as it has done in Europe and. to some extent, in South America Extensive tests of this type of vaccine have been carried out at Pirbright and several large batches have been prepared and tested for safety and potency Other possible sources of tissue in which virus may be grown, such as kidney, are being investigated, since kidneys present a more easily obtainable source of tissue than cattle tongue epithelium

Foot-and-mouth disease must be considered as a world problem Part of the work at Pirbright, therefore, has been directed towards the assessment of the world situation, particularly in regard to the prevalence of the disease and the distribution of the immunological types of virus Earlier reports have referred to the discovery at Pirbright of four new types—*S A T* 1, *S A T*. 2 and *S A T*. 3 from Africa, and Asia 1 in many countries in Asia In the past twelve years considerable progress has been made in determining the broad outlines of type distribution. The international importance of this work was recognized when, in 1958, the Institute was nominated by the Food and Agriculture Organization of the United Nations to undertake the functions of World Reference Laboratory for Foot-and-Mouth Disease

APPLIED GEOGRAPHY

THE application of geography to the study of a wide range of problems has made remarkable progress in recent years, partly during, and partly because of, the Second World War Geography made a substantial contribution to the British War effort both in the planning of campaigns and in the reorganization of the nation's economy, and since the War geographers have been active in the field of planning Some of the fruits of these efforts can now be seen in the increase in the number of geographers who act as advisers in various fields, ranging from problems of conservation to aspects of medical geography there is also the evidence of the results achieved by those who have been largely employed in the study of the application of geography in Britain to national, regional and county affairs during recent years

At the recent meeting of the British Association for the Advancement of Science a whole morning was devoted, largely by the geographers of Section E, to the planning problems of Britain and to those of East Anglia in particular The future administrative organization of Norfolk and Suffolk is at the moment under review by the Boundary Commission, and during the course of the meeting geographers saw, in the field, the significance of the desire of the rapidly growing and increasingly industrialized city of Norwich to expand into its adjoining county areas Others were shown the changing landscape of Thetford, where residential and industrial developments are designed to absorb part of the overspill of Greater London

Among the speakers in the planning symposium were Mr J R. James, deputy chief planner, Ministry of Housing and Local Government, and the principal research officer of the Ministry, Dr E C Willatts, who introduced a new map, at the scale of 10 miles to the inch, showing the major post-war developments in England and Wales that have resulted from, or are affecting, land planning[1] These developments, including the establishment of National Parks, the building of electrical and nuclear power stations, the construction of motorways, the creation of 'new towns' and the extension of other towns, are indicative of both the scale and scope of post-war changes They show, too, how the regional geography of Britain is in process of being transformed by the technological revolution which, in its geographical aspects, was the subject of Prof S. H. Beaver's presidential address to Section E [2]

In the siting of many of these new developments geographers have sometimes played a significant part An assessment of the scope of geographical inquiry and of the fields in which geographers are capable of making important contributions was made by Prof L. Dudley Stamp in a small book, published in 1960, *Applied Geography*[3] Such a book could have had no better author than Prof Stamp in view of his own great efforts in many of the fields of which he writes. these activities have included the Land Utilisation Survey of Britain, carried through under his direction during the 'thirties, a particularly difficult and trying period for British agriculture[4]; the investigations of the Scott Committee, of which he was vice-chairman, into the use of rural land in Great Britain[5], and his later work as an adviser to the Minister of Agriculture on these problems, his initiative in establishing an organization for a World Land Use Survey under the auspices of the International Geographical Union[6], and his work on the geographical background of many new developments oversea both within and outside the tropics

Some of Prof Stamp's topics have been further discussed by other geographers in the recently issued proceedings of the Anglo-Polish seminar held in Nieborów, Poland, in September 1959, and attended by twelve British and seventeen Polish geographers The volume, *Problems of Applied Geography*, has been published in Warsaw by the Institute of Geography of the Polish Academy of Sciences, which organized the seminar in collaboration with the Institute of British Geographers, with the generous support of Unesco and of the British Council limited supplies of the volume are available for sale in Britain[7] The seminar, like that organized a year later for the exchange of views between American and Polish geographers[8], included a series of papers—six British and five Polish—which served as a basis for discussions between the delegations and was followed by a comprehensive tour of both agricultural and industrial areas of Poland

While most of the six British papers have nothing strikingly original about them, they form a valuable and up-to-date summary of the special problems of Britain to-day as seen by a group of geographers As with the Polish delegation, the British team emphasized the achievements and experience of the theory and practice of planning in its own country, while suggesting that many of its conclusions are of use to all concerned in the application of geography to the study of physical, social and economic problems in contemporary society M J Wise's paper outlines the basic problems of national planning in Britain, including the optimum use of resources, the redevelopment of the older industrial areas, the restriction of the growth of conurbations, and the regulation of population movements, and A A L Caesar shows how these problems vary between regions. H C K Henderson describes the basis of the pre-war Land Utilisation Survey of Britain and indicates how its work has been used in the planning of agricultural land use S H Beaver describes the reclamation of industrial waste-land for agricultural and other purposes, illustrating his paper by a series of carefully chosen examples he shows that the area of land in Britain that cannot be reclaimed—less than 1,700 acres a year—is minute compared with the loss of agricultural land to urban development It is this continuing urban encroachment on the countryside that A. E. Smailes in his paper on perhaps the most distinctive feature of British economy and society—the urbanization of Britain—sees as the crux of the physical planning problem of Britain to-day Finally, K. C Edwards, who, as president of the Institute of British Geographers, was leader of the delegation, discusses the 'new towns' of Britain, the results of which, he finds, though "modest in scale and often despairingly slow in realization, at least encouraging"

The Polish contributions have special value because so little information is easily available about modern Poland, certainly in English They have been deliberately designed to match the British papers thus the importance in Poland of regional planning, land use and urban geography are discussed by K Dziewoński, J Kostrowicki and L Kosiński respectively The two other Polish essays are of a very different type, describing the preparation of a geomorphological and hydrographic map and an investigation of local climate their inclusion in the symposium suggests that there is in Poland a greater appreciation of the significance of such detailed and specialized studies in practical affairs than has so far been the case in Great Britain, where research of this nature would, to most administrators and planners, seem not only academic but even irrelevant

But what will strike readers of this volume most forcibly will be the fundamental contrasts between the British and Polish attitudes towards planning and between the nature and scope of the planning envisaged for the two countries In Britain the problems are those of adjustment and reconciliation rather than those of development—the transformation of the population pattern and economic organization developed in one industrial age to what is required in another, and greatly different, age of new and rapidly advancing technology Poland is concerned with the fundamental development problems that call for comprehensive and large-scale regional planning as a basis for the reconstruction and expansion of the national economy, and Dziewoński's paper shows that the methods and results of geographical research in Poland have exerted very considerable influence on the methods and results of planning For this there is not, and probably never will be, any exact British parallel, but Polish experience does heavily underline the diversity of the ways in which the work of geographers can be used for the nation's good, if they are given the opportunity and the encouragement

International seminars of this type broaden the minds of those participating and at the same time help to create deeper understanding and friendship, while the circulation of the report of the proceedings will be of lasting value to all who are interested in applied geography and who are concerned that it should have the closest possible connexion with life and society.

ROBERT W STEEL

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[1] The map prepared by the Ministry of Housing and Local Government is being published by H M Stationery Office

[2] "Technology and Geography", to appear in *The Advancement of Science*, December 1961, and summary in *Nature*, 191, 986 (1961)

[3] Stamp, L Dudley, *Applied Geography* (1960)

[4] See especially Stamp, L. Dudley, *The Land of Britain Its Use and Misuse* (1948)

[5] *Report of the Committee on Land Utilisation in Rural Areas* (Cmd 6378) (H M S O , London, 1942)

[6] Four of the World Land Use Survey's reports have so far been published, the most recent being Niddrie, D L , *Land Use and Population in Tobago* (Geographical Publications Bude, 1961)

[7] *Problems of Applied Geography*, Geographical Studies No 25, Polish Academy of Sciences, Institute of Geography (Warszawa, 1961) Pp 148, with photographs, maps and diagrams Copies (price 25*s*) can be obtained from the treasurer of the Institute of British Geographers, Dr H C K Henderson, Birkbeck College, Malet Street, London, W C 1

[8] See Hartshorne, R , "The American-Polish Geographical Seminar and Excursion, 1960", *Geog Rev* , 51, 443 (1961)

GAS CHROMATOGRAPHY IN MEDICAL RESEARCH

THE Gas Chromatography Discussion Group of the Institute of Petroleum held an informal symposium on September 26, at the Royal College of Surgeons of England in London

In welcoming the members of the Group, Prof R F Woolmer, on behalf of the President and Council, explained that, apart from surgery, the College research and teaching also covered such associated fields as physiology, biochemistry, dental science and anæsthetics It was appropriate that a symposium concerned mainly with the aspects of gas chromatography in medical research should be held at the College, as they believed in co-operation and a full exchange of ideas with other workers

In the opening paper, Dr A T James described the development and operation of a simple detector for the continuous automatic determination of the radioactivity of carbon-14 and tritium Attention had been directed to automatic determination because of the obvious disadvantages of the previously used discontinuous methods Problems of stability at column temperatures as high as 200° C had led to combustion of the eluted material to carbon dioxide and water, and the measurement of the radioactivity at room temperature The detector consisted of a $4 \times \frac{1}{2}$ in copper tube, plated internally and having a central tungsten wire electrode Rhodium plating reduced the tailing exhibited by tritiated materials Argon or helium was used as carrier gas, and carbon dioxide was bled into the feed to the detector to give a concentration of approximately 5 per cent Chromatograms obtained with conventional detectors and the radioactivity detector were shown to superimpose extremely well An integrated record could also be produced

The next paper, on gas-liquid radiochromatography, by Dr S Popják, confirmed the disadvantages of discontinuous procedures for radioactivity measurements The advantages of continuous monitoring over fraction collection had been evident over a period of three years using a scintillation counter In the method described, carrier gas and eluted fractions were passed through a liquid scintillator in a specially designed apparatus, and the radioactivity measured by scintillation counting Two isotopes could be measured simultaneously Full details of the construction of the apparatus, its method of operation and the associated electronic circuitry were given. A stepped record superimposed well on chromatograms obtained with a gas density balance The efficiency of counting was of the order of 40–50 per cent for carbon-14 compounds, and 15–21 per cent for tritiated compounds

Under the title of "Peak Symmetry in Gas-Solid Chromatography", Mr. C G Scott described recent work concerned with the properties of two active solid systems giving symmetrical elution peaks for hydrocarbons at column temperatures below the boiling point of the eluted component When alumina is modified with sodium hydroxide, the energy of adsorption decreases to a constant value, at which stage symmetrical elution peaks are obtained Such peaks can also be obtained from columns packed with solid organic crystals, although, to increase surface area and so reduce column lengths, the compounds are best deposited on 'Celite' It had been found that heats of adsorption were of the same numerical magnitude as the heats of vaporization Entropies of adsorption were calculated for *n*-heptane, benzene, iso-octane and *cyclo*hexane, and values obtained for the first two compared with those obtained by Kemball for adsorption on mercury

Mr D W Hill and Dr R P W. Scott described recent developments in the design of compact cathode-ray tube gas chromatographs Apart from the high speed of analysis for vapours containing anæsthetics, the apparatus should be compact for easy transport and use in an operating theatre, and be capable of giving rapid repetitive results The instrument was designed around a 6-in square, long-persistence tube having a 20–30 sec scan, and contained its own pump An additional port in the sample injection system allowed a 100 1 gas dilution without the high losses of argon given by 'splitter systems' The micro argon detector had been modified to reduce the effects of electron capture so that the strongly electron-capturing halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) gave a positive peak Column packings for the separation of typical anæsthetic mixtures were mentioned The quantitative response of the argon detector was dependent on temperature, and therefore the use of a thermostat or compensation was necessary A simple thermistor compensator unit was described

Dr R A Butler presented a paper entitled "Parts Per Million and All That" in which he first described his work on anæsthetics, indicating the importance of knowing the concentration of anæsthetics in tissue, the rate of its elimination from the tissue, and equilibrium values of the various substances used He queried the accuracy of calibration standards made from volatile substances, and consequently the accuracy of a detector calibration using such standards He felt that some absolute figures often quoted in papers were at the least rather optimistic, and his paper was obviously designed to stimulate a discussion on the quantitative aspects of gas chromatography It was unfortunate and disappointing that it failed to do so

A contribution by Dr T R Phillips described how, during the analysis of Arcton 12 (dichlorodifluoromethane) in Arcton 11 (monochlorotrifluoromethane), the calibration of the apparatus had to be carried out with synthetic mixtures of the substances and the samples taken at a fixed pressure The sensitivity of the apparatus for Arcton 12 expressed as peak-height/partial pressure was shown to vary with the total pressure of the sample and with the partial pressure of Arcton 11 It was thought that this effect could occur in other systems where a small amount of a weakly retained substance was being analysed in the presence of excess of a more strongly retained one Dr Phillips's suggestion that the effect would not be observed if integration of the peak were used instead of the measurement of peak height was confirmed by other contributors to the discussion

The concluding paper on electron capture was given by Mr N L Gregory He described a detector to which a potential of only 10–20 V was applied and which utilized recombination effects to measure compounds having an affinity for free electrons It was extremely sensitive to halogenated compounds, a typical application being to the detection and determination of insecticides in foodstuffs Another application was to the determination of tetraethyl lead in gasoline, there being no response to the hydrocarbons present Apart from halogenated compounds, polycyclic aromatic hydrocarbons, unsaturated fatty-acid esters and quinones were also good electron acceptors The possibility of the affinity of organic compounds for free electrons in biochemical processes was mentioned

Members of the Group were also privileged to view apparatus, watch demonstrations, and discuss gas chromatographic techniques with workers in various departments of the College, and also in the adjoining Nuffield College of Surgical Sciences This was much appreciated by those present.

C E H KNAPMAN

INDUSTRIAL TRAINING IN EUROPE

SPEAKERS from Belgium, Holland, Sweden and Switzerland joined colleagues from Britain at the annual conference of the British Association for Commercial and Industrial Education which was held at the University of Nottingham during September 14–16, when an examination was made of education and training on the continent of Europe, with particular reference to the effects of economic integration The theme of the conference is of special significance since free movement of labour among Common Market countries may lead to standardization of systems of education and training, or at least a levelling up of standards

One common thread ran through all the papers —the need to direct more attention to general education in modern schemes of training All the countries present are now beginning to examine their training systems from this point of view

Among the contributors were Monsieur R Grandbois, inspector-general of the French Ministry of Education, who stated that all differences concerning apprenticeships are a consequence of the past They are the result of local habits and customs ; they represent the embodiment of a way of life, the outcome of an evolution both social and administrative. All the similarities are either a consequence of the present or a result of the pressure of things to come. The various systems of apprenticeship have evolved slowly to their present-day form and crystallized behind innumerable frontiers and in confined surroundings, but they are now and will henceforth be subjected to influences for which barriers and remoteness do not exist It can be assumed that the apprentices of to-morrow will have to be educated in much the same way whatever the country to which they belong

Monsieur G C M Hardebeck, secretary of the Bemetal Foundation in Holland, believes that more attention must be directed to general education because it is our responsibility not only to give young people the necessary technical training but also to prepare them to live in a democracy

In his paper, Mr H A Warren, principal of the South-East London Technical College, explained how in Great Britain at a time in the 1930's, when thousands of man-hours were being idled away in unemployment, the young apprentice was asked to work 48 hours a week and in addition, three evenings at college and two more on homework A few million pounds extra expenditure on day release at that time would have meant that Britain to-day would no longer be suffering from a lack of skilled man-power Warren suggested that the wholly evening course, except for recreation or for advanced refresher course studies, is as out of date as the steam locomotive ; despite this, Britain has the administrative arrangements which, to meet the challenge of the changing nature of skill, stand out as far superior to anything in continental Europe "Nowhere in Western Europe is the sandwich course at the C A T [college of advanced technology] level and standard growing to full and recognized stature as it is in Britain"

The pattern of industrial education might have been greatly strengthened had the City and Guilds of London Institute been entrusted also with the General Certificate course at least up to the Ordinary Level of the General Certificate of Education

The British system is weak because of its lack of incentive Since no end-of-apprenticeship tests are insisted on, the studies for apprentices in a technical college are voluntary both in the sense of attendance, and in the sense of acquiring a qualification When, in addition, little recognition will accrue in wages or promotion, the student not unreasonably asks himself why he is pursuing his studies

Prof G Dunand of the International Labour Organization showed how economic mutual penetration has been practised in Europe on a minor scale for centuries For such exchanges it was accepted and convenient to use mainly one language, Latin, and later French, and more recently English, and also German in Eastern Europe The universal use of French or English for European relations has, however, been increasingly questioned since language has become an essential element of conscious nationhood and indeed nationalism Nowadays, supremacy would not readily be granted to the language of any one of the great powers, as, for example, English or Russian. Hence the multiplication, in particular since 1919, of bilingual treaties and multi-lingual international conferences, which have given birth to the profession of conference interpreter and to an extraordinary development of translation for international relations

From this historical review, Dr O G. Pickard, principal of the Ealing Technical College and School of Art posed the question. "Do we then ask the schools to devote less attention to French and take up other languages in its place ?" This has been tried, during the interim period of the two World Wars there was a determined attempt to introduce a great deal of Spanish to English schools for commercial reasons in place of French The immediate result of this attempt was to antagonize the teachers of French all over Britain, who clearly saw their vested interest in the teaching of this language threatened by the proposals to introduce Spanish in its place

So far as the schools are concerned, the question is "whether a third foreign language should find a place in the school curriculum so that it might be possible for school children to learn say, French, German and Russian, or French, Spanish and Swedish, as a normal practice during their school careers With the growing close association between the economy of Britain and that of Western Europe, it may be necessary to do much more language teaching in schools than has been the practice hitherto [in Britain] This in turn may lead to a lengthening of the secondary school course as happened on the Continent where it is unusual for the secondary school course for the grammar school stream to finish before 19 or 20 years of age". Neither the schools nor the Ministry of Education have taken kindly to this particular view The place for teaching the less common languages may be in further education

Dr M B Brodie of the Administrative Staff College, Henley, and Dr. Walter Goldberg of the Gothenburg School of Business Administration, Sweden, were both concerned with management development Brodie stated that businesses which expand and grow more complex in character require relatively more managers While quantitative estimates must be cautious, since they are likely to transpose present-day notions and assumptions too readily to the future, one estimate which attempts to allow for qualitative changes suggests an increase of about 50 per cent in the number of executives needed over the next ten years

Throughout Europe, the shortage of staff of calibre for teaching and for research in managment is widespread and serious Weakness on the staff side cannot but jeopardize the whole quality and character of any programme "The limitations of educational ventures run with second-rate staff are too manifold to need enumerating "

Dr Goldberg gave details of important Swedish developments in the field of management education. In 1957, for example, an investigation showed that the relative number of academically trained top-level managers since 1944 had increased from 58 5 to 84 5 per cent in industry, and from 43 to 54 5 per cent in other enterprises Sweden proposes to increase its intake of students in business schools to 700 in 1970—more than double the 1955 figures of 325

All the papers have been brought together in a publication, *Continental Comparisons*, which can be obtained from the British Association for Commercial and Industrial Education, Publications Department, 26*a* Buckingham Palace Road, London. S W 1, price 10*s*

PERUVIAN ORCHIDS

THE appearance of Part 4 of Charles Schweinfurth's *Orchids of Peru** marks the completion of a task which has necessitated continuous and concentrated research over a period of many years At last we now have a comprehensive modern account of the orchids of an Andine country, a region which is not only one of the richest in orchids in the world, but which is the original home of the parents of many of our finest cultivated orchid hybrids This excellent account will not only be of service to students or visitors wishing to identify Peruvian orchids but will also facilitate the study of orchids from the neighbouring Andine countries of Bolivia, Ecuador and Colombia which have many species in common with Peru

This final part contains among others the important genera *Odontoglossum*, *Miltonia* and *Oncidium* (including *Cyrtochilum*), the last-named being represented by more than 70 species Like the three preceding parts, Part 4 is well illustrated, containing thirty-three charming line drawings and one half-tone plate of representatives of all the more important genera, thus bringing the number of illustrations in the whole work to 194 Of special interest are the three pictures of the remarkable alpine genus *Telipogon* most members of which are found only above 2,000 m altitude

Closer examination of the text in all these parts shows, however, how much more collecting is necessary in Peru before a really satisfactory final account of its orchid flora can be prepared The author has in many cases not seen any specimens of the species dealt with, having had to be satisfied with descriptions or photographs of the original specimens scattered in various herbaria Here then is an evident field for future work, since it cannot be doubted that with really good field collections available many of the outstanding problems could be solved and a more complete account given even of those species which are comparatively well known V S SUMMERHAYES

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* Chicago Natural History Museum *Fieldiana Botany* Vol 30, No 4 *Orchids of Peru* By Charles Schweinfurth Pp v+787-1005 (Chicago, Ill Chicago Natural History Museum, 1961) 4 50 dollars

AGE OF THE BASALT FLOW AT OLDUVAI, EAST AFRICA

By PROF G. H. R. von KOENIGSWALD
Geological Institute, State University of Utrecht
AND
PROF W GENTNER and H. J. LIPPOLT
Max-Planck-Institut fur Kernphysik, Heidelberg

THE site of Olduvai in Tanganyika, East Africa, is known all over the world because of its remains of early man, the sequence of human industries and rich associated fauna, and we are all greatly impressed by the research, performed under difficult conditions, by Dr and Mrs Leakey Four different beds have been distinguished Bed I is underlain by a massive flow of basalt, and it should be possible to obtain here an age determination by means of the potassium–argon method We are greatly indebted to Mr S E Ellis of the Mineralogical Department of the British Museum (Natural History), who kindly gave us a rock specimen for that purpose (B M 1947/211· collected by K P. Oakley) This sample is called here *O* I As many implements at Olduvai are executed in basalt, we used two pebble tools from Bed I (collected by v Koenigswald, 1951), in order to try to get information for Bed I , these are the samples *O* II and *O* III

Table 1 POTASSIUM–ARGON AGES OF BASALT SAMPLES FROM OLDUVAI
$\lambda_{\beta} = 4·74 \times 10^{-10}\ a^{-1}$, $\lambda_{k} = 0·58 \times 10^{-10}\ a^{-1}$

Sample	Weight (gm)	A^{r}_{40} (10^{-7} c c /gm)	A^{at}_{40} (per cent)	K (per cent)	Age with potassium–argon (10^{6} *a*)
O I	0·52	0·58	88·6	1·13	1·3 ± 0·1
	0·51	0·60	87·9		
	0·51	0·57	90·6		
O II	0·51	1·91	70·2	2·17	2·25 ± 0·16
	0·71	1·93	69·6		
	0·51	1·94	72·4		
O III	0·52	0·93	89·8	1·67	1·4 ± 0·1
	0·60	0·86	89·3		
	0·61	0·96	88·0		
	0·69	0·92	87·9		

If we try to establish the age of Bed I with the help of the fauna, we find that *Elephas planifrons* and *Stegodon*, typical for the Villafranchian of Africa, are missing. There are many archaic types still present such as *Dinotherium* and *Mastodon*, but the dominant species among the elephants is *Elephas recki*, representing the same evolutionary stage as *Elephas antiquus* in Europe Beds I and II have many species in common which do not occur higher up According to Hopwood in ref 1· "There is no faunistic evidence to suggest that the lower part of the Olduvai series is of Lower Pleistocene age". Leakey expressed the opinion that Olduvai "extends from the beginning of the Mindel to the very end of the Riss glaciation" (ref 1, p 160) After the discovery of *Zinjanthropus*, however, Leakey included Bed I in the Lower Pleistocene, the Villafranchian Considering the various views expressed without further discussion we expect that Bed I has an age of 0·4–0·8 m yr (ref 2) and 0·1–0·8 m yr (ref 3) respectively

The basalt bed, however, has a considerably higher age determined by using potassium–argon. The analysis has been carried out on stony material reduced to small pieces. The average diameter of the fragments of the rocks was about 0·8 mm , which guarantees sufficient homogeneity

The basalt below Bed I has an age (determined using potassium–argon) of about 1·3 m yr The pebble tools from Bed I are not younger but older, and therefore not suitable for our purpose The age of *O* III, however, is only a little higher than that for *O* I and might be regarded as confirming the figures for *O* I

We see that the age determined with potassium–argon is about 0·5 m yr higher than we had expected them to be There are three possibilities for this (*a*) The age determined with potassium–argon is too high, because the amount of argon-40 (rad) is accumulated by inherited argon This cause can only be excluded by further work on distant samples (*b*) The sedimentation at Olduvai did not start directly after the formation of the basalt flow but considerably later (*c*) Bed I has an age of about 1·0 m yr and the former estimates were not correct

It is not easy to say which of the three alternatives may give us the right solution Adopting the last suggestion we would be forced to revise considerably our ideas about the duration of the Pleistocene, which might be necessary, but should only be done if more age determinations of Pliocene and Villafranchian rocks are available Therefore, it seems likely to us for the time being that a time-interval does exist between the basalt and the formation of Bed I

Just after Gentner and Lippolt had finished their report on these measurements, an article was published by Leakey *et al* [4] on the age of Bed I at Olduvai Their figures, based on biotite, oligoclase and anorthoclase crystals from various tuffaceous intercalation are higher (1·6–1·9 m yr) than ours, though their samples are geologically younger We thus wonder where this discrepancy comes from

We can exclude that this is conditioned by the technique of measuring, since we are in good agreement with the age determination of a late Trinil basalt from Java measured by Evernden *et al.* Both laboratories obtained an age of 0·5 m yr for this sample, which may be used to date the *Pithecanthropus erectus*

Thus the difficulty comes from the sample itself In connexion with another problem, we investigated different Pleistocene samples (biotite and sanidine) originating from tuffs and found that their ages, determined with potassium–argon, do not necessarily have to agree with the geological sequence, but may be too high because of inherited argon There is every appearance that this error may be present just in tuff minerals of low ages

Furthermore, we have some sedimentological and palæontological questions concerning an extended time-scale

For the top of Bed I the average age is given as 1·23 m yr , for the lower part as 1·75 m yr Consequently, the total of Bed I should have been formed during a period of (at least) 0·50 m.yr. One might expect that such a long period should be subdivided by fauna, sedimentation, dry and wet periods, industry; nothing of that kind has been observed Bed I is about 30 m thick, which would mean an average rate of sedimentation as low as 0 06 mm per year Where are the sediments to support this ? By such a slow rate of sedimentation every bone would completely disintegrate before it would have a chance to survive as a fossil, but Bed I contains a rich fauna. Certain places might have been temporarily dry, to allow occupation sites, but no soil horizons have been formed or marked disconformities Does not the preservation of the bones and the general unity of fauna and industry through the whole of Bed I give the impression of a 'normal' sedimentation ?

Perhaps there is a marked time-interval between Bed I and Bed II If we look at this new time scale, the top of Bed I is estimated to be 1 23 m yr old According to the same authors a post-Chellean II tuff in Bed II has yielded a date of 0 36 m yr If we estimate the bottom of Bed II to be 0 50 m yr. old, the supported interval would last nearly 0 75 m yr Here again we might ask why the faunæ of Beds I and II are roughly the same, having in common even archaic types of elephants, and why both beds, possessing the same grey colour, seem to rest conformably on each other Where are the soils or zones of weathering which would certainly have been formed during this considerable interval of time and should separate the beds ?

As the lower part of Bed I is said to have an age of 1 75 m yr the whole Pleistocene Period would have lasted well over 2 00 m yr This is more than double of what we, cautiously and with good reasons, have estimated

Having considered the many unanswered sedimentological and palæontological questions which make Olduvai a difficult test case, we prefer, as stated here to be cautious until more dates for the lower Pleistocene are available

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THROMBOCYTOSIS EVOKED BY EXERCISE

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AND

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THE mechanism(s) responsible for the rapid, over-all increase in hæmostatic activity in association with physical, emotional and other stress situations[1–4] has not been ascertained[4] Admittedly, the plasma fibrinogen content has been said to be elevated in such circumstances[4], but judging from Selye's survey[2] this response generally takes several days to reach maximum This is in accordance with later work by Rizza[5], who found that acute exercise in human subjects does not raise the plasma-levels of fibrinogen, prothrombin, Christmas factor or Factor V However, in the two normal subjects examined, Rizza observed some increase in the activity of the antihæmophilic globulin and was inclined to believe that the hypercoagulability associated with exercise might possibly be traced to this change Similar increase in the activity of the antihæmophilic globulin has been found after adrenaline infusions[6] On the other hand, it has been mentioned that the platelet count is also increased in stressful situations[4], but according to the evidence given by Selye[2] this response is not wholly consistent While moderate, short-term exercise has been found to raise the platelet count[7,8], it appears that following prolonged[9 10] as well as brief, strenuous exercise[11] no appreciable changes occur in the platelet count Gerheim and Miller[11] expressed the opinion that "the greatly increased circulation velocity during exercise may destroy enough platelets to mask a moderate increase in numbers"

It seems possible, then, that the hypercoagulability associated with exercise and related conditions is ascribable to thrombocytosis rather than to any increase in the levels of the coagulation factors in the plasma At all events, however, the question remains what is the ultimate mechanism of the thrombocytosis that seems to prevail in stressful situations It has been conjectured that the leucocytosis known to accompany exercise and related conditions is effected mainly by the concomitant increase in circulation[12–14] Conversely, the reversible hypothermic leucopænia (associated with thrombocytopænia and bleeding tendencies) has been traced to the reversibly retarded circulation inherent in hypothermia[15] To our knowledge, nevertheless, no attempts have been made to substantiate or quantitate the possible role of the hæmodynamic changes in the establishment of these corpuscular blood changes. Hence, we have examined the relevant hæmatological changes in human subjects undergoing graded muscular exercise as well as the relation of the changes to those in cardiac output and allied parameters

The counts of erythrocytes, leucocytes and thrombocytes (Fonio's method, counted per 2,000 red cells), including the hæmatocrit readings, were determined from the capillary blood and, in a part of the tests, also from the venous blood For work-loading, a bicycle ergometer and marching were employed. The cardiac output was determined by using the dye-dilution technique, and from the dilution curves mean circulation time was reckoned From the circulation time, again, 'relative circulation velocity' was derived (inverse value of the circulation time)

The results obtained, with special reference to the platelet and circulatory changes, may be outlined as follows

In the eight subjects studied, light muscular exercise lasting for some 10 min and capable of raising the cardiac output by 50–200 per cent resulted in an appreciable and consistent rise of the platelet count in the capillary blood. The figures obtained before and immediately after the exercise were 123,000–243,000 per mm ^{3} and 168,000–270,000 per mm ^{3}, respectively The percentage increments were 9-37, and the greatest numerical increment recorded amounted to 58,000 per mm ^{3} The platelet count in the venous blood showed a comparable increment, even if the figures were somewhat higher throughout The 'relative circulation velocity', derived from the mean circulation time, was in all cases increased in direct proportion to the increments in the cardiac output. Of special interest is the observation that the increments in the platelet count and in these two circulatory parameters were fairly commensurate.

More strenuous exercise of about 18 min. duration (testing the physical working capacity by Sjöstrand's method) in the same subjects yielded a more pronounced increase of the platelet count in the capillary blood, the resting values were 138,000–291,000 per mm.^{3}, whereas after the work-load was imposed the figures were 210,000–348,000 per mm.^{3} The highest numerical increment was 107,000 per mm.^{3}, and the percentage increments varied from 17 to 60. The recovery studies performed in this group of tests revealed that after 15 min. rest the platelet count had actually returned to the pre-exercise level (113,000–261,000 per mm.^{3}).

Ten miles marching within some 90 min. resulted in considerably different changes. In the ten subjects studied there were no marked alterations in the platelet count in the capillary blood, in eight individuals the platelet count showed increments of 2–22 per cent, whereas in two instances the count tended to decrease (4 and 8 per cent). After a 90-min. recovery period the platelet count remained substantially unaltered in most instances. Yet, in three cases it was found to be increased no matter what the response to the work-load was.

In this connexion it may be mentioned that the changes in the leucocyte count after these different work-loads and recovery periods were the same in direction as, but usually relatively greater than, those in the platelet count. There was a good correlation between the increments in the leucocyte count and those in the changes in circulation examined. The hæmatocrit values usually rose but only slightly, the greatest increase encountered was 5.5 vol. per cent. The small degree of hæmoconcentration indicates that the increased counts of platelets and leucocytes cannot be accounted for by the concurrent hæmoconcentration.

These preliminary results suggested the tentative conclusion that the leucocytosis and even thrombocytosis that accompany at least light and moderate short-term exercise are actually effected by the concomitantly accelerated circulation. The source of the leucocytes and platelets which appear to be released into the circulation may be the pulmonary vascular bed, particularly its venous side, which is known to remove and discharge leucocytes[13,16] and platelets[13] on changing circulatory conditions. But also, similar release may occur in the peripheral vascular compartments in general, as suggested by the biomicroscopic observations on the microcirculation in hypothermic subjects (cf. Sarajas[15]). Moreover, since the work of Eberth and Schimmelbusch, it is known that on acceleration of circulation leucocytes and platelets enter into the axial flow in increasing numbers (cf. Koller[17]). Especially during prolonged and brief, strenuous exercise other factors, such as an altered fragility and adhesiveness of these cellular blood elements, may come into play, and this would explain the slight and inconsistent leucocyte and platelet changes encountered in our marching tests.

The clear-cut and consistent rise in the platelet count, as observed in the present tests with light and moderate exercise of short duration, makes it evident that the hypercoagulability in association with exercise[1-3] is possibly related to a thrombocytosis and that this thrombocytosis is occasioned by the concomitant circulatory activation. The same mechanisms may be responsible also for the hypercoagulability said to accompany other stressful situations[2-4].

Finally, the question arises whether the thrombocytosis and hypercoagulability of this origin operates particularly in the establishment of coronary thrombosis, which is known to be precipitated by states of emotional and physical stress.

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MODIFIED CUVETTES FOR PHOTOELECTRIC SPECTROPHOTOMETRY

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WITH the development of photoelectric spectroscopy to the point where even colorimetric techniques come closer and closer to being true spectrophotometry, the conventional vessel in which most solution measurements are made, and to which most optical coefficients are referred, has become the optical cuvette with 10 mm. light path. It is the purpose of this article to report a new approach to the problem of making spectral observations over an extended wave-length range, with convenience and accuracy, using modified cuvettes of this type. The discussion is centred around the use of hæmoprotein solutions, for the analyses of which this technique was evolved. It is stressed, however, that the principle is of the widest application and may be used over the whole of the spectrum wherever solutions are to be examined, whenever the solution would normally have to be diluted at some stage of the spectral examination.

It is often possible to arrange to dilute a solution so that it gives a conveniently measurable optical density over the whole of the wave-length range to be studied. There are occasions when this is not feasible, however. For example, many hæmoproteins (other than cytochromes) have relative extinctions at the ultra-violet, near ultra-violet, visible, and near infra-

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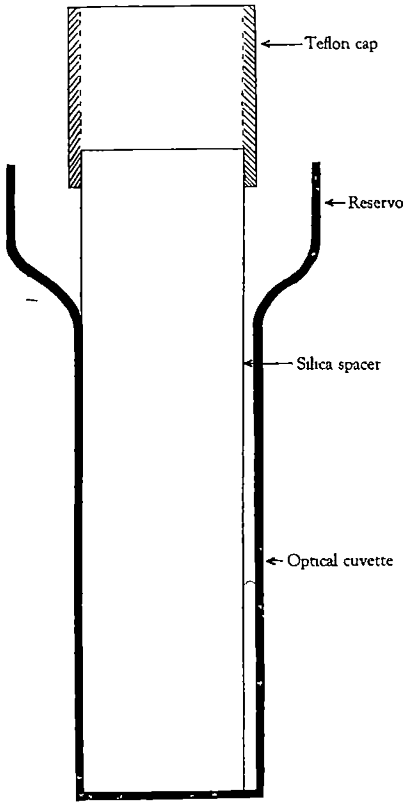

Fig 1 General purpose cell Optical path in plane of paper

red maxima of roughly 30, 100, 10 and 1, a range which is too great for the spectrophotometer to cover with accuracy, and certainly not with suitable clarity in the overall spectrum The desired result can only be obtained by starting with a solution which is of sufficient strength to resolve adequately the lowest peaks of the spectrum, and then reducing the optical density in order to resolve peaks which would otherwise be too intense This may be done in only one of two ways by diluting the solution (assuming that it still obeys Beer's law), or by reducing the optical path of the original solution.

Dilution, as a routine answer, may be criticized on numerous grounds Beer's law may not be obeyed by the solution; the process of dilution is time-consuming and inconvenient, mere dilution may upset equilibria in the experimental solution so that a special dilution medium may have to be prepared, dilution may in itself be undesirable where only small volumes of material are available to start with There remains the possibility of a mechanical reduction of the optical path of the starting solution Three methods are available

(1) *The use of an adjustable cell* These are expensive, need relatively large volumes of solution as compared with the standard cuvette, and must be provided with a reservoir to take the overflow when the path-length is reduced In addition, they are difficult to rinse adequately, and once rinsed they are difficult to dry adequately

(2) *The use of a second cell with a shorter path-length, for example, a 1-mm cell* This is certainly much more satisfactory than (1) There are still disadvantages, however In the first place, with cells as narrow as 1 mm, capillary effects make emptying and filling the cell rather a problem This means using a hypodermic syringe to get the solution in and out of the cell This is reasonably satisfactory, but the very fact that a second cell is employed more than doubles the actual manual operations involved

(3) *The use of a block of suitable material, for example, silica, inserted into the cell* In this case, the cell has to be partly emptied before inserting the spacer or else there is an overflow Furthermore, the amount of fluid to remain is rather critical, both to avoid overflow and to provide sufficient depth of fluid to cover the light beam

It might seem that, as between (2) and (3), (2) is the more satisfactory Nevertheless, it was felt that in principle (3) was the right approach, and steps were therefore taken to remove the disadvantages mentioned above The devices which evolved enable rapid and convenient scanning of extended wavelength ranges without the necessity of diluting the solutions, or even of removing them from the cuvette The descriptions are of actual models which have been in use for more than a year Possible modifications suggested by this experience are noted where appropriate

(a) General Purpose Cell

This is the standard cuvette modified to permit the rapid insertion of a spacer without overflow, and is made by fusing a reservoir on to the end of the cuvette, as shown in Fig 1. The exact dimensions of the reservoir may vary with the design of the spectrophotometer, and any optically suitable material may be employed for the cell as a whole, namely, 'Pyrex', silica, 'Perspex', etc The prototype, intended for use with the Beckman *DK*-1 recording spectro-

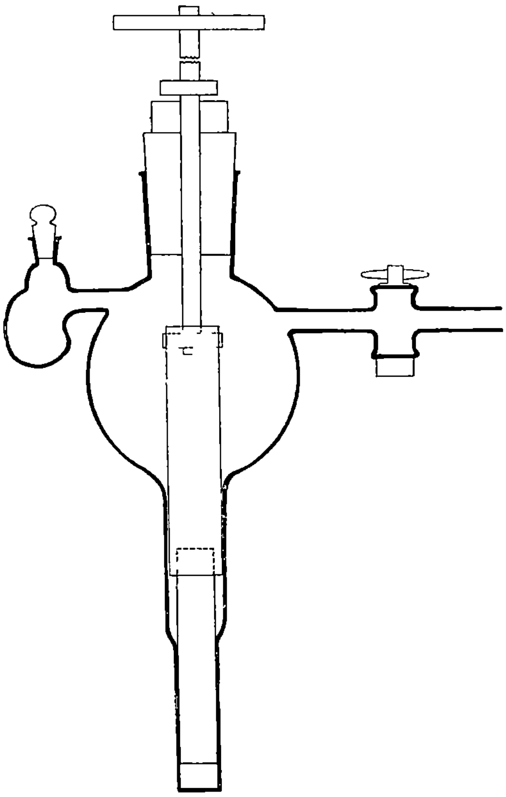

Fig 2 Gas-tight cell Optical path at right angles to plane of paper

photometer, was of 'Pyrex' glass and had a reservoir which was squared-off parallel to the sides of the cuvette The reservoir would take up about 2 ml of fluid A later model of silica has a reservoir of greater cross-sectional area which will take up a full 4 ml For these cells a silica spacer of the conventional type, that is, a block of silica 9 × 9 × 45 mm, was adapted by adding a cap of 'Teflon' to provide a handle for withdrawing the spacer-though, again, any optically suitable material may be utilized This was done by making a tight push-fit with the spacer into a piece of 'Teflon' tubing of the appropriate internal diameter (It may be noted in passing that the complete unit is very similar in principle and in form to the cups used in colorimeters of the Klett type)

(b) A Gas-tight Cell

The idea of attaching an optical cuvette to a gas reservoir, or tonometer, for observing reactions with gas mixtures goes back at least to Conant's work[1] in the late 1920's Since then, the use of a Thunberg tube with a sealed-on cuvette has become standard, since this enables further reagents to be added during the course of the experiment if necessary. The most sophisticated designs of this type appear to be those of Keilin and Wang[2] and of Rossi-Fanelli and Antonini[3], which incorporate a mobile spacer The spacer, however, is not readily controllable from the outside, particularly once the unit is *in situ* in the spectrophotometer The device described here incorporates a spacer which may be raised or lowered at will from the outside It consists (Fig 2) of a standard 'Pyrex' cuvette to which is sealed a glass bulb of about 70 ml internal volume A side-arm bulb of about 2 ml volume is provided for adding further reagents, and a two-way 'Teflon' tap controls filling and evacuation of the whole unit A standard spacer of the type mentioned above was pushed into a length of 'Teflon' tubing suspended from a cross-piece at the bottom of a stainless steel shaft. The 'Teflon' was free to swing on the cross-piece and this provided the slight amount of play necessary to ensure that the spacer never became jammed in the cuvette through error in the alignment of the spacer assembly The stainless steel shaft passed through an 'Asco' gland (A F Smith Co, Rochester, N Y) fitting into a standard ground glass joint at the top of the reservoir The 'Asco' gland is a 'Teflon' stopper which forms a gas-tight seal, outside and inside, by means of rubber O-rings The inner O-ring may be tightened against the shaft by means of an adjusting device The pressure on this O-ring was so arranged that the shaft could be raised or lowered easily, and yet would not move from the set position even with an internal vacuum

If only the vacuum from a water pump is employed, the 'Teflon' stopcock and the rubber O-rings will maintain the desired gas pressure for a considerable time Hence, one can avoid the use of sealing grease, thereby eliminating possible contamination of the solution, and making for quicker and easier cleaning In principle, one should be able to use the cell at much lower pressures, at least down to 0 1 mm. of mercury, but it does not seem possible to quote a lower limit at present owing to individual variations among 'Teflon' stopcocks

With the complete cell one can, for example, determine the spectrum of oxyhæmoglobin from 350 to 700 mμ in one operation, followed immediately by the spectrum of deoxygenated hæmoglobin over the same range, produced by adding a little sodium dithionite from the side-arm Both spectra can be

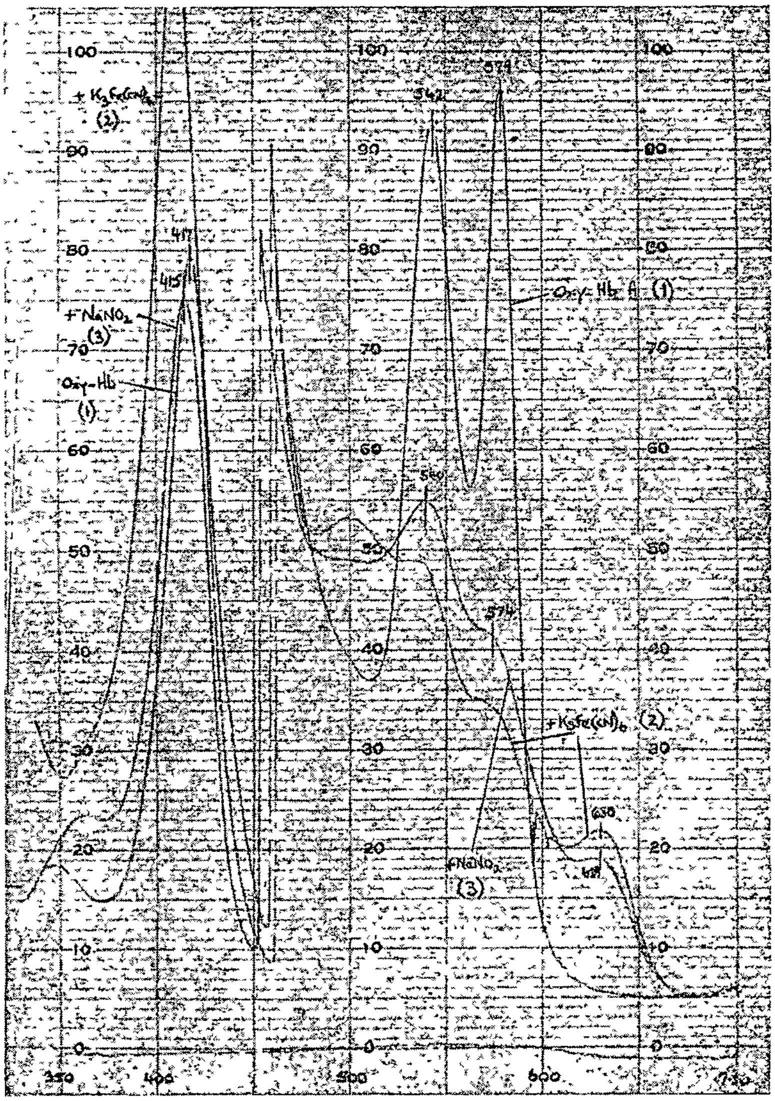

Fig 3 Traces obtained using the general purpose cell in conjunction with a recording spectrophotometer Spacer inserted at about 460 mμ in each case Ordinates optical density Abscissæ wave-length in mμ (1) Human oxyhæmoglobin, (2) acid methæmoglobin from (1) + solid potassium ferricyanide, (3) nitrite methæmoglobin from (2) + solid sodium nitrite

taken under nitrogen, so reducing the liability of oxidation, and this can be replaced by carbon monoxide to give the carbon monoxide–hæmoglobin spectrum without disturbing the solution. The same sequence of operations would be considerably longer and more cumbersome to carry out by the usual procedures, and there would be much greater risk of artefacts due to the presence of oxygen and the mechanical agitation involved.

Practical Points

With both types of cuvette, the use of standard spacers, rather than spacers designed specifically for the purpose, meant that the rise and fall of the spacer had to be carefully checked to ensure that it would enter and leave the light beam completely. This was done by moving the spacer slowly into the light beam and observing the resultant optical density changes. The limits of the beam can be very accurately noted in this way, and were recorded for further work by marking the cuvettes themselves. Also as a result of using standard spacers, in both cells the spacer would no longer go to the bottom of the cuvette and a minimum volume of 2·5 ml. of solution was required. Ideally, one would use longer than normal spacers, which would obviate the need for an added 'handle', and would make possible the use of the cells with very small volumes, that is, a minimum of about 1 ml. of fluid if the cells were raised relative to the light beam.

Fig. 3 shows an actual unretouched trace which demonstrates the capabilities of this technique when used in conjunction with a recording spectrophotometer. Three successive spectra were recorded: oxyhæmoglobin, methæmoglobin formed by the addition of a little solid potassium ferricyanide, and nitrite methæmoglobin formed by the further addition of a little solid sodium nitrite. The traces were recorded using the standard cuvette in a Beckman *DK*-1 recording spectrophotometer. At the point where the optical density was rising sharply in the region of 460 mμ the cell compartment cover was quickly removed and the spacer inserted, the cover then being replaced. The very smooth transition, and the ease with which the complete spectrum may be visualized, should be especially noted. Similar results were obtained with the gas-tight cell, save that it was unnecessary to move the cell compartment cover. The exact procedure to be adopted when inserting the spacer will depend on the instrument being used, and its position in relation to outside light sources. With the *DK*-1, as used in this work, it was possible to have the compartment cover partly open the whole time without being able to detect stray light in the machine. In less-favourable situations, the cell could easily be handled through a black cloth, or placed under a light-tight box standing over the cell compartment.

Both cells have been in fairly continual use for about a year without showing any signs of scratching to either cuvettes or spacers. Neither has there been the slightest difficulty with bubbles adhering to the optical faces. In the original gas-tight cell, the 'Asco' gland used was a size 24/40 with 6-mm. bore and standard taper, the smallest then available. A 10/30 gland with standard taper and 6-mm. bore is now available, and this is a most convenient size. Apart from this, two immediate modifications, in principle rather than design, have been suggested in use. The primary one is that the useful range of the cells would be enormously extended in both the ultra-violet and infra-red regions if silica cuvettes were employed (in conjunction with silica spacers). There is no serious difficulty in doing this, and a standard general purpose cell has already been constructed in this material. Secondly, the usefulness of the cells could be further extended by the provision of spacers with 9 mm. optical path in one direction, and 8 mm. optical path at right angles. In the case of the hæmoproteins mentioned here, the 8-mm. spacing would enable one to examine the protein band around 280 mμ at a more suitable optical density in a continuous scan from 700 mμ down to 220 mμ.

The devices described here were developed and constructed during tenure of National Science Foundation grant *G* 7657 and U.S. Public Health Service grant *A* 3187 (*C*3), and it is a particular pleasure to acknowledge the interest and encouragement of Prof. Philip George.

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EVIDENCE FOR THE PHOTO-ASSOCIATION OF AROMATIC HYDROCARBONS IN FLUID MEDIA*

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THE pattern of singlet and triplet π-electron energy stages of an aromatic molecule A ($^1\Sigma$) shown in Fig. 1*b* closely follows that for an atom with ground state ^1S. A logical development of this pattern should lead us to expect the existence of an electronically excited dimeric state $A_2{}^*$ in fluid media (Fig. 1*b*), corresponding to the stable excited diatomic molecule formed collisionally from excited and unexcited atoms of an inert gas[1], for example

$$He^* + He \rightarrow He_2{}^* \rightarrow He + He + h\nu \text{ (continuum)}$$

(the asterisk denotes electronic excitation). Moreover, the radiative relaxation of the photo-associated species $A_2{}^*$ to a dissociated ground state (process 4 of Fig. 1), like that of its diatomic counterpart, should be accompanied by a structureless emission at longer wave-lengths than the corresponding molecular fluorescence, whereas the reverse process of absorption will be limited by the extent to which association occurs in the ground state.

Such an emission was first reported by Förster and Kasper[2] as a broad, blue, structureless band, which replaces the violet, structured fluorescence of dissolved pyrene as its concentration is increased. No cryo-

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* Presented at the Fifth European Symposium on Molecular Spectroscopy, Amsterdam, May 1961.

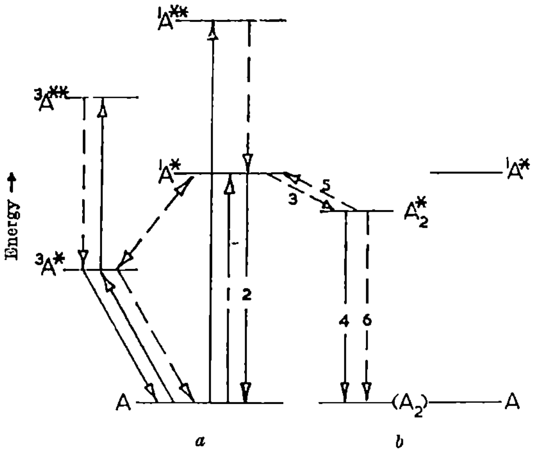

Fig 1 Electronic energy-levels of aromatic hydrocarbon A in monomeric (a) and dimeric (b) forms Radiative and non-radiative transitions are indicated by solid and dashed lines, respectively

scopic evidence was obtained for the association of unexcited molecules even in the most concentrated solutions and the molecular absorption spectrum remains unchanged over the range of concentrations investigated Since the absorbing species is monomeric the operative processes may be represented by the scheme

$$\begin{array}{l} A + h\nu \xrightarrow{1} A^* + A \xrightarrow{3} A_2^* \xrightarrow{4} (A_2) + h\nu\ \text{(blue)} \\ \qquad\qquad \downarrow 2 \qquad\qquad\qquad\qquad\qquad \text{(structureless)} \\ \qquad\qquad A + h\nu\ \text{(violet)} \\ \qquad\qquad \text{(structured)} \end{array}$$

where the asterisk denotes an electronically excited state and the processes are numbered according to Fig 1 The rate constant[3] for process 4 is ~ 10^6 sec $^{-1}$ although a small component of the blue emission with a lifetime of ~ 10^{-3} sec has been observed in degassed solutions[4] 3,4-Benzpyrene[5] and a few other pyrene derivatives[2] show a similar effect to a smaller extent

If the absence of dimer emission in other aromatic systems is due to the relatively high probability of competing processes, the evidence for photo-association must be based on these processes which include

Table 1 EVIDENCE FOR EXCIMER FORMATION IN AROMATIC HYDROCARBONS

Molecule	Emission (process 4)	Delayed fluorescence (process 5)	Self-quenching (process 6)	Sensitized delayed fluorescence (process 7)
Benzene	No (ref 2)		Yes (ref 10)	
Naphthalene			Yes (ref 10)	
Anthracene	No (ref 2)	Yes (refs 7, 8)	Yes (refs 10, 15)	
Phenanthrene		No (ref 14)		Yes (ref 14)
Fluorene		Yes (ref 7)		
Carbazole		Yes (ref 7)		
Naphthacene		No (ref 8)	Yes (ref 11)	
Pyrene	Yes (ref 2)	Yes (ref 6)		Yes (ref 8)
3,4-Benzpyrene	Yes (ref 5)			
Perylene		Yes (ref 6)	Yes (ref 15)	
1,12-Benzperylene		Yes (ref 8)		
Coronene			Yes (refs 12,15)	
Anthanthrene		Yes (ref 8)		
9,10-Diphenylanthracene	No (ref 8)	No (ref 8)	No (refs 13, 15)	

(a) dissociation (process 5) proposed[6] to account for delayed fluorescence observed in the vapours of a number of aromatic compounds recorded in Table 1

$$A_2^* \rightarrow A^* + A \qquad (5)$$

In each case the spectrum of delayed fluorescence is identical with that of the molecular fluorescence originating from the lowest excited molecular singlet state A *, but the lifetime is about 10^{-3} sec , the ratio of delayed to total fluorescence intensities increases with the pressure of the vapour, and the small temperature dependence of the delayed component lifetime is incompatible with a mechanism in which the fluorescent state is thermally re-populated from the lowest triplet level The absence of dimer emission under these conditions indicates that its radiative lifetime is at least 10^{-3} sec and is probably at least ten times longer A similar effect reported by Kautsky[9] in 1935 for very dilute solutions of a number of dyes, hæmatoporphyrin and chlorophyll, was interpreted in the same way

Since process 5 requires an activation energy, it is less predominant at room temperatures than.

(b) internal conversion (process 6) which leads to

$$A_2^* \rightarrow (A_2) \rightarrow A + A \qquad (6)$$

the overall self-quenching of fluorescence in solution[10] exhibited by several of the aromatic hydrocarbons listed in Table 1 In the vapour phase process 5 is dominant in the majority of cases investigated under the conditions of observation, however, a quantitative treatment[13] of the simultaneous self-quenching and delayed fluorescence observed in anthracene vapour has shown that processes 5 and 6 are mutually competitive, which suggests that photo-association is an essential step in the self-quenching mechanism A value of 7 6 kcal /mole obtained[13] for $E_5 - E_6$, the difference in activation energies of processes 5 and 6, provides a lower limit to the heat of photo-association in this case, and since the rate constant for process 6 is ~ 10^3 sec $^{-1}$ at 220° C (ref 13), the excited dimeric state of anthracene should have a lifetime of at least 10^{-3} sec with respect to internal conversion in solution at room temperature The remarkable duration of this electronically excited species should promote

(c) efficient energy transfer to an acceptor B (process 7)

$$A_2^* + B \rightarrow (A_2) + B^* \qquad (7)$$

which has been proposed[14] to account for the identity of the anthracene vapour fluorescence spectrum and the delayed fluorescence spectrum of phenanthrene vapour containing not more than 10^{-4} mm (about 10^{-8} mole/l) of anthracene If the lifetime of phenanthrene in its photo-associated state is $\geqslant 10^{-3}$ sec , this concentration of anthracene acceptor is sufficient to account for the effect observed, which provides an upper limit of 5 4 kcal /mole for the heat of formation of the phenanthrene dimer Furthermore, the efficiency of process 7 in this case is consistent with the conservation of overall spin angular momentum which is only possible if the photo-associated donor is in a singlet state The pyrene-sensitized delayed fluorescence of naphthacene vapour present in trace quantities requires a similar interpretation[8] since delayed fluorescence has not been observed from naphthacene vapour alone where self-quenching is extremely efficient[11] In this case the difference in energies of pyrene and naphthacene molecules in their lowest excited singlet states provides an upper limit of 15 2 kcal /mole for the heat of photo-association of pyrene

If these competing processes are acceptable criteria for the formation of an excited dimeric species by the collisional process 3, the failure of 9,10-diphenylanthracene to exhibit either delayed fluorescence in the vapour phase[8] or self-quenching in solution[15] suggests that the photo-association of this non-planar molecule is sterically hindered However, the observations listed in Table 1 indicate that the photo-association of planar aromatic molecules is of general occurrence, and, despite the absence of direct evidence in the majority of cases, it must be concluded that in fluid media the stable form of planar aromatic molecules in their excited singlet states is dimeric The term 'excimer' has been proposed[4] to distinguish this state from the excited dimer of certain dyes which is produced by absorption from a stable dimeric ground state[16] and which has the quite different properties[4] shown in Table 2 Excimer formation, like triplet state population, is not necessarily limited to aromatic systems

Table 2 PROPERTIES OF EXCIMER AND EXCITED DIMER

	Excimer	Excited dimer
Ground state	dissociated	stable at room temperature
Dimerization	follows act of light absorption	precedes act of light absorption
Radiative relaxation	forbidden	allowed*
Corresponding absorption	not observed	increases with concentration

* According to intensity of corresponding absorption (ref 16)

Although photo-association appears to be of general occurrence unless sterically hindered, the intermolecular binding forces will depend on the electronic properties of the molecules involved and may be of less general origin A resonance splitting of the two-fold degenerate electronic levels of identical molecules in a parallel sandwich configuration has been treated by Hoijtink[17], who concludes that the stable state is a singlet with an energy some tenths of an electron-volt below that of the corresponding state of the monomer, and that owing to its centre of symmetry the radiative relaxation to a dissociated ground state is orbitally forbidden, these conclusions are in qualitative agreement with the experimental observations although the symmetry restriction requires a strictly parallel configuration of the aromatic planes which is unlikely to persist for 1 msec in the vapour phase or even in solution at room temperature

An explanation of excimer emission in solution with $k_4 \sim 10^8$ sec $^{-1}$ and delayed fluorescence in the vapour where $k_5 \sim 10^3$ sec $^{-1}$ appears to require at least two states of the pyrene excimer between which interaction is weak or dependent on temperature Murrell has shown (in a private communication) that the charge-transfer state A^+A^- of pyrene, corresponding to an interplanar distance of $\leqslant 3\ 5$ Å, lies below the first excited singlet state of the monomer, in which case configuration interaction with the resonance state may be responsible for the exceptional radiative properties of this excimer in solution A third possibility is the doubly excited state suggested by Hoijtink (private communication) to explain the remarkable resistance to radiative relaxation exhibited by excimers in the vapour state, Colpa[18] has pointed out that a doubly excited dimeric singlet state correlates with two molecular triplet states, and since the lowest excited singlet energy of molecules such as anthracene and naphthacene is almost exactly twice that of the corresponding triplet-level, the doubly excited state could be populated by a diabatic process following the collision of molecules in the ground and excited singlet states.

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STRUCTURE AND PROPERTIES OF THE FLAVOHÆMOPROTEIN, CYTOCHROME b_2 (L(+)-LACTATE DEHYDROGENASE OF BAKER'S YEAST) AND OF HÆMOPROTEIN, FLAVOPROTEIN AND APOPROTEIN DERIVATIVES

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CYTOCHROME b_2 was first observed in 1942 by Bach, Dixon and Keilin[1], and was partly purified by Bach, Dixon and Zerfas[2,3], who found that the cytochrome was closely associated with the lactate dehydrogenase of aerobic baker's yeast In 1954 Appleby and Morton[4,5] reported the purification and crystallization of cytochrome b_2 and showed that it was identical with yeast L(+)-lactate-cytochrome c reductase (L(+)-lactate dehydrogenase) The crystalline enzyme was found to be a flavo-

Table 1. Nomenclature of Derivatives of Cytochrome b_2*

Name	Non-protein groups	L(+)-Lactate-cytochrome *c* reductase activity	Principal absorption bands (mμ)	Other properties
Type I cytochrome b_2	DNA FMN† Protohæm	Active	α, 557, β, 528, γ, 423.5, u.v., 265	Crystalline, molecular weight, 172,000
Type II cytochrome b_2	FMN Protohæm	Active	α, 557, β, 528, γ, 423.5, u.v., 269.5	Crystalline
Type III hæmoprotein-550	DNA Ferro-porphyrin *c*	Inactive	α, 550, β, 521, γ, 415, u.v., 257	Crystalline, reduced by cytochrome *c* reductases, oxidized by cytochrome *c* oxidase
Type IV hæmoprotein-557	Protohæm	Inactive	α, 557, β, 528, γ, 423.5, u.v., 276	Poorly crystalline, slightly autoxidizable
Type V flavoprotein	DNA (?) FMN	Inactive	Oxidized, 445, 375, u.v., 266	
Type VI apoprotein	DNA	Inactive	u.v., 260 mμ	

* The full name for protein derivatives should include reference to the parent material—thus 'type III cytochrome b_2-hæmoprotein-550'.
† Riboflavin phosphate.

hæmoprotein since it contained equimolecular amounts of riboflavin phosphate and of protohæm as prosthetic groups[4,6,7]. In addition, the enzyme contained a constant proportion of a deoxyribonucleic acid (DNA)[8,9] of small molecular weight as estimated by end-group analysis[9].

The DNA-containing cytochrome b_2 is regarded as the naturally occurring form of the enzyme, and is now known as type I-cytochrome b_2 (ref. 10) (Table 1). The enzymic[4,5,11], chemical[4,7-9,11,12] and physico-chemical[4,8,11,13,14] properties of crystalline type I cytochrome b_2 have been described. The enzyme is homogeneous with respect to sedimentation as indicated by boundary analysis[15] of sedimentation patterns of the enzyme in lactate-pyrophosphate-ethylenediamine tetraacetate buffers, and by sedimentation patterns after treatment with 2.6 *M* and 5 *M* urea[13-15]. Only a single protein component has been detected by moving-boundary electrophoresis[4,8,14]. Although the possible existence of a flavoprotein and a separate hæmoprotein in crystalline type I cytochrome b_2 was recognized by Appleby and Morton[4], continued work since 1954 has produced no evidence for this hypothesis. Boeri *et al.*[16] used a non-crystalline preparation and confirmed the finding of Appleby and Morton[4] that cytochrome b_2 is a flavohæmoprotein identical with yeast L(+)-lactate-cytochrome *c* reductase.

Further information concerning the structure and properties of cytochrome b_2 has been obtained by modification of type I cytochrome b_2. A series of derivative proteins have been prepared, some in the crystalline state. Table 1 gives the nomenclature adopted for the various modified proteins so obtained. Both the hæmoprotein and flavoprotein derivatives are inactive as lactate-cytochrome *c* reductases, as is the apoprotein (the protein derivative free of both prosthetic groups). Lactate-cytochrome *c* reductase activity has been re-established by the re-combination of riboflavin phosphate with the hæmoprotein derivative and by the stepwise addition of protohæm and of riboflavin phosphate to the apoprotein, either alone is ineffective. The two prosthetic groups are attached at the active centre of the one protein, the presence of both groups is essential for enzymic reduction of cytochrome *c*. It is concluded that reduction of cytochrome *c* by the native enzyme involves intra-molecular electron transfer from the flavin to the hæm prosthetic group.

For these investigations, twice-crystallized type I cytochrome b_2 was the usual starting material. Unless otherwise specified, all solutions contained 0.1 m*M* ethylenediamine tetraacetate, and all work was carried out under anaerobic conditions at 0–2° C., with de-aerated solutions equilibrated with oxygen-free nitrogen. Solutions were usually prepared by dissolving crystals of type I cytochrome b_2 in 0.5 *M* sodium lactate–0.04 *M* sodium pyrophosphate–0.1 m*M* ethylenediamine tetraacetate, *p*H 6.8, the protein concentration was 15–25 mgm./ml. Enzymic activities were estimated at 25° C. and as described by Appleby and Morton[5], and by Morton and Shepley[10], rates being calculated from changes in optical density due to the enzyme during the first minute. Protein concentration was calculated from the optical density of the reduced form of the pigment[8] or by the method of Lowry *et al.*[17] Fluorescence was observed visually on irradiation with a 'Hanovia' lamp at about 360 mμ, or measured with an EEL fluorimeter.

Crystalline forms of type I cytochrome b_2. The ferrocytochrome is regularly obtained as crystals which appear as shallow bipyramids belonging to the tetragonal system[4,5]. Crystals have also been obtained by precipitation with acetone, according to the conditions, each of the crystalline forms shown in Fig. 1 may occur. The crystals shown in Fig. 1*B* appear to be macles (hemitropes), formed by co-crystallization of the protein and salt developing on a common crystal face. The ferricytochrome has also been obtained as tan-coloured bipyramids, in contrast to the pink-coloured crystals of the ferrocytochrome. The crystals of the oxidized enzyme give solutions which are burgundy red when concentrated and brown when dilute, the absorption spectrum of a concentrated solution shows a band rising from about 620 mμ to a maximum at 533 mμ, with a minimum at 510 mμ, and a distinctive shoulder at 563 mμ. The peak at 532–535 mμ and the shoulder at 563 mμ are seen as absorption bands in a micro-spectroscope.

The distinctive crystalline forms of type I cytochrome b_2 depend on the presence of the DNA, flavin and hæm group in the protein, dissociation of any one of these will either prevent crystallization under the conditions used or change the crystalline form obtained. By suitable treatments which destroy enzymic activity, but which leave the prosthetic groups intact on the protein, enzymically inactive crystals of cytochrome b_2 have been obtained.

Crystalline type II cytochrome b_2. The deoxyribonucleic acid of native cytochrome b_2 has a marked influence on the electrophoretic mobility, the sedimentation coefficient, and the dependence on concentration of the sedimentation coefficient of the enzyme[14,15]. However, removal of the deoxyribonucleic acid by salt-precipitation of the protein does not interfere with the dehydrogenase activities of the protein[8]. Crystallization of the resultant type II cytochrome b_2 as large bi-pyramidal prisms belonging to the hexagonal system has been described by Morton and Shepley[10]. Type II cytochrome b_2 has equimolecular

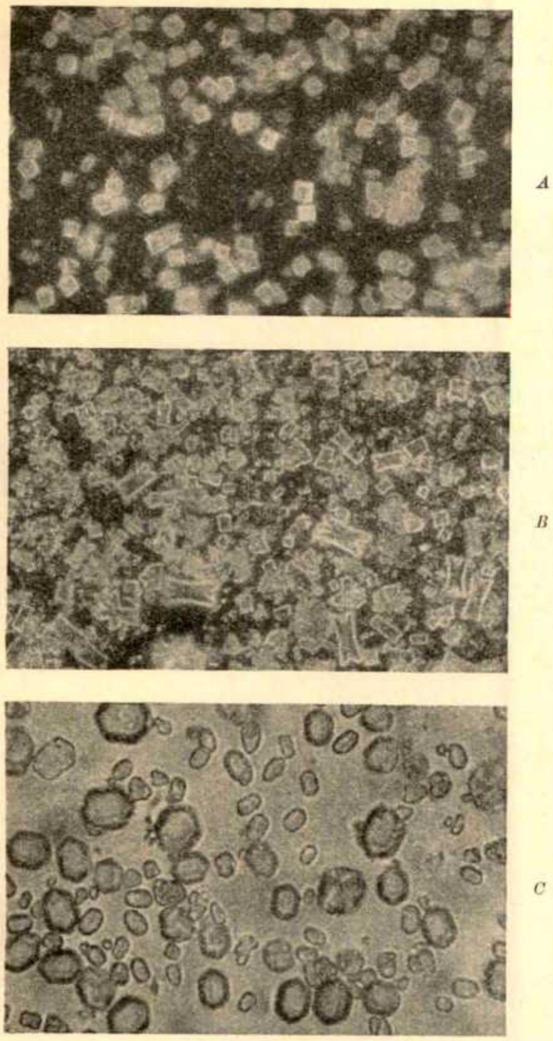

Fig. 1. Different crystalline forms of type I cytochrome b_2 obtained by acetone precipitation; the forms vary according to the conditions used for preparation. *A*, × 200; *B*, × 200; *C*, × 300

proportions of riboflavin phosphate and of protohæm and similar specific activities to type I cytochrome b_2 (refs. 10 and 11).

The occurrence of cytochrome b_2 as a crystalline protein, which may be obtained in a number of characteristic crystalline forms, each having equimolecular proportions of protohæm and of riboflavin phosphate, strongly supports the extensive physico-chemical evidence for the homogeneity of the protein; fortuitous association of a flavoprotein enzyme and of a separate hæmoprotein in cytochrome b_2 obtained by so many different purification and crystallization procedures becomes so improbable that this hypothesis may now be dismissed.

Cytochrome b$_2$ *- hæmoprotein - 550* (*type III hæmoprotein*). This is an enzymically inactive, flavin-free derivative of the enzyme. It was obtained from recrystallized type I cytochrome b_2 fortuitously, by acetone precipitation under conditions to be described elsewhere[18], as somewhat irregular crystalline plates.

The absorption spectrum of both the reduced and oxidized forms are remarkably similar to those of horse-heart and of yeast cytochrome *c*, but the spectra differ considerably in the ultra-violet region, largely due to the DNA-component of type III hæmoprotein. The extinction coefficients, expressed per mole of protein-bound hæm, are substantially the same for the α-, β- and γ-bands of the enzymically reduced cytochrome *c* and the type III hæmoprotein. The type III hæmoprotein derivative resembles cytochrome *c* in the following chemical properties: the pyridine hæmochromes are qualitatively identical for the α-, β- and γ-bands; the prosthetic group is not split by acid-acetone or acid-methylethylketone; treatment with silver sulphate in acetic acid causes distinctive changes in spectra, consistent with cleavage of thio-ether bonds, although the reaction products have not yet been identified. Like cytochrome *c* it is not autoxidizable at *p*H 7 and does not combine with carbon monoxide; it is reduced by ascorbic acid and by cytochrome b_2 (lactate-cytochrome *c* reductase); the reduced hæmoprotein is oxidized by cytochrome *c* oxidase from heart-muscle. However, whereas horse-heart cytochrome *c* has an isoelectric point of 10·2, type III hæmoprotein-550 has similar electrophoretic mobility to type I cytochrome b_2. It migrated very slowly in 12 hr. on electrophoresis at *p*H 6·8 in 0·3 M sodium lactate–0·05 M sodium pyrophosphate–0·1 mM ethylenediamine tetraacetate, *p*H 6·8, ionic strength 0·63, as a single component. The sedimentation coefficient was 3·4 Svedbergs (at 1·3 mgm. protein/ml.), and thus approximately half that of type I cytochrome b_2.

In view of the remarkable similarity to cytochrome *c*, including reduction by cytochrome *c*-linked dehydrogenases, and the available chemical evidence, it is tentatively concluded that cytochrome b_2-hæmoprotein-550 is a derivative of type I cytochrome b_2 containing at least one thio-ether bond from the protein to the hæm prosthetic group. It is likely that (*a*) reactive sulphydryl groups of cytochrome b_2 were exposed, possibly arising from cleavage of disulphide bonds in the original material; (*b*) these sulphydryl groups attacked the vinyl side-chains of the protohæm prosthetic group during acetone treatment of the protein, forming a thio-ether bond to the protein. This work will be described elsewhere[18], since it provides a possible model for physiological formation of cytochrome *c* from a protohæmoprotein precursor. The formation of type III hæmoprotein gives further support for the importance of highly reactive sulphydryl groups in cytochrome b_2[7,13,14]. Moreover, the similarity of the absorption spectra of cytochrome *c* and of type III hæmoprotein in the visible and near ultra-violet regions shows that similar amino-acid residues are co-ordinated with the iron atoms of the hæm residues in cytochrome *c* and in cytochrome b_2. Whereas cytochrome b_2 has two flavin and two hæm groups per molecule, and a molecular weight of 172,000 (refs. 14 and 19) hæmoprotein 550 appears to have one hæm group per molecule, and probably has a much lower molecular weight.

Cytochrome b$_2$ *- hæmoprotein - 557* (*type IV hæmoprotein*). This modification of type I cytochrome b_2 was obtained by removal of the flavin group of the enzyme by ammonium sulphate precipitation at *p*H 3·2. Loss of enzymic activity was proportional to the amount of riboflavin phosphate removed in the supernatant. The final product had about 0·02 times the amount of flavin in type I cytochrome b_2, and was correspondingly inactive with cytochrome *c*, ferricyanide, and dyes as acceptors for the dehydrogenase. The ferro-hæmoprotein has absorption maxima at 557, 528, 423·5 and 350 mμ; it does not combine significantly with carbon monoxide; and is only slightly autoxidizable, indicating a minimum of denaturation during the preparation. Rather poor crystals, which appeared as rectangular plates, were obtained by slow precipitation of this hæmoprotein with acetone. The sedimentation coefficient (1·3 mgm./ml.) was 7·94 Svedbergs; only a single protein component was detected on sedimentation. The prosthetic group is protohæm, as in cytochrome b_2. As shown here, enzymic activity of the enzymically inactive hæmoprotein is partly restored by recombination with riboflavin phosphate.

Cytochrome b_2-hæmoprotein-557 is probably closely related to a hæmoprotein which was crystallized by Okunuki and co-workers in 1957 [20]. The latter material, however, was highly autoxidizable; the effect of carbon monoxide was not reported. Although widely quoted as evidence for the existence of separate flavoprotein and hæmoprotein ('cytochrome b_2') moieties in L(+)-lactate-cytochrome *c* reductase of yeast, it is clear that the material obtained by Yamashita *et al.* was a derivative of native cytochrome b_2 obtained by denaturation of the enzyme during isolation[7,11].

Cytochrome b₂ *- flavoprotein (type V flavoprotein).* The lability of the flavin group of cytochrome b_2 creates considerable difficulty in selective removal of the hæm group without simultaneous depletion of riboflavin phosphate. However, it was found that the hæm group was quantitatively removed without extraction of the riboflavin phosphate by the following procedure. About 5 mgm. of freshly prepared crystals of type I cytochrome b_2 were dissolved in approx. 3 ml. of 0·1 *M* potassium cyanide at 0° C., and acetone pre-cooled to – 20° C. was slowly added to the solution at – 2° to – 5° C. until all the protein was precipitated. The yellow precipitate was washed with 60 per cent (v/v) acetone at – 15° C. and dissolved in about 2 ml. of 0·1 *M* sodium lactate, *p*H 7·5. Some insoluble protein was removed by centrifuging. The solution was dialysed against 0·1 *M* sodium lactate – 0·05 *M* sodium pyrophosphate – 0·1 m*M* ethylenediamine tetraacetate for 4 hr. Some insoluble protein was removed by centrifuging. Application of this procedure for removal of the prosthetic group of other hæmoproteins is being examined.

The flavoprotein derivative so obtained has 1 mole of riboflavin phosphate per 80,000 gm. as does native cytochrome b_2; however, the flavin group of the derivative, unlike that of native cytochrome b_2, is fully fluorescent. The absorption spectrum of the type V-flavoprotein shows maxima at 266, 375, and 445 mμ; there is a slight shoulder at 418 mμ, probably due to contaminant hæm material. These maxima correspond with those observed in the difference spectrum between native cytochrome b_2 and a preparation treated so that the hæm group was reduced but the flavin oxidized; a similar difference spectrum was found by Hasegawa and Ogura[21].

The type V flavoprotein wss enzymically inactive with lactate as substrate and with cytochrome *c*, ferricyanide, or 2 : 6-dichlorophenol indophenol as acceptors; enzymic activity was not restored by incubation at 0° C. with protohæmin. The material is readily denatured with simultaneous dissociation of the flavin group.

Cytochrome b₂ *- apoprotein (type VI - apoprotein).* Simultaneous removal of both the flavin and hæm prosthetic groups was accomplished by treatment of type I cytochrome b_2 crystals with acid-acetone at – 40 to – 60° C. The colourless apoprotein has a single absorption peak at about 260 mμ. Although there was considerable amount of denaturation, some of the protein was readily soluble in dilute lactate-pyrophosphate buffer. With this material, recombination of flavin and hæm groups partly re-established enzymic activity, but a fraction of the protein only soluble in more concentrated buffer was completely inactive.

Restoration of lactate dehydrogenase activity in hæmoprotein-557 and in apoprotein from cytochrome b₂. Table 2 shows that the inactive, type IV hæmoprotein-557 may be re-activated by incubating with riboflavin phosphate. The resultant protein catalyses reduction of cytochrome *c*, ferricyanide, 2 : 6-dichlorophenol indophenol and phenazine methosulphate. Whereas about 12 per cent of the ferricyanide reductase activity of type I cytochrome b_2 was recovered, addition of the flavin group restored only about 1 per cent of the cytochrome *c* reductase activity. Thus, as compared with reduction of ferricyanide, reduction of cytochrome *c* involves a more specific orientation of the two prosthetic groups.

Table 2. RESTORATION OF LACTATE DEHYDROGENASE ACTIVITY OF TYPE IV HÆMOPROTEIN-557

Acceptor	Hæmoprotein-557	Hæmoprotein-557 plus riboflavin phosphate
Cytochrome *c*	20	120
Ferricyanide	< 10	1,640
2 : 6-Dichlorophenol indophenol	7	90
2 : 6-Dichlorophenol indophenol plus phenazine methosulphate	10	140

The hæmoprotein solution was incubated with riboflavin phosphate (approx. 3 moles per 80,000 gm. of protein) at *p*H 7, and at 0° C., for about 2 hr. Activities were estimated as described by Morton and Shepley (ref. 10); they are given as μmoles of acceptor reduced per hr. per ml. of solution of hæmoprotein.

Further information concerning the functions of the two prosthetic groups was obtained from attempts to re-establish activity with a type VI apoprotein. The inactive material was incubated at *p*H 7·5 with either 1·5 moles of protohæmin per 80,000 gm. of protein or with 3 moles of riboflavin phosphate per 80,000 gm. of protein, or successively with each of these for 6–8 hr. at 0° C. At the end of this period (about 16 hr.), activities of the various materials, alone and in several combinations, were tested.

The apoprotein-hæmin and apoprotein-flavin incubates were inactive. Moreover, there was no reduction of any acceptor in the presence of lactate when portions of both the apoprotein-hæmin and the apoprotein-flavin preparations were added to the reaction mixtures. However, the preparation which had been incubated with hæmin for 6 hr. and afterwards with riboflavin phosphate showed a considerable activity with each acceptor. Fig. 2 shows the restoration of activity with cytochrome *c* as acceptor; similar results were obtained with ferricyanide and 2 : 6-dichlorophenol indophenol. Significantly, no activity was found in the preparation in which the addition of hæmin followed the addition of riboflavin phosphate. In a separate experiment, it was shown that some activity was recovered after incubation with both hæmin and riboflavin phosphate at the one time.

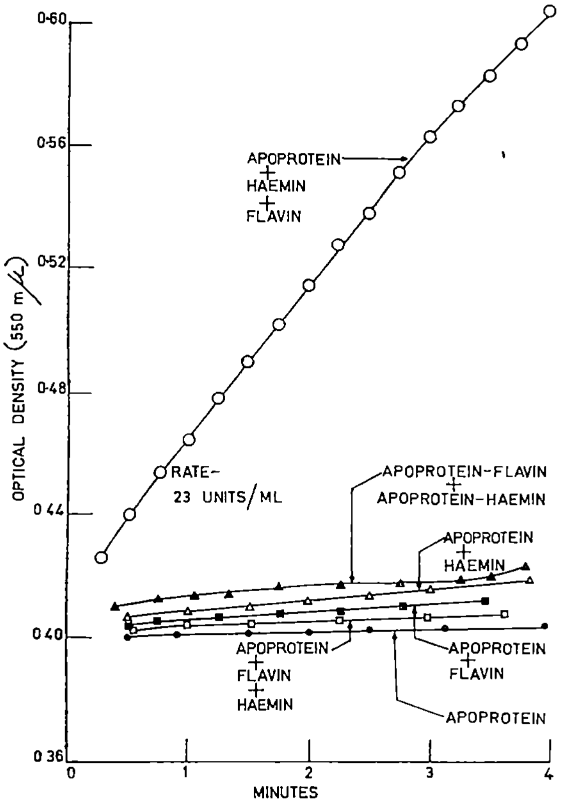

Fig 2 Restoration of lactate-cytochrome *c* reductase activity of type VI apoprotein by successive incubation with protohæm and riboflavin phosphate. Activity tests were carried out as described by Morton and Shepley (ref 10), approximately 5 μgm of protein were used in each test

The recombination of the apoprotein and hæmin forms a hæmoprotein with an absorption spectrum which closely resembles that of type IV hæmoprotein-557. The re-activated protein formed by the further addition of riboflavin phosphate differs from the native type I cytochrome b_2 in that it is strongly fluorescent, and only a portion of the added hæmin is reduced by lactate. This is consistent with the finding of about 1 per cent of the cytochrome *c* reductase activity of cytochrome b_2 in the recombined material. In view of the established lability of cytochrome b_2[3,7], and the rather drastic conditions needed for the removal of the hæm group from the enzyme, it is unlikely that recovery of activity can be further increased. Details of these and related investigations will be reported elsewhere[22].

Selective inhibition of lactate-cytochrome c *reductase activity of cytochrome* b₂. Of a number of compounds recently tested by Hallett and Morton[23], most (such as arsenite and *p*-chloromercuribenzoate) caused similar inhibition of enzymic reduction of ferricyanide and of cytochrome *c* catalysed by type I cytochrome b_2. However, amytal at about 2·6 mM concentration caused a substantial inhibition of reduction of cytochrome *c* but had no similar effect on reduction of ferricyanide. Although the possibility of combination of amytal with cytochrome *c* has not been eliminated, this seems most unlikely. This selective inhibition supports kinetic studies of Armstrong and Morton[14,15] showing that the reduction of ferricyanide and of cytochrome *c* involves different mechanisms.

Discussion

Warburg and Christian[24] in 1932 isolated the first flavoprotein enzyme, and these workers, and Theorell[25], showed that the flavin group could be removed under acid conditions with loss of enzymic activity, which was recovered by recombination of flavin with the apoprotein.

The acid-acetone treatment developed by Hill and Holden[26] for preparation of globin from hæmoglobin was used by Theorell[27] in 1940 to remove the hæm prosthetic group of peroxidase. Recombination of apoprotein and hæmin was accomplished after incubation at room temperature for some hours, with recovery of enzymic activity[27].

In the case of cytochrome b_2, an enzyme discovered in 1942 by Dixon[1–3], activity has now been shown to depend on the presence of both flavin and hæm prosthetic groups. Since apoprotein-hæmin material added with apoprotein-flavin material is inactive, arguments for a flavoprotein-hæmoprotein pair in cytochrome b_2 (ref 4) can be dismissed.

Kinetic[11], inhibitor[23], and reactivation[22] investigations are consistent and confirm that reduction of ferricyanide (an artificial acceptor) and of cytochrome *c* proceed through different pathways, as proposed in 1955[6]. However, enzymic activity with any acceptor depends on the presence of the two prosthetic groups, which so clearly interact, and not on either group alone.

Cytochrome b_2 of yeast mitochondria is a distinctive type of dehydrogenase, having two molecules of riboflavin phosphate and two molecules of protohæm per enzyme molecule, together, riboflavin phosphate and protohæm constitute the essential prosthetic group of this enzyme

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RESOLUTION OF PATHOLOGICAL HUMAN GAMMA-GLOBULINS BY STARCH-GEL ELECTROPHORESIS

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THE heterogeneity of the 7S normal human γ-globulins has been demonstrated by several techniques, including electrophoresis, convection, chromatography and biological assays[1-7] On starch-gel electrophoresis employing the continuous buffer system of Smithies[8], the γ-globulins are known to migrate towards the cathode as a broad zone In the discontinuous buffer system described by Poulik[9] most of these globulins move in the opposite direction The γ-globulins of pathological human sera, analysed by the 'continuous' gel electrophoresis technique, have been shown to migrate essentially as a single zone located in different positions within the area of the normal γ-globulins[10,11]

We report here the resolution by starch-gel electrophoresis of the γ-globulins of certain pathological sera into several distinct bands

The principal results of this work are presented in Fig 1 The distribution of the γ-globulins of the five representative pathological sera differed significantly from one other In the continuous borate buffer system these globulins of four of the five specimens migrated towards the cathode like the normal γ-globulins. The first pattern (Fig 1*a*) shows the starch-gel electrophoretic analysis of the serum from a patient with cancer of the ovary and widespread metastasis The remarkable finding is that the γ-globulins separated into seven bands The next pattern (Fig. 1*b*) represents the gel electrophoresis of the serum of a patient with probable chronic myeloid leukæmia The γ-globulins resolved into three zones of which the middle one appeared to be further separated into four sub-bands The pattern of the serum of a patient with hypergamma-globulinæmia of four years duration but without clinically detectable disease is shown in Fig 1*c* The γ-globulin zone appeared very dense compared with that of normal serum (Fig. 1*N*) although their areas were about equal The γ-globulins of another patient with unknown diagnosis displayed a similar pattern with three narrow heavy bands superimposed The γ-globulins of a further patient (Fig 1*d*) moved as a single rather narrow band to the cathode side of the normal γ-globulin area Comparable findings have been reported earlier[10,11] The fifth specimen was obtained from a patient with chronic normochromic normocytic anæmia of sixteen years duration It was interesting to observe that the γ-globulins migrated towards the anode, that is, in the direction opposite to that of the movement of the normal γ-globulins, and exhibited a pattern (Fig 1*e*) characterized by a very intensely coloured band in the slow α_2 ($S\alpha_2$) region (It is worth mentioning that this patient is not unusually prone to infections and, therefore, must still have the benefit of antibody protection) In addition, there were three minor zones distributed almost equally between the slot of application and the major γ-globulin band mentioned

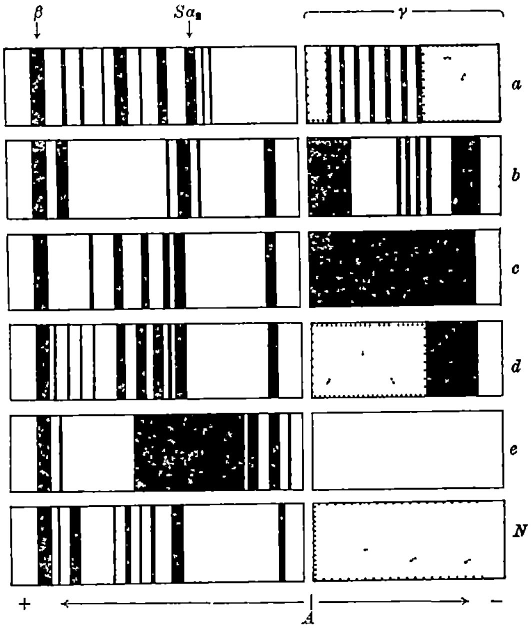

Fig 1 Starch-gel electrophoretic analysis of five pathological sera (*a–e*) For comparison the corresponding pattern of normal human serum (*N*) is included, and the positions of the γ- (γ) and slow α_2- ($S\alpha_2$) globulins and transferrin (β) are given The two arrows indicate the directions of movement of the proteins '*A*' points to the slots of application The starch-gel electrophoresis was performed in the cold room at 3° in *p*H 8 6 borate buffer at 20 m amp for 17 hr The part of the patterns that shows the transferrin–pre-albumin region is not reproduced

Use of the discontinuous system of buffers led to the following findings The γ-globulins of serum 1*a* entered the anodic side of the application slot and again separated into four bands which were located in the $S\alpha_2$ region It appeared that additional γ-globulin bands were present in the haptoglobin area Only traces of protein moved towards the cathode Under the same conditions normal γ-globulin (Cohn's Fraction II) formed a broad zone in the $S\alpha_2$-haptoglobin area and further bands in the post-albumin region[12] The γ-globulins of serum 1c displayed a pattern which was very similar to that observed in the continuous buffer system, except that a relatively smaller amount of protein seemed to be present, probably because the normal γ-globulins of this serum moved in the opposite direction The γ-globulins of patient 1*d* migrated as in borate buffer towards the cathode, but a separation took place which led to one minor band preceding and two following the main zone Moreover, additional lines were observed in the haptoglobin region The γ-globulins of patient 1*e* displayed a pattern which was essentially the same as the protein distribution observed in the continuous system Thus, judging from the behaviour of the γ-globulins of this limited series of sera, and as pointed out earlier by Poulik[13], the discontinuous buffer system leads to a higher resolu-

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tion than that resulting from the continuous system Further, all γ-globulin bands obtained by the discontinuous system were located closer to the albumin zone as noted previously[13] Nevertheless, certain pathological γ-globulins (1*b*, *c*, *d*) still migrated towards the cathode

Paper electrophoresis at *p*H 8 6 in citrate barbiturate buffer of the sera investigated demonstrated in each case a significant increase of the proteins which migrated with mobilities corresponding to those of the γ-globulins The increase over the normal value was 23–34 relative per cent As judged by ultracentrifugal analysis the 7S-globulins of the same sera varied between 30 and 40 per cent The 19S-component appeared approximately normal in concentration Abnormal plasma proteins with sedimentation coefficients of 10 and 14S, respectively, were observed in one case (1*e*) only

The present starch-gel electrophoretic investigation on pathological γ-globulins demonstrates that this procedure offers an advantage over paper electrophoresis in that certain abnormal γ-globulins can be resolved into several bands This resolution seems to indicate that these pathological γ-globulins display a discontinuous spectrum with respect to apparent mobility in contrast to the continuous spectrum of the normal γ-globulins If the mobility of the 7S-γ-globulins may be related to the net charge of these molecules, then this observation can be interpreted to mean that certain pathological γ-globulins are synthesized in such a way that they carry relatively unlike electrostatic net charges

This work was supported by grants from the Lilly Research Laboratories, Eli Lilly and Co , and from the National Institutes of Health [*A*-3456(*C*1)], United States Public Health Service

Note added in proof After this communication had been submitted, an article by Owen, Got and Silberman (*Clin Chim Acta*, 3, 605, 1958) came to our attention, in which the heterogeneity of the γ-globulins of patients with myeloma is described

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GAMMA-GLOBULIN AND ACQUIRED IMMUNITY TO HUMAN MALARIA

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MALARIAL infection is followed by the development of immunity which is specific for the homologous parasite, but ineffective against heterologous strains and species[1–4] The epidemiological effects of acquired immunity in man are seen among the inhabitants of hyperendemic malarious regions who show a consistent pattern of susceptibility to infection Infants born in such regions are relatively resistant during the first three months of life and thereafter all children suffer severe and recurrent attacks of the disease Clinical malaria becomes comparatively infrequent in later childhood and among adults is rarely seen in acute form[5,6].

The response of phagocytic cells in malarial infection was shown in early histological work[7], and for many years resistance to the disease was considered to be exclusively cellular in nature In 1937, Coggeshall and Kumm[8] demonstrated the passive transfer of malarial immunity in rhesus monkeys and so established the presence of protective antibodies However, the therapeutic effect of large doses of immune serum was evident only against minimal numbers of parasites and has not been demonstrated convincingly in man The belief is therefore still prevalent that circulating antibodies play a relatively unimportant part in acquired malarial immunity[9]

Passive Transfer of Human Malarial Immunity

In this investigation the role of circulating antibody in malarial immunity has been studied in subjects living in The Gambia which is a hyperendemic area of West Africa The therapeutic effect of γ-globulin prepared from the serum of apparently immune adults has been tested in young African children suffering from severe clinical malaria with dense parasitæmia The γ-globulin prepared by chromatography on columns of diethylaminoethyl cellulose[10] was homogeneous on electrophoresis and in the ultracentrifuge appeared as a main peak with a sedimentation coefficient ($S^0_{20,W}$) = 6 78, an additional heavier component (10*S*) comprised less than 5 per cent of the total protein The remainder of the protein eluted from the columns (referred to here as γ-free serum) was shown by serological tests to contain about 70 mgm 7S γ-globulin and 20 mgm 19S γ-globulin/gm total protein

Twelve children aged 4 months–2½ years (weight 5 4–12 6 kgm.) admitted with a clinical diagnosis of malignant tertian malaria and an initial parasitæmia of 10,000–230,000/c mm received γ-globulin by intramuscular injection at intervals of 8–24 hr for 3 days The total dose was 1 2–2 5 gm /child which

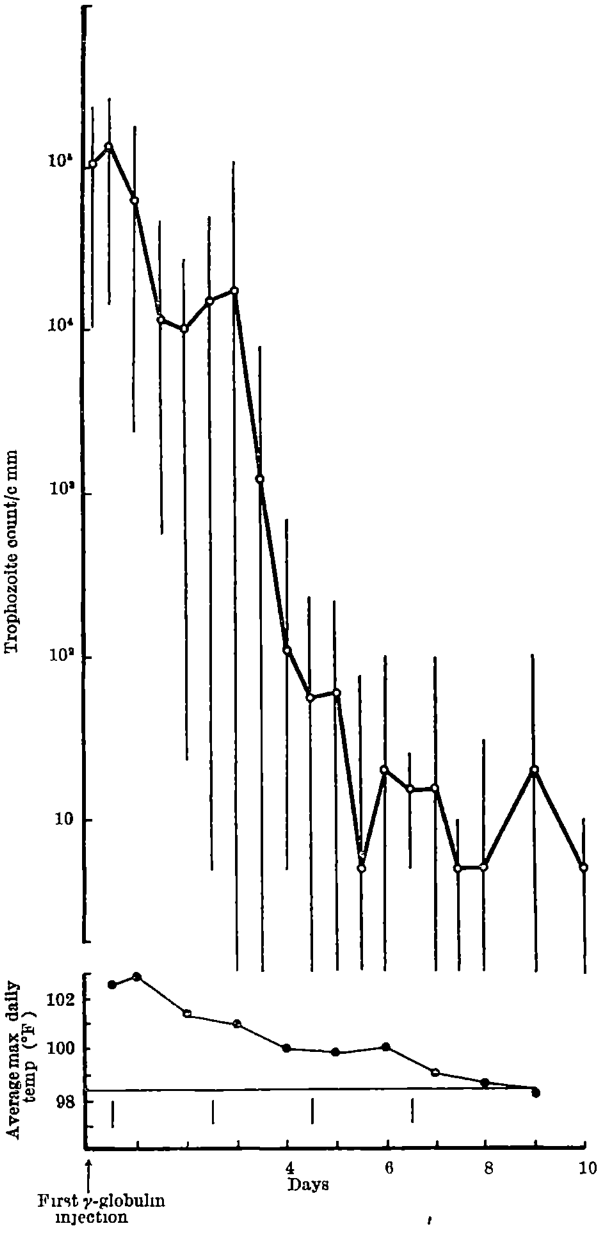

Fig 1 Trophozoite counts (mean and range shown on a logarithmic scale) and average maximum daily temperatures in 12 children with acute *Plasmodium falciparum* malaria treated with immune γ-globulin The time-scale refers to days after the first γ-globulin injection Vertical lines below indicate approximate times of expected schizogony

was equivalent to 10–20 per cent of the recipient's own γ-globulin

The infections were predominantly synchronous and γ-globulin therapy was usually started when the peripheral blood contained a high density of young ring forms Blood examinations were made at 12-hourly intervals and the course of parasitaemia is shown in Fig 1 By the fourth day after the inception of treatment parasite counts were always less than 1 per cent of the initial values, by the ninth day trophozoites were not detectable in 8 out of 12 cases and the maximum count was 80/c mm (Fig 1) The fall in parasitaemia was accompanied by progressive alleviation of clinical illness although temperatures did not usually return to normal before the seventh day (Fig 1) No alterations in parasite morphology have been observed during treatment; however, in malignant tertian infections only immature parasites are detectable in peripheral blood The immune γ-globulin appears to be effective both against *Plasmodium falciparum* and *P malariae* (Fig 2), but probably has no action against gametocytes (Fig 3)

Two children aged 2–3 years failed to respond to the injection of 1 2 gm γ-globulin They had been admitted with pneumonia and measles respectively,

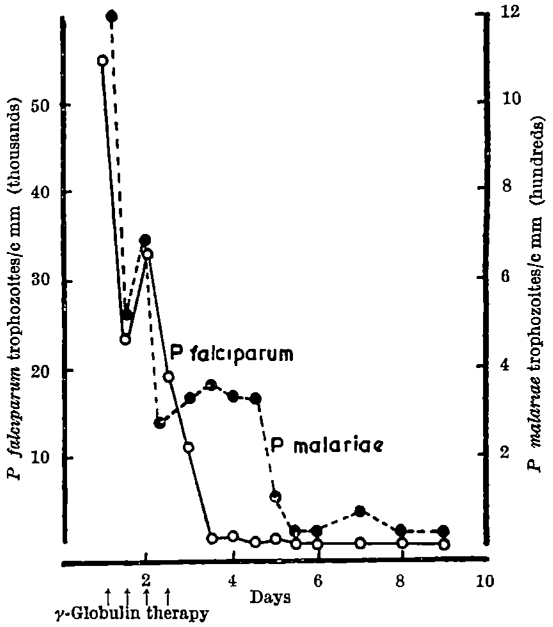

Fig 2 Trophozoite counts of *Plasmodium falciparum* and *P malariae* in a child with a mixed infection treated with immune γ-globulin, arrows show times of administration of γ-globulin by intra-muscular injection

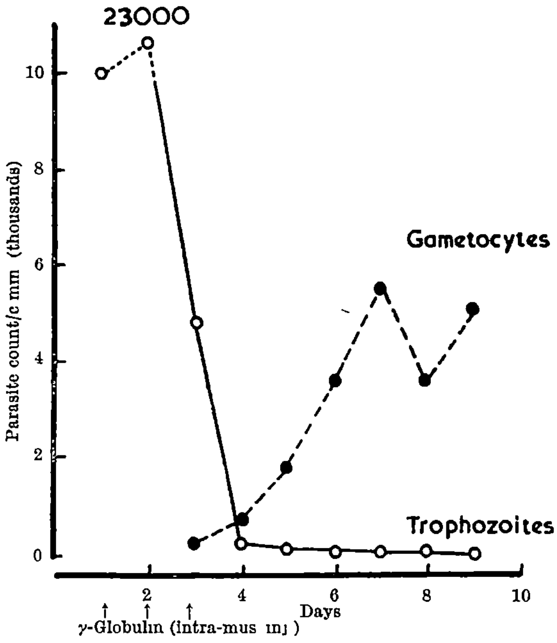

Fig 3 Trophozoite and gametocyte counts in a child with acute *Plasmodium falciparum* malaria treated with immune γ-globulin Arrows show time of administration of γ-globulin by intra-muscular injection

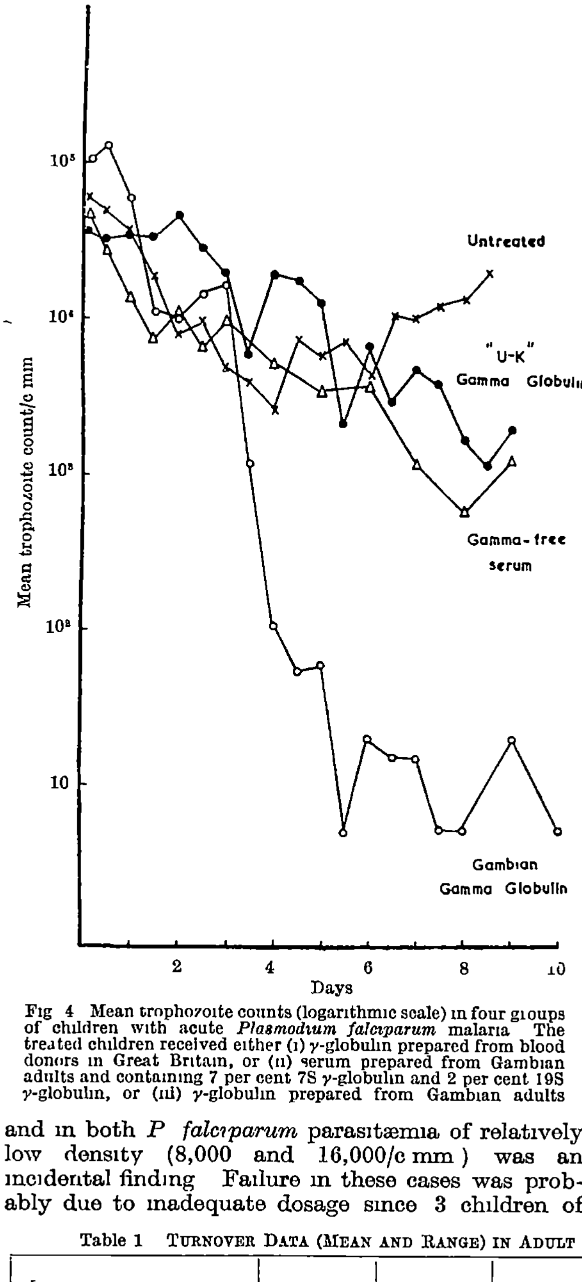

Fig 4 Mean trophozoite counts (logarithmic scale) in four groups of children with acute *Plasmodium falciparum* malaria The treated children received either (i) γ-globulin prepared from blood donors in Great Britain, or (ii) serum prepared from Gambian adults and containing 7 per cent 7S γ-globulin and 2 per cent 19S γ-globulin, or (iii) γ-globulin prepared from Gambian adults

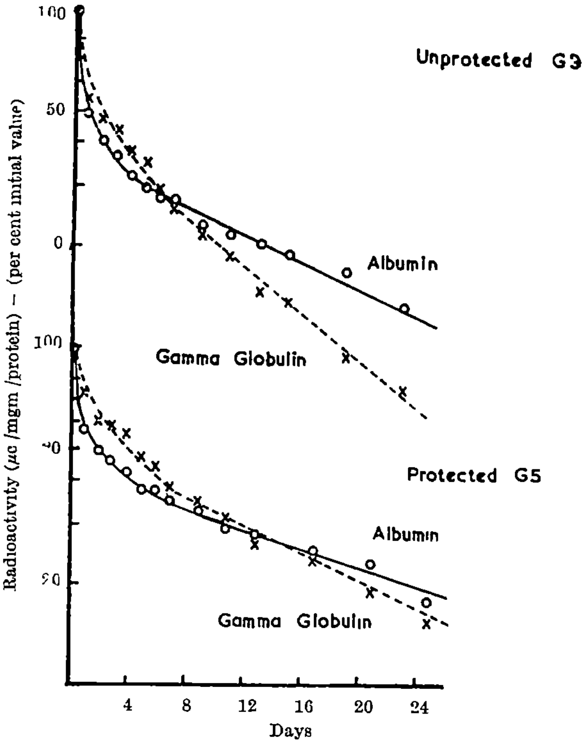

Fig 5 Plasma elimination of ^{131}I-albumin and ^{125}I-γ-globulin in an unprotected adult Gambian and in a similar subject who had received 'Pyrimethamine' (25 mgm /week) for 6 years

and in both *P falciparum* parasitæmia of relatively low density (8,000 and 16,000/c mm) was an incidental finding Failure in these cases was probably due to inadequate dosage since 3 children of comparable weight (10–12 kgm) responded to the injection of 1 8–2 5 gm γ-globulin

In further experiments, 7 children with acute *P falciparum* malaria received the γ-free fraction of Gambian serum (0 8–1 4 gm) A single batch was used in all experiments and no child has developed signs of serum hepatitis In addition, 4 children received γ-globulin prepared from the sera of blood donors in Great Britain (Lister Institute, Elstree, Herts) and 2 were kept under observation without anti-malarial therapy Trophozoite counts usually decreased in all these children However, the response was irregular, and between the fourth and tenth days the mean trophozoite counts were considerably greater than those observed after treatment with adult African γ-globulin (Fig 4)

Table 1 TURNOVER DATA (MEAN AND RANGE) IN ADULT SUBJECTS INJECTED WITH LABELLED ALBUMIN AND γ-GLOBULIN

Subjects	No	Total protein	gm (per cent)	Intra-vascular pool (gm)	Distribution ratio	Turnover-rate		
						Per cent I–V pool/d	gm /d	mgm /kgm /d
			Albumin					
Unprotected Gambians	4	8 1 (7 6–8 7)	3 6 (3 1–4 2)	86 (61–110)	1 3 (1 2–1 4)	10 8 (8 6–12 3)	9 1 (6 8–12 0)	170 (150–222)
Protected Gambians	5	8 3 (7 8–8 6)	4 1 (3 8–4 3)	91 (86–102)	1 2 (1 1–1 5)	10 5 (7 1–14 3)	9 4 (6 7–12 3)	176 (124–241)
W Africans in Great Britain	4	8 6 (8 4–8 7)	4 6 (4 2–4 9)	113 (108–115)	1 3 (1 1–1 5)	10 4 (9 9–11 0)	11 7 (11 4–12 2)	159 (149–177)
Europeans in Great Britain (ref 12)	11	7 8 (7 2–8 4)	4 5 (4 1–5 8)	123 (95–145)	1 4 (1 1–1 2)	10 4 (9 1–12 1)	12 7 (10 5–17 1)	185 (136–257)
			γ-Globulin					
Unprotected Gambians	5	8 6 (8 3–9 2)	2 7 (2 2–2 9)	66 (57–82)	0 8 (0 7–1 0)	13 5 (11 8–15 7)	8 8 (7 9–9 7)	169 (139–203)
Pregnant unprotected Gambians	1	7 6	1 8	50	0 8	9 6	4 8	84
Protected Gambians	5	8 3 (7 8–8 6)	2 1 (1 5–2 4)	47 (30–67)	0 7 (0 6–0 8)	11 5 (8 5–15 4)	5 2 (4 6–5 7)	98 (80–113)
W Africans in Great Britain	4	8 6 (8 4–8 7)	1 8 (1 6–2 0)	45 (38–55)	0 8 (0 7–0 9)	8 2 (7 8–9 3)	3 7 (2 7–4 3)	50 (42–57)
Europeans in Great Britain (ref 11)	5		1 1 (1 0–1 1)	37 (28–44)	0 8 (0 6–0 9)	5 1 (4 0–5 7)	2 1 (1 5–2 4)	23 (18–28)

Distribution ratio = ratio extra- to intra-vascular protein mass

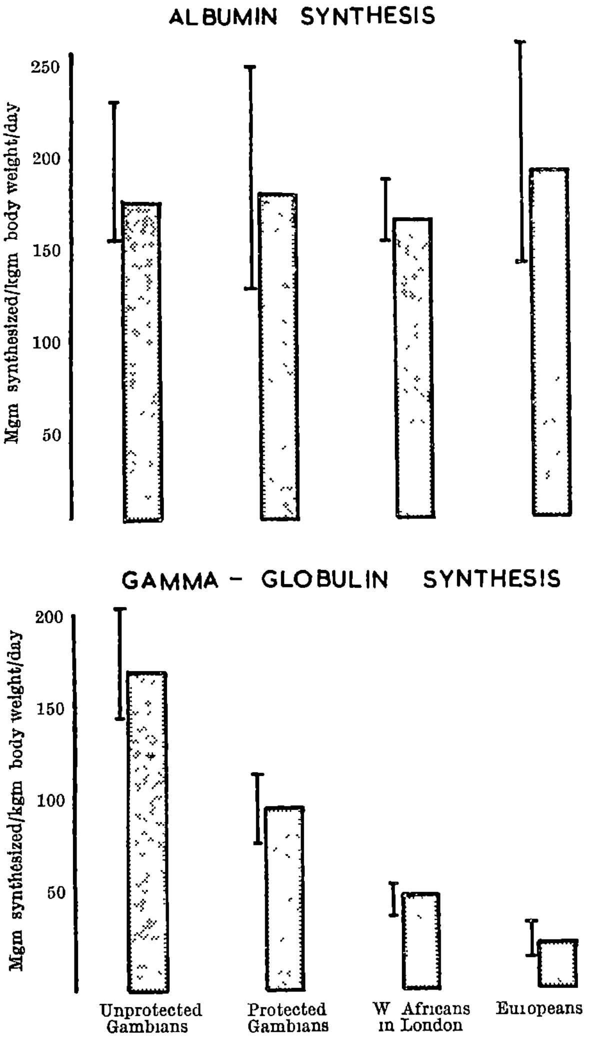

Fig. 6 Comparison of the average rates (and range) of albumin and γ-globulin synthesis in malarious and non-infected Africans and in healthy Europeans living in Great Britain

γ-Globulin Synthesis in Relation to Malarial Infection

The distribution and turnover of separate plasma protein fractions can be measured simultaneously by using two isotopes of iodine (iodine-131 and iodine-125) having different γ-emission spectra[11] Using this method, rates of albumin and γ-globulin synthesis have been measured in apparently healthy Gambian adults exposed to malarial infection but not showing detectable parasitæmia Similar studies were carried out on 5 Gambian adults who had received an anti-malarial prophylactic weekly for 4–5 years (Fig 5) and also on 4 West African medical students who had suffered from malaria in childhood, but had been resident in England for 3–10 years The experimental procedure has previously been described[11,12], results were analysed according to the method of Matthews[13]

The rate of albumin synthesis was similar in the three groups of Africans and within the range for healthy Europeans On the other hand, the rate of γ-globulin synthesis in unprotected Gambians was about 7 times that observed in Europeans, protection by anti-malarial therapy considerably reduced the rate of γ-globulin synthesis (Table 1 and Fig 6) The rapid turnover of γ-globulin in unprotected adults cannot be attributed to the selective catabolism of isotopically labelled malarial antibody since γ-globulins prepared from European and adult Gambian donors have identical elimination-rates (Fig 7) Considerably less γ-globulin was synthesized by an unprotected subject who was three months pregnant at the time of study (Table 1), this finding is of interest in relation to the increased susceptibility to malarial infection described in pregnant women living in hyperendemic areas[14,15]

West Africans resident for some years in the United Kingdom synthesized less γ-globulin than Gambian subjects maintained on anti-malarial therapy (Fig 6), this difference must be attributed to environmental factors other than malarial infection After several years in Great Britain, West Africans continue to synthesize γ-globulin at almost twice the rate observed in healthy Europeans, this may be due to environmental influences of early life or to genetically determined differences in γ-globulin metabolism.

Mechanism of Acquired Malarial Immunity

The preparation of γ-globulin from pooled sera of apparently hyperimmune donors and the use of susceptible children as recipients likely to have been exposed to homologous parasite strains, have provided optimum conditions for demonstrating the potency of naturally occurring malarial antibodies active against *Plasmodium falciparum* and probably also against *P malariae* The results show that the mechanism of acquired malarial immunity is basically similar to that observed with many other infections, being primarily dependent on the presence of protective antibodies which are associated with 7S γ-globulin The response to immune γ-globulin

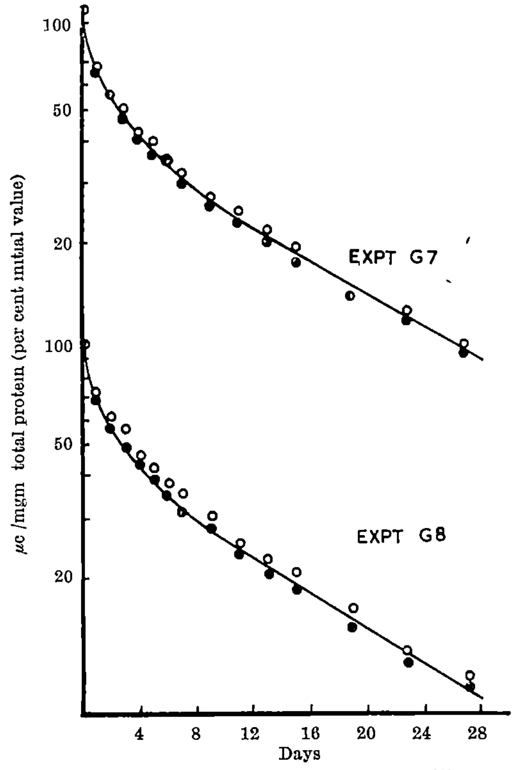

Fig 7 Comparison in two unprotected African adults of the plasma elimination of γ-globulin prepared from European and Gambian donors and labelled with iodine-125 and iodine-131 respectively. ●, European γ-globulin, O, Gambian γ-globulin

considered in relationship to the cycle of parasite development as well as the absence of morphological changes in the parasites of peripheral blood, suggest that antibody acts either on mature intracellular forms or on merozoites liberated from red blood cells Protection is of limited duration and a treated subject was again susceptible three months after receiving γ-globulin Other fractions of immune serum including 19S γ-globulin did not appear to have a protective effect nor did the γ-globulin of European blood donors

These findings support the hypothesis that the relative resistance of infants born in hyperendemic areas is due to passive transfer of protective antibodies from the mother This process may, however, be rendered relatively ineffective by partial loss of malarial immunity during pregnancy[14,15], which appears to be associated with a reduction in the rate of γ-globulin synthesis Other factors, such as selective vector biting[16], deficiency of *p*-aminobenzoic acid in milk-fed infants[17,18] and the presence of a high proportion of fœtal hæmoglobin in circulating red cells[19] may contribute to the suppression of malarial infection during early life

In areas of hyperendemic malaria, immunity is established only after long exposure to intense infection This slow response is probably attributable both to the inherently poor antigenicity of the parasite and to the serological diversity of naturally occurring plasmodia Acquired immunity is associated with hypergammaglobulinæmia[20,21] and with a striking increase in the rate of γ-globulin synthesis Protection of Gambian adults against malaria over a period of several years reduced the average daily synthesis of γ-globulin by 3 5 gm , which is more than the total daily production in healthy Europeans Since plasmodial infection leads to the production of complement-fixing antibodies[22] and precipitins[23], neither of which is species specific, it is likely that a considerable part of the γ-globulin synthesized in response to malaria is not protective antibody

We wish to thank Sir Charles Harington, whose interest led to the initiation of this work, and Prof R R Porter for much helpful discussion We are grateful to Dr D Harling for clinical supervision of patients, Dr W d'A Maycock for supplies of U K γ-globulin and Miss Margaret Polley for serological estimations of 19*S* γ-globulin One of us (S C) is in receipt of a Medical Research Council grant

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MOISTURE EXPANSION RELATIONSHIPS FOR A FIRED KAOLINITE-HYDROUS MICA-QUARTZ CLAY

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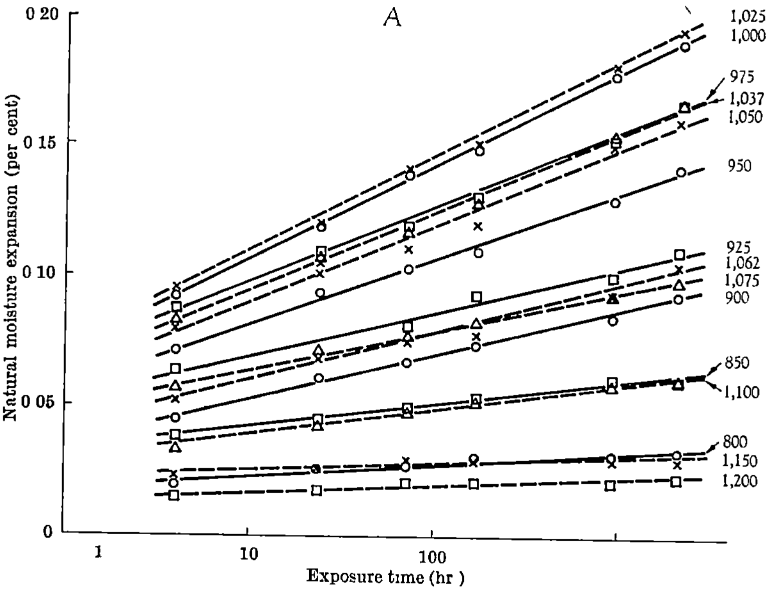
IN carrying out a detailed study of the moisture expansion characteristics of a clay containing 30 per cent kaolinite (poorly organized), 30 per cent hydrous mica (poorly organized) of the following approximate composition

$$[Si_{6\ 81}Al_{1\ 20}]\ [Al_{2\ 42}Fe_{1\ 16}Mg_{0\ 04}]$$
$$[Ca_{0\ 30}K_{0\ 25}Na_{0\ 15}H_3O_{1\ 42}]\ O_{20}(OH)_4$$

35 per cent quartz, 2 per cent hæmatite, and 1 per cent anatase, I have established relationships that might be found useful in interpreting the results from other clays and clay products

The curves of moisture expansion versus firing temperature for specimens exposed to air for 90 days, and for specimens autoclaved at 220 lb /sq in for 2, 8, 30 and 200 hr (measurements beginning from the same datum, specimens removed from the muffle at 300° C and cooled for 2 hr in a dry desiccator before initial measurement) show that the autoclave expansion curves parallel the natural expansion curve and that at a firing temperature of about 1,000° C all have a maximum in expansion If, however autoclave expansion is plotted directly against natural expansion (Fig 1), non-linear relationships exist over the firing range 950–1,200° C for all periods of autoclaving, and the points below and above 1 000° C separate into two curves for 30 and 200 hr of autoclaving (Linear relationships exist if autoclave expansion is plotted against the log of natural expansion) The non-linear relationship expresses the fact that for this clay the ratio of autoclave expansion to natural expansion varies widely with firing temperature, and implies that there must be several reactive components produced over the range of firing temperature covered by the specimens Mineralogical analysis has established that these reactive components are amorphous matter, glass, and anhydrous clay minerals Amorphous matter, which is responsible for the coincident moisture expansion peak at about 1,000° C in autoclaved and naturally exposed specimens, is equally reactive to water vapour in the air or to steam under pressure, but anhydrous clay minerals and glass are far more active to steam than to water vapour in the air Thus, for fired clay samples containing varying amounts of amorphous matter, anhydrous clay minerals, and glass, non-linear relationships must arise between autoclave expansion and natural expansion Furthermore, the nature of the anhydrous clay minerals and of the glass will determine whether the autoclave and natural moisture expansion peaks occur at the same temperature

It is well known that both natural moisture expansion[1,2] and autoclave moisture expansion[2-5] increase rapidly at first but in the later stages the increases are much slower The interesting feature of the new results is that a plot of natural expansion against logarithm of time (Fig 2*A*) is linear for all firing temperatures from 800 to 1,200° C and exposure times from 4 hr to 90 days A plot of the autoclave expansions against the log of the time of autoclaving (Fig 2*B*) shows also that a linear relationship exists for specimens fired at 1,000–1,150° C for autoclave times for 2–200 hr Similar relationships can be found from the data in the literature[2], if plots are made against log time rather than against time However, data on other clays from the Division[6] suggest that at times longer than those investigated here the logarithmic relationship breaks down and that the complete curve of expansion against logarithm of time is frequently *S*-shaped Consequently caution is needed in using the log-time or any other time relationship in predicting future values of moisture expansion from known results



Weight measurements made at the same time as expansion measurements show that as the temperature of the natural moisture expansion peak is approached from low firing temperatures, progressively less water is required to produce unit expansion, but that at temperatures beyond that of the moisture expansion peak the same weight of water produces the same amount of expansion The same is true for specimens expanded in the autoclave Furthermore, the amounts of water per unit expansion for specimens fired above the temperature of the moisture expansion peak are much the same for the natural (1 52 per cent increase in weight for a 1 per cent increase in length) and autoclave (1 92 per cent increase in weight for a 1 per cent increase in length) expansion, that is, the moisture absorbed in the two processes must be held in the same way However, there is a small amount of

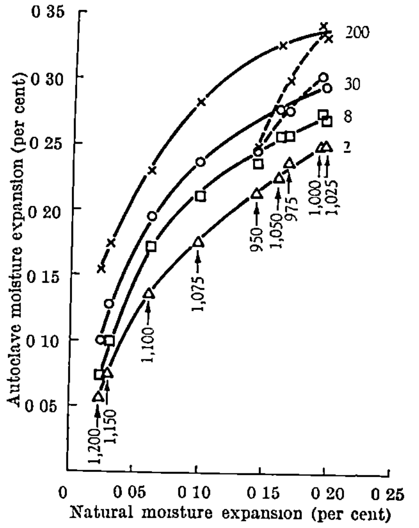


Fig 1 Autoclave moisture expansion on a dry basis after 2 8, 30 and 200 hr of autoclaving versus natural expansion after 90 days of exposure for specimens fired at 950–1,200° C The results for specimens autoclaved for 30 and 200 hr after being fired at 950, 975 and 1,000° C form separate curves which are shown by dotted lines

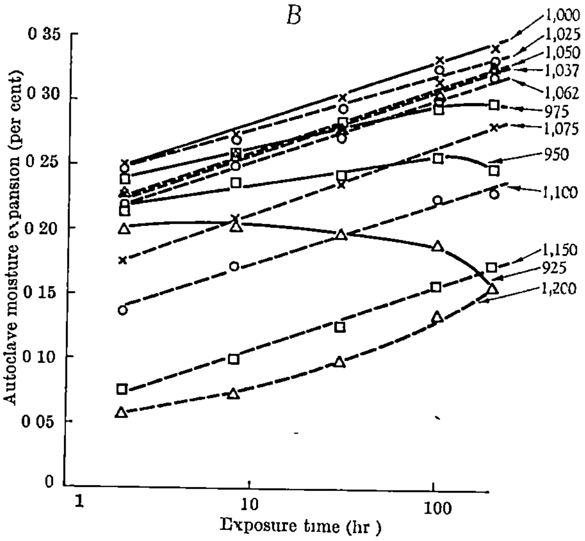


Fig 2 *A* Natural moisture expansion for specimens fired at 800–1,200° C plotted against the logarithm of time of standing in air Data for temperatures of 1,000° C or less are represented by full lines, data for other temperature by dotted lines
B Autoclave moisture expansion after drying at 110° C for specimens fired at 925–1,200° C plotted against the logarithm of the time of autoclaving Data for temperatures of 1,000° C or less are represented by full lines, data for other temperatures by dotted lines

water taken up in the autoclave which must be presumed to cause no expansion at all.

When specimens were desorbed up to a temperature of 500° C by re-heating, they lost weight and contracted, but at about this temperature those that were low-fired (600–900° C) showed an expansion with loss in weight which was not reversed until much higher temperatures of re-heating were reached. The magnitude of the expansion depended on the original temperature of firing and was only zero at a firing temperature of 950° C, that is, when the anhydrous mica was destroyed. Thus the expansion can be associated with the dehydration of the mica mineral regenerated from the anhydrous form by water sorbed after firing.

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VISUAL EFFECTS OF NON-REDUNDANT STIMULATION

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OPTICAL fields modulated randomly with respect to space and time ('visual noise') appear to offer a powerful new tool for the investigation of visual perceptual processes. In earlier work[1,2] I have described the use of such 'non-redundant' stimuli to shock-excite the visual system during the inspection of regular spatially repetitive patterns, so as to reveal the 'complementary image' that is generated in the visual system by such patterns. The present article is intended as an interim report on a wider range of new visual phenomena revealed by the same technique. It is not concerned with exposure to random fields as a form of 'sensory deprivation', which has interesting effects in a different category[3].

A typical source of 'visual noise' is offered by a de-tuned television receiver with high gain. Most readers will be familiar with the 'snowstorm' effect thus produced on the screen. An alternative, which gives greater flexibility[4] is to make up a ciné film-loop from successive samples of a random distribution of white (or black) spots. (Photographs of sandpaper, or sprinkled confetti, are remarkably adequate.) With any such source, the following effects may readily be observed.

(1) *Discontinuity between foveal and peripheral fields.* The normal impression first created on inspection of visual noise is that of a homogeneous field of 'particles' in violent agitation. Individual 'particles' can be followed for a second or more in pseudo-Brownian motion. On prolonged inspection, however (especially if monocular), the foveal area of the field is clearly distinguishable from the remainder. Its motion is more coherent, as of a liquid boiling up from a restricted central source, and its average amplitude of pulsation is greater than that of the peripheral field. As the gaze is directed to different regions of the random stimulus, the 'boiling' foveal area can be clearly identified by the fact that it travels with the eye.

(2) *Monocular versus binocular viewing.* Considerable theoretical interest attaches to the contrast between monocular and binocular excitation. Most subjects spontaneously report that the monocular field appears the more densely populated, and scintillates more rapidly. With two eyes the field is seen as less 'sparkling', the motion more 'oily', the 'particles' fewer and more persistent. On most theories of binocular fusion it would not have been expected that the simultaneous and identical excitation of corresponding retinal areas should reduce the apparent density and frequency of what is perceived.

(3) *Effect of intensity.* One is led to ask whether simply halving or doubling the intensity of stimulation could produce the same effect as changing from binocular to monocular viewing or vice versa. But in fact rather the opposite is the case. At weak intensities the field appears much more 'oily' and is traversed by wriggling, interlocking snake-like shadow-patterns that may each extend over much of its area. At the normal intensity of a television screen, however, a change by a factor of 2 (or more) makes little difference. Binocular fusion here involves clearly more than the central summation of retinal impulses from 'corresponding points'. This is in line with the discovery by Jung[5] and others of separate cortical representations of each retina (in the cat).

(4) *Steady illumination in one eye.* It might be suggested that the occlusion of one eye causes centrifugal feedback, to both retinæ, which enhances the sensitivity of the unoccluded eye. As a partial test of this hypothesis the unused eye can be flooded with uniform illumination adjustable in brightness above or below the average level of the random source. The effect, however, is negligible, and not at all comparable with that of using both eyes to view the source.

(5) *The 'frame-adhesion' effect.* One of the most pleasing effects occurs when a portion of the random field is 'framed' within a movable contour such as the outline of a loop of wire. When the wire frame is moved and followed with the eye, the whole assemblage of 'Brownian particles' within it (and those immediately outside) are unmistakably seen to move with it. If the motion of the frame is rapid and oscillatory, the particles show a kind of inertia, lagging behind and swirling just as if immersed in a fluid.

When, however, a stationary object such as a finger-tip is placed within the framed area, practically the whole is suddenly stabilized, 'adhering' to the finger-tip. If the finger in turn is moved, it appears to be 'pursued' by a swarm of particles.

That the interactions concerned are central, rather than retinal, can be seen by presenting the noise to one eye and the frame to the other, when the effects are much the same, apart from a somewhat more noticeable time-lag in the coupling between frame and noise field.

(6) *Complementary images* As already reported[1] the effects of superimposing random noise on spatially repetitive patterns are especially striking When a source of noise is viewed through a transparency such as Fig 1, the otherwise chaotic disposition of the 'particles' is at once organized into one or several superimposed geometrical figures (the 'complementary image' (ref 1)) the principal directions of which run at right angles to the lines of the repetitive pattern A similar but transient reorganization' is seen if the random source is viewed alone, immediately after inspection of the repetitive pattern

The phenomenon is extremely tolerant of the optical definition of the random source Even with a thin sheet of paper between the pattern and the television screen, the complementary image is clearly visible An impression of a typical figure complementary to the stimulus of Fig 1 is shown in Fig 2 If the stimulus figure is a 'grid' formed by superposing sets of parallel lines at right angles, the complementary image lines run diagonally

The complementary figures so generated are generally seen in motion With Fig 1, for example, the subject typically reports two or more superimposed 'rosettes' of the type of Fig 2, which may be seen as contra-rotating, or all rotating clockwise or anticlockwise, more or less at will If Fig 1 is itself rotated, the complementary image rotates in the opposite direction

When Fig 3 is used, a petalloid radial complementary image is seen, with strong radial streaming along the petals In addition, however, there is a slow and steady rotation of the noise field, with an angular velocity, varying with the radius, of approximately one radian a second This turns out to be a quite distinct phenomenon, not dependent on spatial repetitiveness , for if visual noise is viewed through even a single annular aperture, it is still seen to rotate in each direction alternately

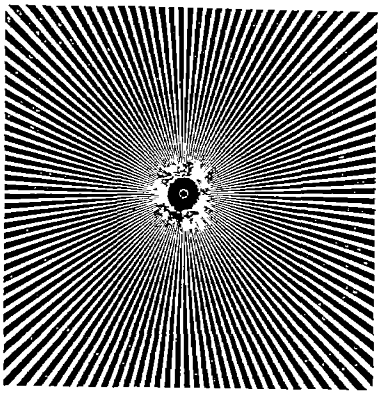

Fig 1

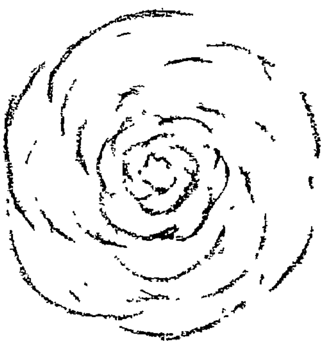

Fig 2

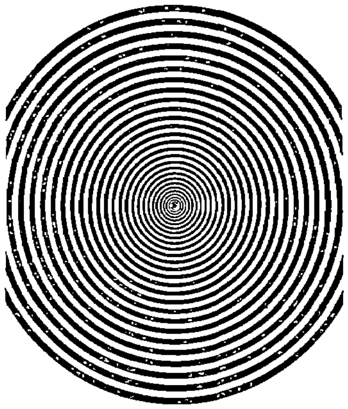

Fig 3

(7) *Implications* It is not my purpose here to advance interpretations of the phenomena described, on which we are working at present , but if they demonstrate anything clearly, it would seem to be the strong synthetic element in perception, which is present even when 'associations' in the usual sense might be expected to be absent What is seen under excitation by random noise bears only an approximate relation to the stimulus Instead, even against a blank background, it seems to be a fairly strongly structured synthetic 'imitation'—that is, a distribution of activity matched presumably in respect of certain statistical features, but sufficiently well structured for changes in its position to be readily attributable and easily perceived (Exp 5) With a background of a regularly periodic nature, the structure seen bears still less resemblance to that of the stimulus

The foregoing are only samples of the new data revealed by this way of 'shock-exciting' the visual system, which will be discussed more fully elsewhere The present article is intended mainly to direct attention to the power and potential value of this class of stimulus In communication engineering, random noise is often used as a 'neutral' test-signal to reveal the characteristic modes of response (resonances and the like) of an information-system In the study of the visual system it seems clear from the foregoing examples that visual 'noise' offers a tool with a corresponding ability to uncover some characteristic modes of response of perceptual mechanisms Formally, it can be regarded as the neutral member of a family of stimuli at the extremes of which are stroboscopic illumination (spatially uniform, temporally periodic) and our parallel-line patterns (temporally uniform, spatially periodic) At each of these extremes characteristic responses are also evoked[1,6], and it can scarcely be doubted that all three classes of phenomenon have something to contribute to our theorizing about perceptual processes

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LETTERS TO THE EDITORS

ASTROPHYSICS

Presence and Correlation of Fine Structure in Jovian Decametric Radiation

JUPITER's non-thermal emission in the decameter region of the spectrum takes the form of sporadic radio noise storms having durations ranging from minutes to several hours[1]. Observers agree that the storms show fine structure, in part composed of bursts which typically have durations ranging from several tenths of a second to several seconds. However, there has not been agreement as to the presence of still finer structure or the intrinsic origin of any of the fine structure.

In 1956 Kraus[2] and Gallet[3] reported very short bursts having durations of tens of m sec or less, which they associated with Jupiter, but other observers[4-7] have been unable to confirm this. As for the origin of fine structure, radio sources of angular size much greater than Jupiter often show pronounced ionospheric scintillation with components occasionally as short as several seconds[8,9], the Jupiter source must be expected to show at least similar effects. Recordings made by Gardner and Shain[4] on three nights in 1956, using receivers spaced 25 km east–west, indicated little correlation of the noise fine structure over this base-line, the lack of correlation suggesting that ionospheric irregularities might account for much if not all of the burst structure. On the other hand, a spaced-receiver observation by us in 1958 (ref 10), while confirming the existence of considerable amplitude distortion, showed 80 per cent correlation in the time of arrival of bursts at stations separated by as much as 30 miles east–west. Florida–Chile observations in 1960 by A. G. Smith and Carr[11] also indicated partial correlation over a very long base-line, suggesting the reality of some intrinsic fine structure.

To resolve these questions a new series of base-line correlation observations was initiated by us in March 1961. Four identical crystal-controlled total-power receivers working at 22 200 Mc./s with 6 kc./s band-width were fed with identical antennas. The locations chosen were the Yale Observatory's Bethany Observing Station (central site), Hendrie Hall at New Haven (15 km south of Bethany), Van Vleck Observatory at Middletown (30 km east of Bethany), and Pomfret School (100 km east of Bethany). The audio outputs of the receivers were transmitted to Bethany on telephone lines. During Jupiter storms all four channels plus timing marks were recorded on a high-speed photographic recorder with a time constant of 30 msec. Records obtained on 19 nights up to August 1 exhibited various degrees of correlation, including 6 nights on which correlation was essentially perfect, without amplitude distortion. Correlation tended to improve through the Jupiter observing season from March onwards, suggesting that the phase with respect to Jovian opposition (July) or to the terrestrial season (summer) may be significant.

A small portion of a storm with correlated events (July 18) is reproduced in Fig. 1 (the New Haven receiver was not functioning during this run). Storms with similar portions of closely correlated events were observed on June 5 and July 11, 20, 27, and 30. No form of ionospheric effect is known that preserves its correlation over 100 km., accordingly we conclude that fine structure such as that of Fig. 1 is characteristic either of the source at Jupiter or of the interplanetary medium, but that this intrinsic structure is often largely obliterated by local ionospheric effects.

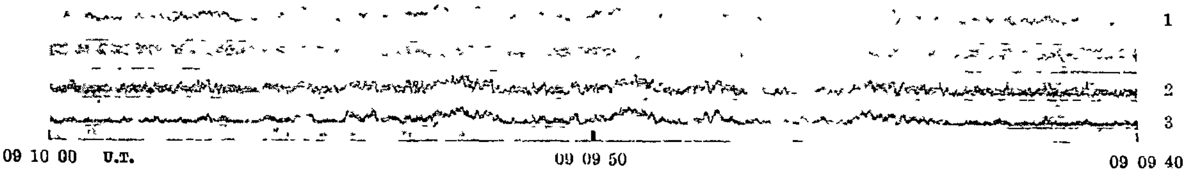

Fig. 1. A 20-sec portion of the high-speed recording of the Jupiter storm of July 18, 1961, showing close correlation of fine-structure events received at Middletown (3) and Pomfret (2) stations separated by 30 and 100 km. respectively from the Bethany Station (1)

During this observing season we noted the presence of numerous correlated fine-structure components having durations of hundredths rather than tenths of seconds. In comparison, our observations during 1960 showed no events having durations less than 0.1 sec. We must regard this extreme fine structure as real, in view of its correlation over long base-line and association with Jupiter storms (not present before or after). Thus the occasional existence of Jupiter bursts of extremely short duration as reported by Kraus and Gallet seems to be confirmed.

The co-operation and assistance particularly of E. Milone of Yale Observatory, Dr. F. Zabriskie of Van Vleck Observatory, J. McCullough of Pomfret School, and financial support from the National Science Foundation are gratefully acknowledged.

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GEOPHYSICS

Confirmation of Outer Atmospheric Asymmetry postulated to explain the False Zodiacal Light

IN 1952 and afterwards[1-3], I suggested that the false zodiacal light—an early morning pyramid of light above the western horizon, resembling the zodiacal light which at that time would be on the opposite side of the sky—might be explained if Earth's gaseous tail were driven off mainly from the western twilight rim of the globe I can now point to some recent findings that support this idea[4,5]

A terrestrial gaseous tail has been repeatedly postulated (by Lomonosov in 1753, Houzeau in 1888, Evershed in 1902, Astapovich[6], Fesenkov[6], Divari[6], and many others) to explain the counterglow or gegenschein, an antisolar luminosity with a variable brightness and a gaseous emission spectrum (Karimov[6], Tikhov), which Soviet parallax measurements place at an apparent distance of ~ 20 Earth radii The gases forming such a tail could only be of atmospheric origin, presumably solar radiation pressure carries off atoms or molecules thermally expelled in high trajectories from the underlying denser atmosphere I pictured "the solar heating steadily unwinding a ribbon of gas from the upper atmosphere into the ecliptic plane" (ref 2, p xviii)

The false zodiacal light, observed and named by Divari and Fesenkov in 1946 (ref 6, p 22) but previously described by Sykes[7] and by Schmid[7], is presumably a luminosity in the lower part of the tail, below the counterglow The counterglow luminescence is thought to be excited by solar corpuscular emission in the region beyond the terrestrial magnetosphere (Astapovich[6]) To-day one may suspect that the nearer luminosity of the false zodiacal light is connected with the van Allen radiation, though other theories are possible[1,2,6]

Most remarkable is the asymmetry of the false zodiacal light it is seen in the western sky as a post-midnight opening-out of the counterglow ellipse, but it is never seen in the eastern sky If the gaseous tail itself is asymmetric, the reasons could be that (*a*) the rotation of Earth assists the solar radiation pressure at the evening twilight rim of Earth, but opposes it at the morning twilight rim, (*b*) at the evening twilight rim the atmosphere has been heated by the Sun, so that the supply of thermally accelerated exospheric particles, that is, the density of the exterior atmosphere, would here be significantly greater than on the morning rim (ref 2, p xvi)

Point (*b*) is now confirmed by the exospheric densities calculated from the orbital behaviour of artificial satellites Beginning at ~ 200 km height above the terrestrial ellipsoid, there is a minimum of density at 6 hr true local time and a maximum at 14 hr The difference between maximum and minimum steadily increases with altitude, and at 1,700 km, according to the model used by Romer[5], it exceeds two orders of magnitude At 18 hr the pressure remains high, at 700 km height the supply of gas particles is ~ 7 5 times greater on the evening rim than on the morning rim of Earth (ref 4, Fig 4), at 1,700 km it is ~ 12 times greater (ref 5, Fig 2)

The exterior atmosphere is chiefly maintained by solar energy absorbed and stored at lower (*F*-layer) levels This explains the lag of the density maximum toward the afternoon and the continuing higher density in the evening

The false zodiacal light, seen only in the west, must not be confused with the zodiacal twilight[8,9]—the atmospheric component of the ordinary zodiacal light—which according to Divari's specific finding appears with equal intensity on the western and eastern horizons Moreover the zodiacal twilight is concentrated toward the horizon, while the false zodiacal light, opening out from the counterglow (Divari[6], Fig 10), is strong at higher angles of elevation

It is also obvious that the false zodiacal light, extending from the counterglow, is unrelated to the cosmic dust-cloud component of the ordinary zodiacal light Aside from the nearness, the variability and the emission spectrum of the counterglow as demonstrated by the Soviet authors, there is Roach and Rees's demonstration[10] that the counterglow and the cosmic zodiacal light lie in slightly different planes The former is, as expected, in the ecliptic, while the cosmic dust cloud is symmetrical with a plane through the Sun determined mainly by Jupiter's gravitation[11] (Long-lasting confusion has been caused by the reports of those who profess themselves unable to find the slightest asymmetry or variability of the zodiacal light with respect to the ecliptic These reports may be dismissed as pointless, because there is no imaginable reason why the zodiacal dust cloud around the Sun should lie precisely in the ecliptic, which is merely the orbital plane of the Earth)

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BIOPHYSICS

Extendability of Tendons undergoing Contraction-Relaxation

EQUILIBRIUM melting of tendons[1] and collagenous tissues[2] follows the theoretical behaviour expected for a first-order phase transition Thus, the ordered crystalline regions of polypeptide chains disintegrate into randomly oriented coils of the amorphous phase Rat-tail tendons subjected to melting by lyotropic agents undergo rapid contraction followed by relaxation to rupture, and these physical events are easily measured characteristics of the melting process Stress-strain curves of thermally shrunk tendons have been shown to obey equilibrium elastic theory for amorphous polymers[3], for example, stress is proportional to $(\alpha - \alpha^{-2})$, where α is the strain This communication deals, however, with a kinetic analysis of melting of rat-tail tendons in concentrated aqueous urea at *p*H 7 0 and 37° C

When the velocity of length-time observations (Fig. 1) was analysed kinetically, equation 1 was found to agree with the data The terms involved are dL/dt, the velocity of change of length at any time (t), $(dL/dt)_c$, the initial velocity of contraction, $(dL/dt)_r$, the terminal velocity of relaxation, and K_{cr}, the specific rate-constant

$$\text{Log}\,[dL/dt - (dL/dt)_r] = \log\,[(dL/dt)_c - (dL/dt)_r] - K_{cr}t \quad (1)$$

Temperature had a pronounced influence on the rate process, but it systematically followed equation 2

$$\text{Log}\,K_{cr} = A + B/T \quad (2)$$

The resistance to disruption of ageing tendons in 10 M urea solution, furthermore, increased according to equation 3

$$\text{Log (time-to-break)} = C + D(\text{age, or weight of animal}) \quad (3)$$

Generally, it is believed that the attack on collagen by urea breaks stabilizing hydrogen bonds[4] A complication in the selectiveness of this protein–urea interaction is that a condensation can occur between free amino groups of the protein with a slight amount of ammonium cyanate which exists in equilibrium with aqueous urea[5] This should not, however, interfere with hydrogen bond rupture, in fact it could promote it by assisting to liberate one conjugor (amine side-chains of lysine or hydroxylysine) from a previously existing hydrogen bond with those groups It has also been shown that aqueous urea condenses with long hydrocarbon side-chains to form 'adduct' compounds[6] Since collagen in tendons has few such long hydrocarbon side-chains, it does not appear that adduction of urea presents any great complication

Accepting that action of urea results in rupture of secondary, tertiary, and higher-order —OH stabilizing bridges[4], we investigated next how this depolymerization influenced tendon elasticity To answer this question experiments were conducted in which the momentary extension of a tendon was measured by adding and removing a weight. A change in length ($\Delta L'$), illustrated in Fig 1, was determined periodically and superimposed on the contraction–relaxation sequence Fig 2 shows a plot of strain $(1 + \Delta L'/L)$ as a function of contraction–relaxation time

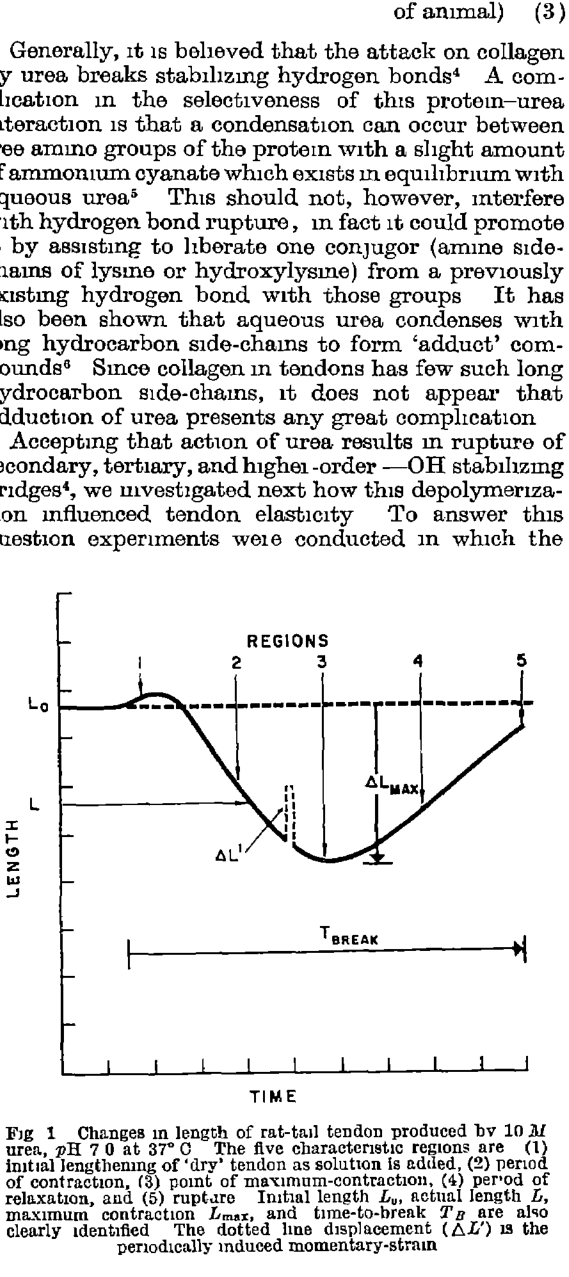

Fig 1 Changes in length of rat-tail tendon produced by 10 M urea, pH 7 0 at 37° C The five characteristic regions are (1) initial lengthening of 'dry' tendon as solution is added, (2) period of contraction, (3) point of maximum-contraction, (4) period of relaxation, and (5) rupture Initial length L_0, actual length L, maximum contraction L_{max}, and time-to-break T_B are also clearly identified The dotted line displacement ($\Delta L'$) is the periodically induced momentary-strain

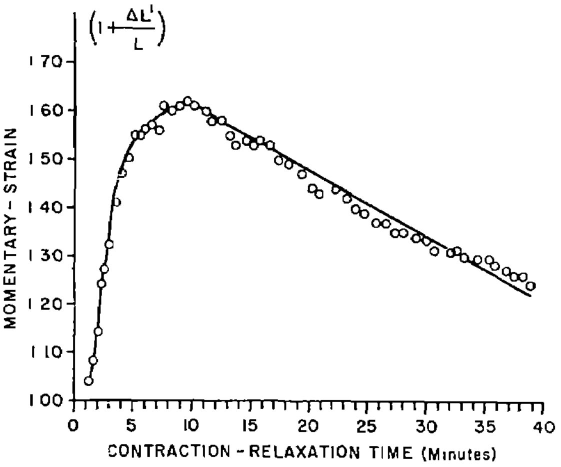

Fig 2 Dependence of momentary-strain $\left(1 + \frac{\Delta L'}{L}\right)$ on time of contraction–relaxation

Figs 1 and 2 reveal that a tendon becomes more easily extendable as a result of the action of urea which also produces shortening and re-extension Maximum extendability occurs near the point of maximum contraction, and it decreases with subsequent lengthening of the tendon It is obvious, therefore, that extendability and contraction–relaxation are inversely related

The change of strain with time $\frac{d}{dt}(\Delta L'/L)$ was evaluated and plotted as a function of time Numerous graphs of these data suggested that there was a semi-logarithmic relationship between velocity of strain and time A final graph was made of the difference between velocity of strain at time (t) and that constant velocity of strain which characterized the relaxation period A linear relationship was observed with this function, and equation 4 was derived on the basis of these numerical analyses

$$\text{Log}\left[\frac{d}{dt}(\Delta L'/L) - \frac{d}{dt}(\Delta L'/L)_r\right] = \log\left[\frac{d}{dt}(\Delta L'/L)_c - \frac{d}{dt}(\Delta L'/L)_r\right] - K'_{cr}t \quad (4)$$

These measurements of the rate of increased tendon extendability during contraction support the interpretation that the latter reflects a crystalline/amorphous phase transition, or melting It is not clear, however, why extendability and re-extension are inversely related because the tendon is continuing to degrade to the point of rupture A maximum degree of shortening would appear to be theoretically possible but this may not be realized due to possible independence of $(dL/dt)_c$ and $(dL/dt)_r$ Consideration of factors responsible for incomplete shortening and relaxation might lead to a better understanding of the kinetic mechanism of contraction–relaxation Finally, this could give a more precise explanation of effects of ageing on rate of tissue dissolution such as shown by equation 3

Partial financial support from the Greater Miami Heart Association, and grant *A*–1155, National Institutes of Health, Bethesda, is appreciated

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METALLURGY

New Ternary *R*-Phases with Silicon

THE *R*-phase was first found[1] in the chromium–molybdenum–cobalt ternary system. Its crystal structure has been determined by Shoemaker *et al* [2] Additional *R*-phases were later found in the molybdenum–manganese–cobalt and molybdenum–manganese–iron ternary systems[3], as well as in the titanium–manganese binary system[4] Recently, in the investigation of several ternary systems with silicon a number of new *R*-phases have been identified Table 1 gives the approximate composition and the lattice parameters of these new *R*-phases

Table 1 COMPOSITION (APPROXIMATELY) AND LATTICE PARAMFTERS OF NEW *R*-PHASES

Alloy	Annealing temperature (° C)	*c* (Å)	*a* (Å)	*c*/*a*
$Ti_2Mn_{79}Si_{19}$	1,000	19 23	10 87	1 77
$Nb_5Mn_{79}Si_{16}$	1,000	19 28	10 89	1 77
$Ta_5Mn_{79}Si_{16}$	1,000	19 19	10 86	1 77
$Mo_3Mn_{78}Si_{19}$	1,000	19 18	10 85	1 77
$W_3Mn_{78}Si_{19}$	1,000	19 22	10 86	1 77
$V_{37}Fe_{41}Si_{22}$	1,100	19 23	10 79	1 78
$V_{45}Co_{40}Si_{15}$	1,100	19 14	10 78	1 78
$V_{45}Ni_{40}Si_{15}$	1,100	19 10	10 82	1 77

It is interesting to note that in all alloys as yet known to consist of the *R*-phase one of the component elements has a relatively large co-ordination number 12 atomic radius in relation to that of the others For example, in all the *R*-phases listed in Table 1, the ratio of the co-ordination number 12 radii of the two transition elements lies between 1 07 and 1 12 In the case of the ternary *R*-phases with manganese and silicon the concentration of the element with the large atomic radius needed in order to stabilize the *R*-phase may be quite small, for example, 2 *a*/*o* titanium, 5 *a*/*o* niobium or tantalum, 3 *a*/*o* molybdenum or tungsten However, the amount of vanadium required in the ternary systems with iron, cobalt or nickel and silicon is very much larger

It has now been established that the unidentified phases previously designated as I, III and V co-existing with the σ-phase in the vanadium–iron–silicon, vanadium–cobalt–silicon and vanadium–nickel–silicon systems[5] are identical with the corresponding *R*-phases listed above Previously unidentified phases II and IV also co-existing with the σ-phase in the vanadium–iron–silicon and vanadium–cobalt–silicon systems have now been identified as having the α-Mn structure An α-Mn phase also occurs in the vanadium–nickel–silicon system Details of these phase relations will be published

This work was supported by the U S Army Research Office, contract number *DA*-11-022-*ORD*-1175

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α-Manganese Phases containing Technetium-99

THE existence of α-manganese phases in the zirconium–technetium, niobium–technetium and molybdenum–technetium systems was recently reported by V B Compton *et al* [1] During the course of our investigation of alloy phases involving technetium-99 with transition metals, α-manganese phases were found in the binary systems scandium–technetium, titanium–technetium, hafnium–technetium and tantalum–technetium Pertinent information is given in Table 1 The alloys were made by arc-melting transition metals of high purity with technetium-99 X-ray data were obtained from specimens in the as-cast condition with a powder camera of 114 6 mm diameter using filtered copper radiation

Table 1 LATTICE PARAMETERS OF SEVERAL α-MANGANESF PHASES CONTAINING TECHNETIUM-99

System	Alloy composition, atomic per cent	X-ray data a_0 (Å)
Sc–Tc	87 5 Tc, 12 5 Sc	9 509 ± 0 001
Ti–Tc	87 5 Tc, 12 5 Ti	9 579 ± 0 001
Hf–Tc	87 5 Tc, 12 5 Hf	9 603 ± 0 001
Ta–Tc	83 3 Tc, 16 7 Ta	9 565 ± 0 001

Crystal structure and lattice dimensions of technetium-99 have already been reported by R C L Mooney[2], using a microgram specimen He found a hexagonal close-packed structure with $a_0 = 2\ 735 \pm 0\ 001$ Å, $c_0 = 4\ 388 \pm 0\ 001$ Å, $c/a = 1\ 604$ In the present investigation lattice dimensions of technetium-99 were obtained from an annealed needle of high-purity technetium-metal The dimensions are as follows $a_0 = 2\ 743 \pm 0\ 001$ Å, $c_0 = 4\ 400 \pm 0\ 001$ Å, and $c/a = 1\ 604$ Since Mooney reported a slight impurity in his mixed sample, the lattice parameters reported here are probably more accurate

This work was performed under the auspices of the U S Atomic Energy Commission

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A Cold Crucible for High-Temperature Melting Processes

Exploratory research in the solid-state field has resulted in the development of a new melting technique for the processing of silicon. Although this method has been associated primarily with the purification of silicon and its growth as single crystals, it has important applications in the more general field of metallurgy.

In our earlier work[1], a container was made from a silver tube by pressing a longitudinal dent into its surface for most of the length. This silver 'boat' was internally water-cooled and contained a silicon bar in the depression. When power was applied to a short radio-frequency coil surrounding a small part of the boat, a narrow molten zone was formed in the bar. This zone was traversed along the bar to zone-refine the silicon and leave only the impurity boron, which does not segregate appreciably in silicon. The silver boat acts as a radio-frequency transformer, and current is induced into the silicon bar from the silver underneath it, as well as from the primary coil above it. The liquid silicon does not wet the cold silver surface; it should be emphasized, however, that melting is complete, without any skin or shell of solid silicon.

The results of pinch effect caused by mutually opposing electric currents can be observed following changes in the power setting or movements of the coil. Partial levitation of the melt can be obtained with suitably designed coils, particularly at a frequency of 500 kc./s. or lower; but at frequencies of 2 Mc./s. and above, these magnetic effects are very much reduced.

Another form of horizontal boat which has certain advantages in special cases can be made by using several straight parallel tubes mounted in a semicircle and closely spaced, so that the melt is confined in the boat by its surface tension.

The growth of silicon single crystals has been accomplished in the past by pulling from silica crucibles or by means of a crucible-free method (see, for example, ref. 2). The methods suffer from some disadvantages; in the former method, oxygen is introduced into the growing crystals due to chemical reaction between molten silicon and silica; in the latter procedure the density of dislocations tends to be high, and the diameter of the crystal is limited in size. Some attempts were made to grow single crystals horizontally in the silver boat, but these failed mainly on account of the large temperature gradient at the solid/liquid growing interface.

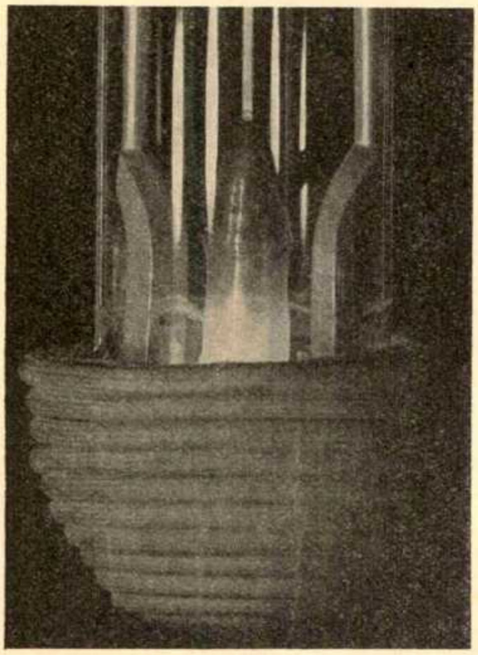

Fig. 1. A silicon single crystal grown from a cooled silver crucible

In order to obtain a more favourable thermal symmetry and a correct temperature distribution for the growth of single crystals, several novel types of crucible[3] have been designed. In one particular form (Fig. 1) a number of tapered and water-cooled silver tubes is arranged vertically, and curved inward at the bottom to join at their narrow ends and form a basket. This crucible is water-cooled internally, and mounted inside a radio-frequency primary coil which has the shape of an upturned skep. Power is induced through the spaces between the tubes, and also by induction due to the current which flows circumferentially in each tube. The tubes are placed sufficiently close together to prevent the melt, when formed, from escaping. Again it is important to note that melting is complete, and that no thin skin of silicon remains as in 'skull melting'.

Single crystals of silicon which are oxygen-free and dislocation-free have been grown by the Czochralski method from crucibles of this and similar types, using silicon which was obtained by the thermal decomposition of pure silane gas[4]. Life-times up to 1 msec. have been measured and resistivities greater than 1,000 ohm cm. have been preserved. Electrical and radiochemical tests have not detected the presence of silver in these crystals.

The cold-metal crucible has been also successfully used as a non-contaminating container for the melting and processing of such materials as ferrites and allied oxides, rare earth metals, molybdenum, titanium, uranium and boron; it is not restricted to high-temperature melting applications only.

We wish to acknowledge the work of Mr. F. J. Raymond, who is a co-inventor, and also other assistance given by colleagues of Standard Telecommunications Laboratories, Ltd., who are associated with this project. We thank the management of these Laboratories for permission to publish this communication.

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RADIOCHEMISTRY

Organic Extraction from Fused Uranium Chlorides

Fused plutonium and uranium chlorides may be a good nuclear fuel for a fast breeder reactor[1–4]. The aim of the present work is to investigate some chemical properties of fused chloride components, in particular, the presence of dissociated or associated or non-ionic molecules, and the possibility of using the liquid salt–liquid organic extraction. Some recent results concerning the extraction processes with fused

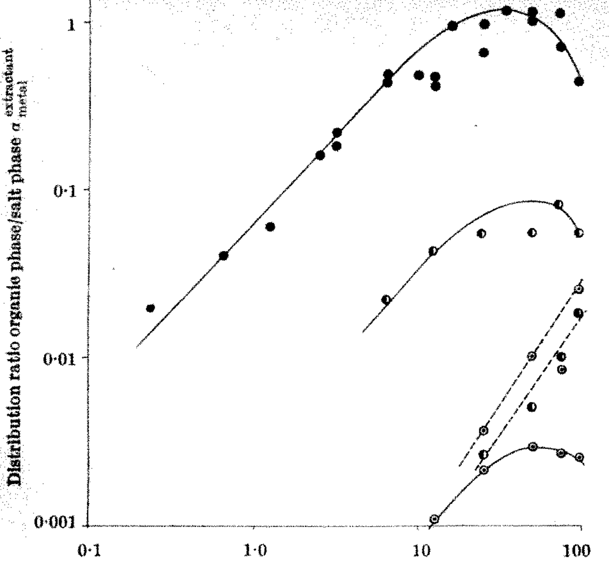

Fig. 1. ●, Uranium; ◐, copper; ⊙, potassium ——, TOA; - - -, TBP

salts are presented in ref. 5. In the present investigation the salt phase contains: diluent (nuclear non-active): eutectic mixture of KCl–CuCl, 1 : 2 molar ratio, and fissionable material: uranium tetrachloride (prepared by the reaction, uranium dioxide + carbon tetrachloride at 450° C.) with a concentration of uranium tetrachloride of 1·5 per cent w/w. The salts were dried under vacuum by heating and fusing in the presence of dry hydrochloric acid and dry argon, at a temperature of 180° C. The organic phase contains: diphenyl as diluent and one of the following extractants: tri-*n*-octylamine (TOA), tri-*n*-butylphosphate (TBP), di-*n*-butylphosphoric acid (HDBP); dried in vacuum by heating. All components of fused salts were used as labelled atoms: copper-64, potassium-42, chlorine-36, uranium enriched, registered by suitable radiometry α, β, γ. The extraction process was carried out in a small volume, 1·6 ml., in 180° C., for 30 min. (15 min. with shaking): after separation of both phases (using different solidification temperature) they are dissolved: salt phase in nitric acid solution, organic phase in toluene or benzene. All operations were performed in glove boxes with dried atmosphere. The distribution ratio $\alpha^{\text{extractant}}_{\text{metal}}$ for each metal is measured as ratio of total radioactivity of organic phase to salt phase (the volume ratio of the two phases is approximately 1). The results are shown in Fig. 1.

The experimental distribution ratios are as follows:

uranium: $\alpha^{\text{TBP}}_{\text{U}} \geqslant \alpha^{\text{HDBP}}_{\text{U}} \geqslant \alpha^{\text{TOA}}_{\text{U}}$

copper: $\alpha^{\text{TOA}}_{\text{Cu}} \gg \alpha^{\text{TBP}}_{\text{Cu}} > \alpha^{\text{HDBP}}_{\text{Cu}}$

potassium: $\alpha^{\text{TBP}}_{\text{K}} > \alpha^{\text{TOA}}_{\text{K}}$

TOA: $\alpha^{\text{TOA}}_{\text{U}} \gg \alpha^{\text{TOA}}_{\text{Cu}} \gg \alpha^{\text{TOA}}_{\text{K}}$

TBP: $\alpha^{\text{TBP}}_{\text{U}} \gg \alpha^{\text{TBP}}_{\text{K}} > \alpha^{\text{TBP}}_{\text{Cu}}$

From these results, it may be concluded that: in fused chlorides there are cations K^+ and Cu^+; non-ionic molecules, CuCl, Cu_2Cl_2 and potassium chloride; anions, $Cu_2Cl_3^-$, UCl_5^-.

For the CuCl–TOA extraction system, with a small concentration of TOA: $0·1\ M \leqslant$ concentration TOA $\leqslant 0·3\ M$, the ratio of molar concentration, copper/TOA in the organic phase is about 1 : 3, which corresponds to the formula $[TOACu^+]\ [Cu_2Cl_3^-]$. It also seems that for uranium tetrachloride–TOA extraction the extractable species is an ion-pair, namely, $[TOACu^+]\ [UCl_5^-]$.

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A Simple Microdiffusion Technique for the Radioiodination of Proteins

A NUMBER of methods for the radioiodination of proteins and amino-acids have been described[1,2]. Some have relied on transfer of elemental iodine from an oxidizing medium by extraction with an organic solvent ; in others oxidation of iodide to iodine has been effected in the presence of the material to be iodinated with the associated risk of damage to the latter by the oxidizing reagent in the mixture.

Many of these techniques are somewhat cumbersome; moreover, some are such that little confidence can be felt either in the purity and integrity of the product or in the safety of the experimenter.

Faced with the task of labelling insulin with iodine-131 at high specific activity (~ 100 mc./mgm.) we have used Conway's microdiffusion technique[3], using in the first instance No. 2*a* diffusion units and more recently modified cells of smaller (~ 1 cm.). diameter. The technique has also been successfully applied to the radioiodination of beta- and gamma-globulins, albumin and tyrosine.

The material to be iodinated, dissolved in 0·2 *M* borate buffer (*p*H 8·0), is placed in the central chamber of the unit ; radioiodine diluted with the desired amount of inactive carrier is placed in the outer compartment together with an appropriate volume of acidified dichromate. Diffusion of iodine vapour into the central compartment is largely complete in a matter of 2 hr. (80–90 per cent is a typical recovery in most cases) although the exposure may be terminated at any desired time by addition of excess alkali to the outer chamber. Adequate distribution of iodine through the protein solution is effected either by agitation of the unit or by use of a small magnetic stirrer in the central compartment.

The maximum permissible volume of the oxidizing mixture is critically dependent on the weight of carrier iodine available for transfer. Where μgm. quantities of protein are to be iodinated, adequate transfer-rates of the μgm. amounts of iodine usually desired in these circumstances can only be obtained using oxidizing mixture volumes of a few microlitres. This places a lower limit on the amounts of protein of less than 5 μgm. which may conveniently be used, and hence indirectly on the specific activities which may be obtained with tolerable starting amounts of iodine-131. Nevertheless, specific activities of the order of 100 mc./mgm. insulin can readily be achieved using about 3–5 mc. iodine-131.

Radiochemical purity of the iodinated protein has been checked by electrophoresis and, in the case of insulin, by chromatography in a secondary butanol/

acetic acid solvent system[4]. No loss of biological activity of the iodinated insulin was observed using the rat epididymal fat pad bioassay technique.

Overall recovery of radioactivity in the final product has been of the order of 25 per cent. Though this implies considerable wastage of iodine-131, it has been felt that the advantages in this method of the mildest possible oxidative environment for the protein and the minimal radiation damage to which the latter is exposed compared with other techniques merit its application in many circumstances.

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CHEMISTRY

Pyrolysis and Gas Chromatography for the Detection of the Benzene Ring in Organic Compounds

In a systematic investigation concerning the correlation between structure and pyrolysis products, it was found that pyrolysis of compounds eluted from gas chromatographic columns could be used for the detection of the benzene ring when present in organic compounds.

A conventional gas chromatograph was used in the experiments. The top of the column was provided with a heated quartz tube filled with 'Chromosorb' (about 2 gm.). Silicon oil was used as stationary phase.

The quartz tube was connected to a short thick-walled glass capillary with silicone rubber tubing. The capillary was closed with a serum-cap and the carrier gas was led into the column through a side tube just behind the serum-cap. The quartz pyrolysis tube was surrounded by a small furnace of which the temperature was measured with a thermocouple between the walls of the furnace and the pyrolysis tube. The compound under investigation was injected through the serum-cap in the usual way on to the hot 'Chromosorb'. Of liquid compounds 1 µl. was injected. Of solid compounds 5–10 µl. of a solution of the compound in methanol was used.

Some 100 different aromatic compounds containing a benzene ring were tested, and experiments with benzyl alcohol, benzylbenzoate and benzyl phenylacetate are shown in Fig. 1 as characteristic examples. It is seen that two peaks are present in the chromatograms. One peak was identified by infra-red spectroscopy as benzene, the other as toluene. This behaviour is characteristic for compounds that contain the benzene ring and a side-chain or groups such as —OCH_3 or —$OOCH_3$. The formation of toluene in these cases may be explained on the basis of free radical mechanism in which methyl radicals are formed from the —OCH_3 and —$OOCH_3$ groups respectively. This is made evident by the following experiment. When a saturated solution of benzoic acid in methanol is pyrolysed a small toluene peak is observed following a large benzene peak. When, however, a saturated solution of benzoic acid in chloroform instead of methanol is pyrolysed no toluene peak is found. A small peak with the correct retention time of chlorobenzene now appears next to benzene. For the compounds tested the ratio of the peak areas is a typical constant for the compound which is pyrolysed when the experimental conditions are kept identical (Table 1).

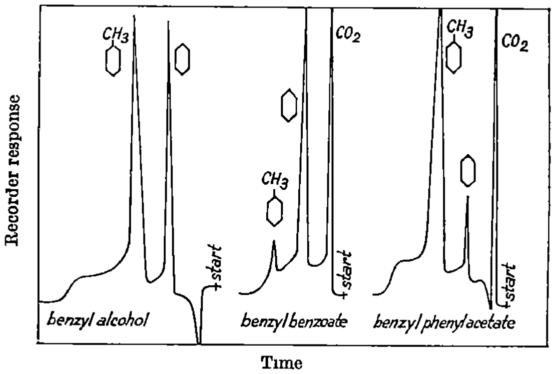

Fig. 1. Chromatograms of pyrolysis products of benzyl alcohol, benzyl benzoate and benzyl phenylacetate

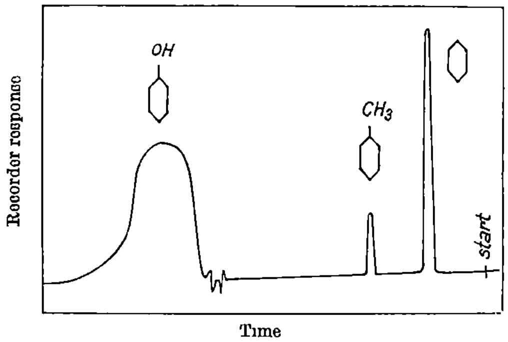

Fig. 2. Chromatogram of the pyrolysis products of anisole

The result of the pyrolysis of anisole is shown in Fig. 2. The formation of phenols was always observed in the pyrolysis of compounds containing the C_6H_5O-group such as salicylic acid (pyrolysed in methanol solution), *p*-cresylacetate, anisole and others.

The formation of benzene and simple benzene derivatives on pyrolysis of aromatic compounds was thus found to be a very general reaction for these compounds. Although it is known that benzene can be synthesized from simple aliphatic compounds by pyrolysis, no benzene peaks were observed when aliphatic compounds were tested under normal conditions. Aromatization clearly is negligible under the conditions used.

During the experiments recorded here separate series of tests on minute amounts of simpler organic

Table 1. Ratio of Peak Areas at Pyrolysis Temperature 550° C.

Compound	Area of toluene peak / Area of benzene peak
Toluene	7·5
Toluene	6·9
Toluene	7·9
Benzyl alcohol	1·7
Benzyl alcohol	1·7
Benzyl alcohol	1·8
Benzyl phenylacetate	5·4
Benzyl phenylacetate	4·9
Benzyl benzoate	*c.* 0·12
Benzyl benzoate	*c.* 0·12
Anisole	*c.* 0·127
Dioctyl phthalate	*c.* 0·08

compounds such as aliphatic alcohols, amines, acids and esters were pyrolysed and analysed in the way described From all these compounds characteristic chromatograms of the pyrolysis products were obtained which made it possible to differentiate between compound groups, individual members of homologous series and between isomers such as normal- and iso-alcohols

It is believed that a valuable technique for identification of the small amounts of material eluting from gas chromatographic columns can be based on this method of pyrolysis

The method does not seem to be restricted to volatile compounds[1]

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A Coating with Antifouling Properties from the Reaction between Cuprous Oxide and Sodium Silicate

Films prepared from dispersions of zinc dust in sodium silica media of a high silica ratio of about Na_2O $SiO_2/1$ 3 8 by weight (Pass, A , and Meason, M J F , private communication) have been reported to become substantially insoluble in water within a few hours without the aid of curing agents previously needed to produce satisfactory air-drying zinc silicate paints[1] The mechanism of the formation of these films is only partially understood, and in the course of work undertaken to elucidate it, changes in viscosity occurring when zinc and other pigments are dispersed in silicate media are being examined

Various pigments were dispersed in aqueous solutions containing 16 6 per cent of sodium silicate having an Na_2O SiO_2 ratio of 1 · 3 55 by weight, and the changes in viscosity of the dispersions with time were measured at a shear-rate of 54 sec $^{-1}$ using a Couette co-axial cylinder type viscometer

Fig 1 shows curves which illustrate the viscosity/time relationships characteristic of the two main groups of pigments which were studied Dispersions of non-reactive pigments such as talc, micaceous hæmatite, titanium dioxide and iron oxide decrease in viscosity with time due to an improvement in dispersion brought about by the action of the viscometer Films produced from these dispersions will only become insoluble in water on long exposure in air or by the application of heat or curing agents In contrast, the viscosity of dispersions of some other pigments including zinc dust, cuprous oxide and mercuric oxide increases gradually with time evidently because the pigments react with or cause a reaction in the medium Of particular interest was the indication that, as with zinc, films of cuprous oxide in sodium silicate media of a high silica ratio might cure unaided at room temperature and this has been confirmed by experiment

The proportion of cuprous oxide in the dispersion and the Na_2O SiO_2 ratio in the medium can be varied over quite a wide range, and the film will still become insoluble in water within, at most, an overnight drying period Methyl cellulose, bentonite, micronized mica and similar viscosity control agents can be included to improve the application and film-forming properties of the dispersion A typical composition having satisfactory film forming and curing properties is made by dispersing 225 parts by weight of cuprous oxide and one part of methyl cellulose in 140 parts of an aqueous solution containing 18 6 per cent of sodium silicate having an Na_2O SiO_2 ratio of 1 3 8

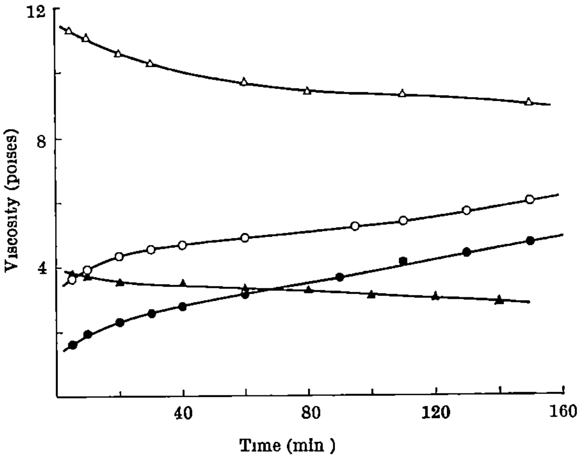

Fig 1 Viscosity at constant shear-rate as a function of time △, Talc, ○, cuprous oxide, ●, zinc dust, ▲, micaceous hæmatite The quantities of pigment added to 120 ml of sodium silicate solution were 56 gm , 225 gm , 450 gm and 300 gm respectively

Films of this composition and of variants of it have shown no fouling during a period of 95 weeks immersion in Sydney Harbour, Australia The most abundant macrofouling organisms recorded at this severe exposure site are *Balanus* (*Balanus*) *amphitrite* var *cirratus* Darwin, *Bugula avicularia* L , *Bugula neritina* L , *Hydroides norvegica* Gunnerus, *Spirorbis* sp , *Watersipora cucullata* (Busk), *Conopeum reticulum* (Linne), *Mytilus planulatus* Lamarck and *Crassostrea commercialis* Iredale and Roughley, and settlement of at least three of these species took place on inert control panels during each month of the immersion period (ref 2 and Wisely, B , private communication) The average fouling-free life of 46 commercial paints immersed along with the silicate compositions was 26 weeks (Laurie, H A , private communication)

There are indications that the inclusion of zinc dust up to the extent of about 10 per cent of the cuprous oxide content of such dispersions may prolong the fouling-free life of the film by depressing the solution-rate of the copper in the early stages of immersion

We thank the Department of the Navy, Australia, for the use of its immersion facilities in Sydney Harbour and Mr. H A Laurie for the periodic examination of panels The work forms part of a programme sponsored by the Department of the Navy, and this communication is published by permission of the Chief Scientist, Department of Supply, Australian Defence Scientific Service, Melbourne, Victoria, Australia

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Evolution of Hydrocarbons from Impure Titanium Getters

When titanium getters are heated and evaporated in vacuum hydrocarbons are formed from carbon and hydrogen impurities in the metal[1] The evolution of hydrocarbon gases from two grades of titanium has been investigated in this laboratory with an 'Omegatron' mass spectrometer and found to be related to the partial pressure of hydrogen in the system and the carbon impurity in the getter

Two grades of titanium were used having the following chemical analyses and termed here grade *A* 0 055 per cent carbon, 0 0065 per cent hydrogen and grade *B* 0 007 per cent carbon, 0 016 per cent hydrogen Getter filaments were made by twisting together a titanium wire of 1 mm diameter and 5 cm long with three tungsten wires each of 0 5 mm diameter The getter filaments were mounted within glass cylinders (8 5 cm diam × 8 cm high) inside the stainless steel vessel of a bakeable high-vacuum plant The vessel was exhausted by a diffusion pump charged with silicone 704 and fitted with a liquid-nitrogen trap All the vacuum seals were made with metal gaskets and after baking the system an ultimate pressure of 10^{-8} torr was obtained To raise the partial pressures of the gases evolved by the getter, and thereby aid their detection by the 'Omegatron' spectrometer, the speed of pumping the vessel was limited to 10 l /sec by a diaphragm in the mouth of the liquid-nitrogen trap

When either grade of titanium was evaporated in high vacuum the principal hydrocarbon gas formed had a mass of 15 (CH_3) However, when the titanium was volatilized in hydrogen at a pressure of 10^{-7}–10^{-6} torr (nitrogen equivalent) the predominant hydrocarbon formed had a mass of 16 corresponding to the saturated compound of methane Subsequent experiments were then made with hydrogen leaked into the vessel during pre-heating of the getter, evaporation of titanium and after film deposition Mass spectra were recorded of the atmosphere in the system during each of these three stages A typical experiment was completed in a total time of 70 min with a pre-heating time of 40 min and an evaporation time of 15 min

To ensure that impurities in the tungsten heater did not influence the experiments bare tungsten filaments were heated in the same manner as titanium-loaded filaments, and the resulting mass spectra

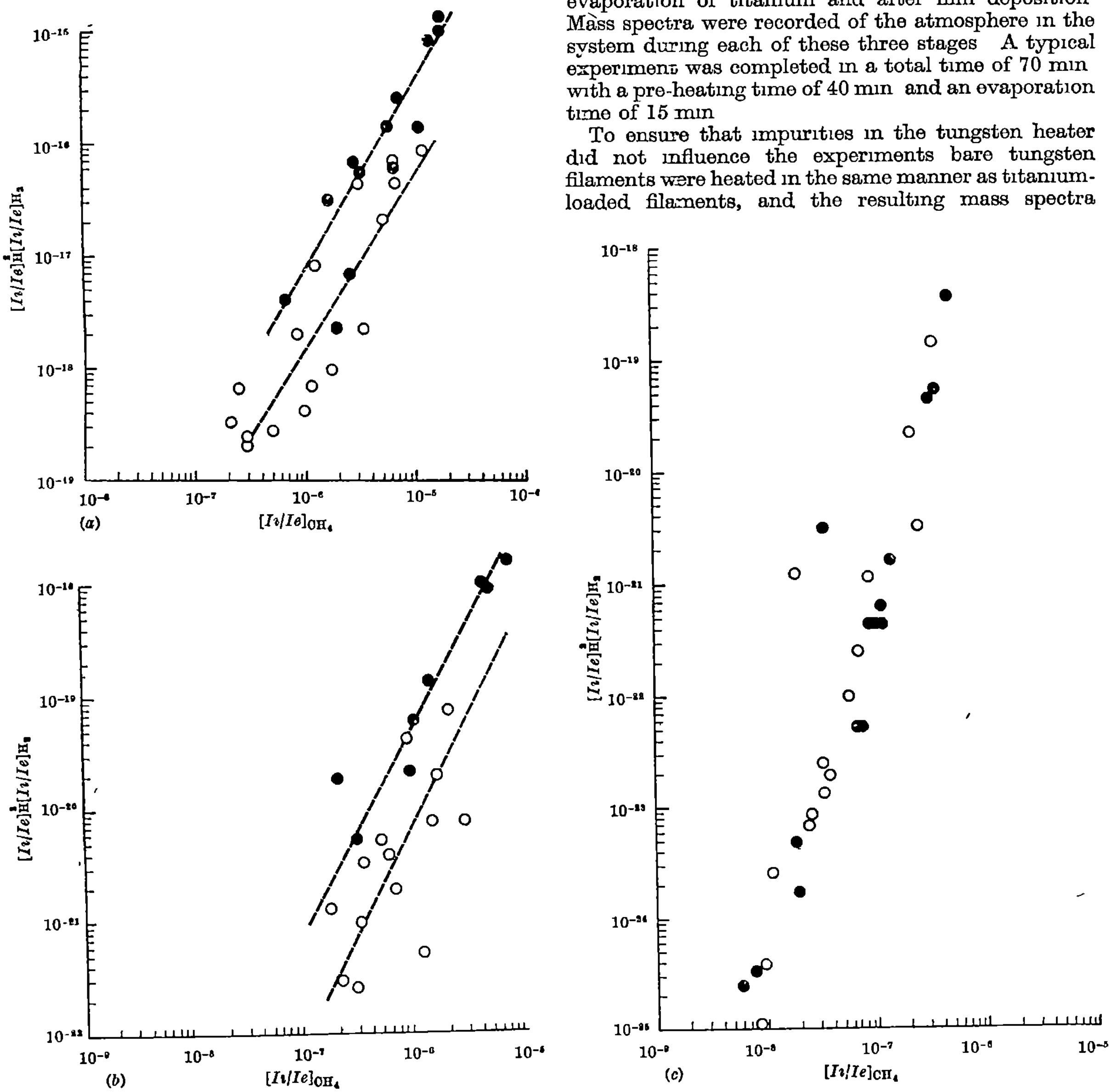

Fig 1 *a* ○, Type *A*, before evaporation, ●, type *B*, before evaporation *b* ○ Type *A*, during evaporation, ● type *B*, during evaporation *c* ○, type *A*, after evaporation, ●, type *B*, after evaporation $[I_i/I_e]$ is the ratio of ion to electron current showed that negligible amounts of hydrocarbon gas were evolved.

To find the relation between the partial pressures of hydrogen and hydrocarbon gas in the titanium experiments, the peak heights of atomic hydrogen, diatomic hydrogen and methane in the mass spectra were measured. Corrections were made to the measured values for variations in rate of evaporation during film growth and for changes in hydrogen concentration during the recording of the mass-spectra. The hydrocarbon evolution during the pre-heating and evaporation of the getter indicated that it followed the law of mass action if one assumed a simple equation of the kind $C + 2H + H_2 \rightleftharpoons CH_4$. Thus by plotting methane concentration $[CH_4]$ as a function of $[H^2][H_2]$, Figs. 1*a* and 1*b* were obtained for the pre-heating and evaporation stages respectively and empirical lines drawn through the points showed an approximately linear relation between $[CH_4]$ and $[H^2][H_2]$. The plotted values are divided into two groups in each graph, dependent on the carbon content of the getter metal. The partial pressure of hydrocarbon gas measured during the post-evaporation stage (Fig. 1*c*) did not show a dependence on the carbon impurity in the titanium. Also, the curve following the values in Fig. 1*c* departs more markedly from a linear relation between $[CH_4]$ and $[H^2][H_2]$ and the mass action law is not considered to apply.

During pre-heating the reversible chemical reaction between carbon and hydrogen must occur at the surface of the hot titanium. However, when evaporation begins it is uncertain whether the reaction can still proceed at the high filament temperature ($\geqslant$ 1,625° C.). It is possible that the reversible reaction now occurs at the surface of the condensing titanium film. At the pressure of operation ($\simeq 3 \times 10^{-7}$ torr) hydrogen molecules strike the titanium film at a rate of $4·5 \times 10^{14}$ molecules sec.$^{-1}$ cm.$^{-2}$ and rough calculations of the rates of arrival of titanium atoms give values between 1·5 and $5·0 \times 10^{15}$ atoms sec.$^{-1}$ cm.$^{-2}$. Thus the ratio of impinging titanium atoms to hydrogen molecules varies from 3 : 1 to 10 : 1. The heat released at the surface by the condensing titanium and the heat radiation from the getter filament may both be responsible for a reversible reaction at the getter surface.

Although there are many aspects of the foregoing work which are unsatisfactory, for example, the sole consideration of methane production ignoring other hydrocarbon species, it does show that metal film getters will only be completely effective in the sorption of hydrogen if they are prepared free from carbon.

Acknowledgments are made to Mr. Elwell, Division chief analyst of Imperial Chemicals Industry, Metals Division, and to Mr. Webb, chief analyst of Johnson Matthey, for their kind assistance in the chemical analysis of the titanium used in these experiments. The experimental work reported here was sponsored by C. T. R. Division, Atomic Energy Research Establishment, Harwell.

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Detection of Carboxyl Groups in Conjugation with the Porphyrin Ring System

THE band positions in the visible region of a porphyrin spectrum are influenced by the electrophilic nature of the substituents conjugated with the porphyrin ring system, the more electrophilic the substituent the farther the absorption maxima lie towards longer wave-lengths. Thus, when a reagent reacts specifically with a particular group to produce a derivative which has significantly different electrophilic characteristics, a corresponding change in the spectrum is observed, and this change indicates the presence of the group in question. For example, porphyrins with carbonyl groups conjugated with the ring system react with hydroxylamine to produce oximes which have their absorption maxima at wave-lengths shorter than those of the original carbonyl compounds. Such a spectroscopic change indicates the presence of a carbonyl group in conjugation with the tetrapyrrolic ring system. Methods of this type, which require only a trace of material, have played an important part in the structural determination of naturally occurring porphyrins.

My recent work has required a similar procedure for the detection of a carboxyl group in conjugation with the porphyrin ring system. It has been argued that since the position of the absorption maxima of porphyrins is dependent on the electrophilic nature of their substituents, the visible spectrum of a carboxylic acid, when in conjugation with a tetrapyrrolic ring system, should be different from that of its anion. Since the spectra of porphyrins in alkaline solution are often ill-defined, it has been found advantageous to carry out the present investigation on a series of pyridine hæmochromes (ferrous complexes of porphyrins, co-ordinated through iron to pyridine). These derivatives have well-defined two-banded spectra in alkaline media, and the position of the longer wave-length band (α-band) is particularly sensitive to changes in the electrophilic nature of the particular substituent conjugated with the porphyrin ring system. However, hæmochromes are stable only in alkaline solutions, and to provide a spectrum which would approximate that of the un-ionized acid, the methyl ester was used instead of the free acid, which under the alkaline conditions would be ionized.

The pyridine hæmochromes have been prepared by dissolving the porphyrin iron complex in pyridine 0·1 *N* sodium hydroxide (1 : 1) and reducing the mixture with sodium dithionite. Table 1 records the α-band position of pyridine hæmochromes derived from porphyrins containing a conjugated carboxylic acid group and compares them with values obtained from the corresponding methyl esters. A difference of more than 10 mμ between these values is observed, which provides a reliable diagnostic test for the presence of a carboxylic group in conjugation with the tetrapyrrolic ring system.

Table 1

Compound	α-Band of pyridine hæmochrome (mμ)	
	Acid	Ester
2-Carboxyl-deuteroporphyrin *	549	563
2-Carboxyl-4-vinyl-deuteroporphyrin *	555	567
2,4-Dicarboxyl-deuteroporphyrin *	554	567
4-Acrylic acid-deuteroporphyrin (ref. 2)	561	572
Rhodoporphyrin (ref. 3)	553	566
Porphyrin *a* acid (ref. 4)	560	572

* These compounds were prepared by oxidation of the corresponding formyl porphyrin, using Jones's reagent (ref. 5).

Fischer and Orth[1] quote a value of 563 mμ for the α-band of a hæmochrome derived from rhodoporphyrin In view of the values reported here their result appears anomalous and seems to approximate that of the ester rather than of the acid

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Examination of Side-Chain Interactions in Poly-L-Tryptophan

WHILE hypochromism has been examined quantitatively by Tinoco[1] for polynucleotides and Imahori and Tanaka[2] have reported decreased extinction of the peptide absorption band at 1900 Å in poly-L-glutamic acid in the helical conformation, it was of interest to examine interactions of the aromatic side-chains of poly-L-tryptophan The results of absorption and fluorescence studies of the polyamino-acid compared with a suitable model for the monomer are discussed in this communication

Poly-L-tryptophan, having an average molecular weight of 17,800, was supplied by Dr Michael Sela Acetyltryptophan is commercially available and tryptophan ethyl ester was prepared by a modification of the method of Brenner and Huber[3] Solvents were distilled prior to use except for dimethylformamide, which was 'Spectroquality' reagent purchased from Matheson, Coleman and Bell Absorption spectra were taken with a Cary recording spectrophotometer model 14, and intensities of emitted light were measured with the Aminco-Bowman fluorometer with the monochrometers set at constant excitation and emission wave-lengths

The limited solubility of polytryptophan did not permit examination over a wide range of solvent polarities In this work the solvents used were dimethylformamide, dimethyl sulphoxide and *n*-butylamine/water (9 1 by vol) Poly-L-tryptophan absorption obeys Beer's law in these solvents over the twenty-fold range of concentration studied The absorption maxima and their extinction coefficients are shown in Table 1 together with the results of Hurd and Bauer[4] for polytryptophan (average molecular weight 4,000) in ethanol

The total areas under the absorption curves as a measure of the oscillator strength, *f*, were determined after extrapolation below 2600 Å , where accurate measurements were not obtainable because of solvent absorption The *f*'s are very nearly the same for acetyl- and poly-tryptophan in the solvents used in this investigation Similarly, temperature variation between 25° C and 80° C had no exclusive effect on the absorption spectrum of the monomer or polymer

Table 1 ABSORPTION MAXIMA AND EXTINCTION COEFFICIENTS OF *N*-ACETYL- AND POLY-TRYPTOPHAN

Solvent	*N*-Acetyltryptophan		Poly-L-tryptophan	
	λ_{max} (Å)	$\varepsilon \times 10^{-3}$*	λ_{max} (Å)	$\varepsilon \times 10^{-3}$
Dimethylformamide	2830 2910	5 99 5 35	2830 2910	5 84 5 13
Dimethyl sulphoxide	2835 2910	5 68 5 16	2835 2910	5 74 5 15
n-Butylamine/water (9 1 by volume)	2840 2915	5 58 5 07	2840 2915	5 43 5 10
Ethanol	2825 (ref 5) 2905	5 62 4 90	2820 2905	5 49† 4 68

* These values are the average of 10–15 determinations and are ± 0 05

† Values from Hurd and Bauer (ref 4) for polytryptophan, the average molecular weight of which is 4,000

Although there are no significant differences between the absorption properties of acetyl- and poly-tryptophan, tryptophan–tryptophan interactions may be detected more readily by the fluorescence properties of the aromatic amino-acid side-chain Emission intensities in the 3400 Å region were measured for solutions having optical densities of 0 050–0 250 at the absorption maximum In all solvents, the polymer was consistently 35–45 per cent less fluorescent than either acetyltryptophan or tryptophan ethyl ester in the same solvent. The decreased fluorescence of the polymer may be a reflexion of quenching as a consequence of the high local concentration of the aromatic groups.

The absence of hypo- or hyper-chromic effects in the absorption of polytryptophan suggests that either the transition moments of the freely rotating aromatic side-chains are not oriented or interactions between identical transitions cannot be detected by absorption studies The present work also indicates that the high quantum yield of some tryptophan-containing proteins is not explicable in terms of tryptophan stacking as has been suggested[6] Borrowing from or lending energy to other groups in the protein may account for unusual fluorescent properties of some albumins as recently reported by Weber[7]

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Absence of Correlation between Odour and Molecular Vibration

IN 1928 Dyson[1] proposed a relationship between odour and molecular vibration This hypothesis was worked out in more detail by Wright[2–6], who correlated the odour of a compound with its low-frequency vibrations (below 800 cm $^{-1}$)

Wright postulates that odour-perception is caused by the interaction between the odorous molecule and a pigment molecule present in the olfactory cell. The former is attached to the latter by "forces of adsorption, or something of the sort"[5] The pigment molecule is permanently in an excited electronic state and can only fall back to its electronic ground-state if the relevant selection rule is relaxed by a perturbation due to the odorous molecule in "a process akin to Fermi-resonance"[6]. This perturbation is thus assumed to occur only if the odorous molecule and the receptor molecule have one or more identical vibration frequencies The electronic transition then triggers an electrical process in the cell membrane Leaving the electronic transition and its selection rules out of discussion in this communication, we will now examine the vibrational interactions more closely

It is easily seen that no correlation can be found between the vibrations of a free molecule and its odour if the molecule forms a complex with the receptor molecule, as the vibrational frequencies of the complex are never the same as those of the constituent parts As a consequence any molecule attached to the pigment molecule would give rise to odour-perception if the mechanism proposed by Wright applies

A second possibility exists in very weak interactions, which, for example, give rise to coupling of vibrations of molecules in crystals As the perturbation is weak, it is very questionable, however, whether it can change the selection rule for the electronic transition The strongest objection to this vibrational coupling lies in the odours of hydrogen sulphide and deuterium sulphide There are no grounds to believe that these compounds behave differently from all other substances From the fact that the vibrational (and also rotational) frequencies of hydrogen sulphide and deuterium sulphide are completely different, it follows that there should be a distinct difference between the odours of these two compounds

In an experiment similar to that described by Wright as inconclusive, the odours of hydrogen sulphide, deuterium oxide and deuterium sulphide were compared The fundamental frequencies[7] of hydrogen sulphide, deuterium oxide and deuterium sulphide are listed in Table 1 As an influence of molecular rotations cannot be excluded, the rotational constants[8,9] are also given

Table 1 THE FUNDAMENTAL FREQUENCIES AND ROTATIONAL CONSTANTS IN CM^{-1} OF DEUTERIUM SULPHIDE (D_2S), DEUTERIUM OXIDE (D_2O) AND HYDROGEN SULPHIDE (H_2S)

	D_2S	D_2O	H_2S
ν_2	934	1 179	1,290
ν_1	1,892	2,666	2,611
ν_3	1 999	2,789	2,684
A	5 47	15 38	10 39
B	4 49	7 25	9 04
C	2 44	4 83	4 72

Judged from the vibrational frequencies, the odours of hydrogen sulphide and deuterium sulphide should be quite different, deuterium oxide should have an odour falling between that of hydrogen sulphide and deuterium sulphide The rotational constants also indicate that the odours of hydrogen sulphide and deuterium sulphide would be different if molecular rotations play a part.

The odour of deuterium sulphide was tested in the following way Deuterium sulphide was synthesized by adding anhydrous aluminium chloride to a solution of sodium sulphide in pure deuterium oxide The trace of gas (the smell of which was near the threshold of detection) was kept in a stoppered bottle which was opened only long enough for each person to recognize the odour. As the deuterium in deuterium sulphide exchanges very rapidly, especially when in contact with the mucous membrane of the nose, this membrane was thoroughly 'deuterated' immediately before each experiment by spraying deuterium oxide into the nose, especially in the region of the olfactory epithelium Recognition of the odour of *p*-dichlorobenzene before and after deuteration showed that this treatment does not affect the observer's sense of smell. The four observers all agreed on the following

(1) Deuterium oxide is odourless (2) Deuterium sulphide cannot be distinguished from hydrogen sulphide (3) The intensity of the smell of deuterium sulphide is independent of the precautions taken to ensure that no hydrogen–deuterium exchange takes place This proves that the actual odour of deuterium sulphide was smelt and not that of traces of hydrogen sulphide which may have been present

This experiment thus seems to indicate that odour-perception is not linked with the vibrations of the free molecules

If relaxation of the selection rules for an electronic transition is assumed to trigger the nerve impulse, it is very plausible that the formation of a weak complex with the accompanying redistribution of charge in the participating substances is the first step in the process of odour-perception It is clear that there are other less-complicated processes, such as induction effects, which are able to trigger nerve impulses after complex-formation Unfortunately little is known at present about the pigment molecules in the olfactory cells. It seems fruitful, however, to investigate the possibility of correlations between odour and molecular properties such as electron distribution.

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Large-Scale Polymer Column Fractionation

THE gradient elution method for polymer fractionation was introduced by Williams[1-3] To fractionate larger amounts of polymer, a scaled-up apparatus was necessary Previous experience in column design in this laboratory advised against an increase in the dimensions of a single column to avoid difficulties in temperature control and channelling. Consequently, an apparatus was built with six parallel columns, each 1 m in length and 2 5 cm in inside diameter A sketch of the multicolumn unit is shown in Fig 1 The upper ends of the aluminium columns are welded to the bottom of a common solvent-mixing container The metal container is 7 litres in capacity and is fitted with a heater, regulator, stirrer, and sight glass The solvent mixture is heated to the proper temperature for the desired column temperature gradient The lower ends of the columns are connected to a common outlet and immersed in a water thermostat The temperature gradient along the insulated columns is found to be nearly linear, as measured by four evenly spaced thermocouples The apparatus is charged and operated similarly to single columns previously described[1-3] Two ganged 'Minipumps' (Milton Roy Company, Philadelphia, Pennsylvania), each with an adjustable 12–120 ml /hr pumping capacity, regulate the rate of withdrawal of polymer solution from the common outlet The pumps also replenish the solvent at the head of the column at the same rate, thus establishing the solvent gradient and degree of fractionation The evaporation of the solvent for recovery of fractions is done automatically, *in situ*, using a rotary vacuum evaporator, fitted with a nitrogen inlet, vacuum line, and water-bath. A low evaporation temperature coupled with the nitrogen ensures polymer stability

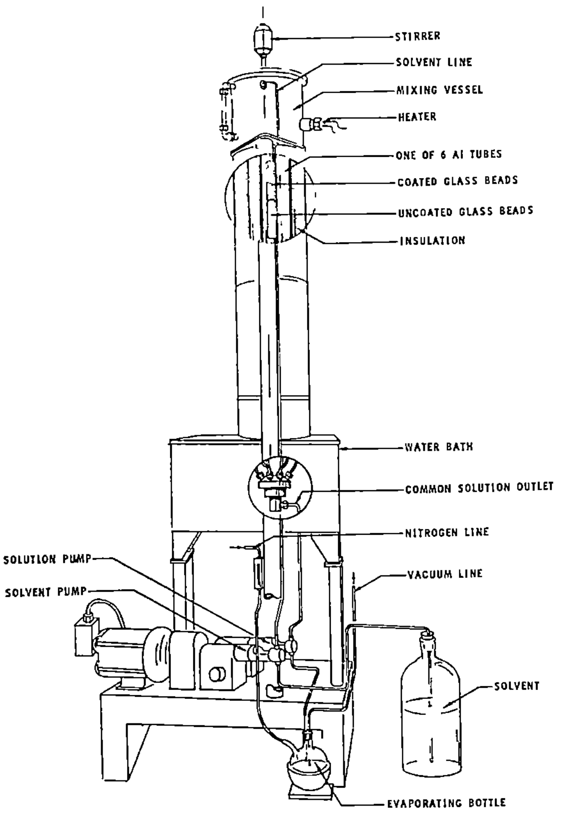

Fig 1. Multi-column unit

As an example of apparatus performance, Table 1 gives results on the fractionation of 34 gm. of 'Vistanex *LM–MS*', a commercial polyisobutylene The columns were packed with glass beads, average diameter 0 1 mm The non-solvent employed was acetone, the solvent benzene The temperature decrease down the column was from 50° to 28° C At a throughput of 80 ml /hr , the fractionation required eight days Polymer recovery was 95 per cent The molecular weights were determined by viscosity measurements in carbon tetrachloride at 30° C and evaluated according to the relation given by Fox and Flory[4] Table 1 shows good fractionation in light of the low degree of original inhomogeneity. for the unfractionated polymer, $M_w/M_n = 2\ 6$ As the apparatus requires little attention after starting up, fractionations require appreciably less effort than conventional precipitation methods and no more time than single-column operation Successful fractionations have been made with sample sizes of 30–50 gm , depending on polymer molecular weight and type

Table 1 Large-scale Fractionation Data for Polyisobutylene

Fraction	Gm	Weight (per cent)	(η)	Molecular weight
A	0 3026	0 89	0 035	1,150
B	1 1217	3 28	0 058	2,500
C	1 6848	4 93	0 103	5,700
D	2 8666	8 38	0 155	10,300
E	2 8142	8 23	0 219	17,100
F	2 1895	6 40	0 233	18,800
G	2 0337	5 95	0 277	24,200
H	2 1108	6 17	0 340	31,500
I	2 4199	7 08	0 367	36,500
J	3 6250	10 60	0 411	43 100
K	2 0348	5 95	0 481	54,400
L	2 1725	6 35	0 550	66,100
M	2 0653	6 04	0 628	80 500
N	1 7823	5 21	0 730	100,000
O	2 1330	6 24	0 834	122 000
P	0 7442	2 18	0 982	155,000
Q	0 3314	0 99	1 029	166,000
	32 4323	94 87		

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BIOCHEMISTRY

Determination of the Biological Availability of Methionine and Cystine

There is no adequate method to determine the biological availability of the sulphur-containing amino-acids in the chick, but Schweigert and Guthneck[1] have described a technique for the rat A method has therefore been developed for the bio-assay of methionine using White Leghorn chicks at 11–18 days of age it can readily be modified to determine methionine plus cystine in terms of methionine equivalence By difference an indication can be obtained of available cystine The basal ration contains (per cent) 48 6 maize starch, 35 extracted soybean meal, 5 dried whey, 5 beef tallow, 6 mineral and vitamin mixture[2], 0 2 choline chloride and 0 2 glycine All supplements to this ration replace part of the maize starch Where test materials are calculated to contribute less 'metabolizable' energy than starch additional tallow is included to correct for the difference

It was first established that methionine was the only amino-acid limiting growth and food utilization This was accomplished by supplementing the diet with all other amino-acids singly and in various groups all in the presence of sufficient methionine to correct the known deficiency In no case was any growth improvement observed, but it was shown that part of the methionine deficiency could be satisfied by L-cystine A standard supplement of 0 4 per cent L-cystine was therefore included in all diets to eliminate the effect of any cystine in test proteins When the methionine deficiency was corrected, chick growth was limited only by the protein-level of the diet A relatively low protein-level was chosen since this tends to maximize the response to methionine supplements when these are expressed as a proportion of the protein

In the presence of excess L-cystine the growth response to additions of methionine is essentially linear on a logarithmic dose basis within the range of 0 01–0 1 per cent of the diet. Similarly, in the absence of added cystine the linear range is approximately 0 04–0 2 per cent (Fig 1) These are the potential ranges for the bio-assay of methionine and methionine plus cystine respectively.

In order to satisfy the requirements for a bio-assay procedure it has been established that chick growth is a function only of the methionine or cystine content, that a constant growth response is obtained on repeated assay and that a linear and parallel response is obtained on the inclusion of graded amounts of standard and test materials in the diet Using this technique, assays of biologically available methionine and cystine have been made on several

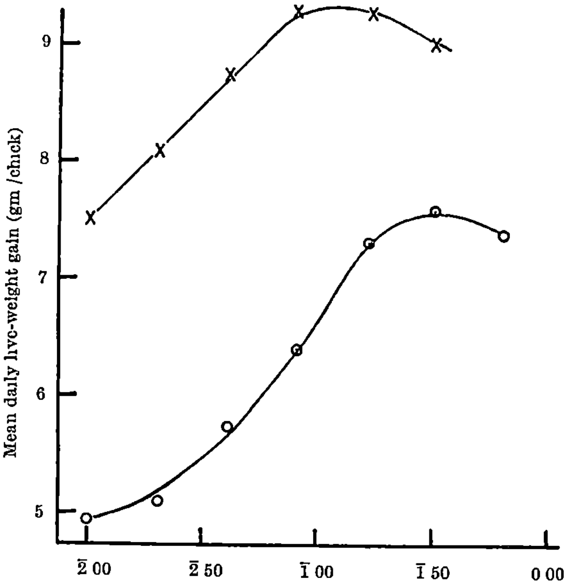

Fig. 1. Growth response of male White Leghorn chicks to graded supplements of DL-methionine. O, DL-methionine; ×, DL-methionine plus 0.4 per cent L-cystine

soybean samples (Table 1). The second experiment quoted refers to three extracted soybean meals obtained from the samples examined for protein quality in animal feeding-stuffs by the Agricultural Research Council. The values for both available methionine and cystine are uniformly high in relation to chemical determinations. These samples had received varying heat treatments but none was shown to exhibit more than slight urease activity. On the other hand, a somewhat lower availability of these amino-acids was found in C-1 assay protein. This agrees with the observations of Turk *et al.*[3] and Ousterhout *et al.*[4] and also with our preliminary results, which suggest low availability of cystine in 'underheated' soybean meal samples (high urease activity).

Table 1. ASSAYS OF AVAILABLE METHIONINE AND METHIONINE PLUS CYSTINE (BASED ON LIVE-WEIGHT DATA)

In all cases cystine is expressed as methionine equivalence. Chemical determinations by a Moore and Stein technique. The fiducial limits are quoted at 5 per cent level of probability. All values expressed as gm./16 gm. nitrogen

	Methionine Assay	Methionine Fiducial limits	Methionine + cystine Assay	Methionine + cystine Fiducial limits	Cystine (by difference)
Experiment 1					
C-1 assay protein (soybean, 14.1% N)	0.97	1.22 0.74	1.68	2.14 1.36	0.71
Chemically determined	1.39		—		1.02
Availability (per cent)	70		—		69
Experiment 2					
Soybean 22 (8.01% N)	1.61	2.11 1.23	3.30	4.27 2.62	1.69
Soybean 23 (8.24% N)	1.74	2.30 1.33	3.16	4.08 2.51	1.42
Soybean 24 (7.81% N)	1.44	1.89 1.09	2.97	3.81 2.36	1.53
Mean of three samples	1.60		3.14		1.54
Chemically determined	1.78		—		1.80
Availability (per cent)	90		—		86

Table 2. EFFECTS OF L-GLUTAMIC ACID AND AN AMINO-ACID MIXTURE ON THE RESPONSE TO GRADED SUPPLEMENTS OF DL-METHIONINE

Amino-acids added so that the total nitrogen is approximately equal to that of test proteins. Fiducial limits at 5 per cent probability

	Relative potency* per unit DL-methionine: In presence of 0.4 per cent L-cystine	Fiducial limits	In absence of cystine	Fiducial limits
Methionine + L-glutamic acid	0.98	1.23 0.78	0.92	1.12 0.75
Methionine + amino-acid mixture	0.92	1.16 0.74	1.01	1.23 0.83

* Computed according to Finney (ref. 6).

In view of the observations of Fisher *et al.*[5] it was considered necessary to examine the possible effect of additional amino-acid inclusion on the response to standard supplements (Table 2). Neither supplements of L-glutamic acid nor a complete amino-acid mixture[5] significantly modified the growth pattern. This shows that under the conditions of this experiment alteration of amino-nitrogen or essential amino-acid status did not alter the response to methionine supplements.

Thanks are due to the Agricultural Research Council for financial support and to J. G. Rowell, Agricultural Research Council Statistics Group, Cambridge, for assistance with statistical computations.

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Cation Toxicity and the Stability of Transition-Metal Complexes

DESPITE the many important contributions that have been made to the literature during the past century, no completely satisfactory theory of cation toxicity has been formulated. Blake[1] first concluded that there was a correlation between degree of toxicity and atomic weight (hence 'heavy-metal' poisons). Matthews[2] and Jones[3] later pointed out a relationship between metal ion toxicity and the 'solution pressure' (standard electrode potential) of the corresponding metals. Seifriz[4] considered various other chemical properties of the ions in his attempt to find a satisfactory chemical explanation for the relative toxicity of cations. Shaw[5-7], basing his arguments on results obtained with enzymes, postulated that metal ions killed aquatic organisms by combining with an essential group (probably —SH) on a key enzyme. Correlation with metal sulphide insolubility was demonstrated. An attempt was also made[6] to relate the relative inhibitory efficiency of bivalent metal ions toward the enzyme urease with metal complex stability. At that time (early 1953), however, the field of co-ordination chemistry had not reached a stage of sufficient maturity for such a comparison to be successfully made. The explosive growth that has occurred during the past decade in our knowledge of the chemistry of co-ordination compounds necessitates a re-examination of the intriguing possibility that there exists a relationship between metal complex stability and the toxicity of metal ions to living organisms.

In their classic paper, Irving and Williams[8] collected and critically examined available data on

Table 1 TOXICITY OF TRANSITION-METAL IONS

Organism or enzyme	Ref	Toxicity* of Mn(II)	Fe(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)
Daphnia magna	14	3.4	4.0	4.6	5.3	6.8	6.0
Urease	12	2.5	4.1$_9$	4.2$_3$	4.5	6.5	5.7
Stickleback	15	3.1	—	3.8	4.9	6.6	5.3
Guppy	7	2.2	—	3.3	4.7	6.5	5.7
Tadpole	7	2.1	—	4.0	4.1	6.5	5.0
Polycelis nigra	3	1.9	—	2.8	3.1	5.2	3.3
Diastase	16	0.79	—	2.0	3.0	3.9	1.8
Paramecium	17	1.0	—	1.2	1.2$_{[illegible]}$	2.7	0.90
Fundulus eggs	2	0.30	1.0	1.1	1.2	4.2	0.60

* Toxicities are reported on a *pI** scale (refs 5–7) that is, as the negative logarithm of the reported metal-ion concentration necessary to produce the particular level of biological response (LD_{50}, etc) arbitrarily established by the researcher *pI* values for enzymes have been discussed elsewhere (refs 5 and 12)

the stability of complexes formed by bivalent ions of the first transition series The Irving–Williams or 'natural' order. Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II) was found to hold for the stability of nearly all complexes irrespective of the nature of the ligand Theoretical justification for this important finding has been presented[8-10] The relevance of the Irving–Williams order to biological processes in general has been eloquently emphasized by Williams[10,11] Most of the examples used by him to illustrate his thesis were based on work on enzymes requiring metal ions and on metal-ion-catalysed reactions of model systems Work on the applicability of the Irving–Williams order to the inhibition of enzymes by metal ions are not numerous, but recent work[12] has demonstrated that metal ions in the absence of phosphate buffer inhibit urease in the order Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II) Since it seems reasonable to assume[6,7,13] that metal ions are toxic to living organisms because they combine with some ligand (probably itself part of an enzyme) necessary for life, one might naïvely expect the toxicity of transition-metal ions to follow a sequence similar to the Irving–Williams order. A more sophisticated view, however, would lead one to expect that complications involving competition of the host of ligands present in living systems for the metal ions might completely obscure such a simple relation[11]. This sophisticated view does not seem to be correct (Table 1), and the amazing fact is that all the data obtained throughout the years from the many different laboratories where work on the toxicity of metal ions to aquatic organisms has been done follows the toxicity sequence Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II) The relationship holds also for urease, diastase, and other enzymes (Table 1) In media containing concentrated phosphate or other ligands with strong affinity for metal ions the sequence may, of course, be broken

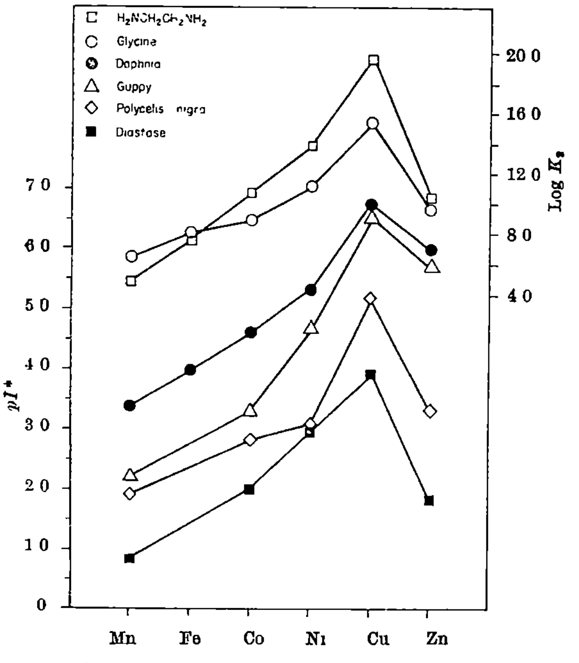

Fig 1 Correlation of transition-metal complex stability (ref 11) (two upper curves, right ordinate) with transition-metal ion toxicity (lower curves, left ordinate) Toxicities in *pI** units were obtained from Table 1

A comparison of toxicity with complex stability[11] is shown in Fig 1

The results of this investigation (Table 1, Fig. 1) establish the toxicity sequence proposed by me Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II) as one that is of very general significance in aquatic biology and one that is also firmly based on the principles of co-ordination chemistry

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A Simplified Culture Medium for the Production of Collagenase

PRESENT methods for the production of *Clostridium histolyticum* collagenase depend chiefly on growth of the organism in complex culture media[1,2] It is the purpose of this communication to describe a relatively simple, partially defined medium for the production of large quantities of highly active fermentation broths of *Cl. histolyticum* collagenase The medium described appeared to facilitate the isolation and purification of collagenases which were essentially free of non-specific proteinases[3]

The organism used in these experiments was *Cl histolyticum* 47Q–5 obtained from the Army Medical Service Graduate School, Washington, D C A metabolically constant source of collagenase was prepared by pre-treating the organism in the following manner A 24-hr. culture was injected intragluteally into Swiss mice through five serial passages Isolation (muscle exudate) was accomplished and growth continued in a seed medium of composition casein hydrolysate, enzymatic (General Biochemicals, Chagrin Falls, Ohio), 16 gm , sodium succinate 5 gm , sodium pantothenate, 0.1 mgm ; pyridoxamine, 0·2 mgm.; nicotinamide, 0·8 mgm., uracil, 1·5 mgm., riboflavin, 10 μgm., biotin, 0·1 μgm., vitamin B_{12}, 63·0 μgm., and distilled water to 1,000 ml. (*p*H 7·6). Stock cultures of *Cl. histolyticum* were maintained in 'Pyrex' tubes (25 × 200 mm.) containing 40 ml. of this medium to which 1 gm. of dry, fat-free bovine heart was added. The stock cultures were transferred at monthly intervals and were refrigerated in the interim. The culture medium used in the production of collagenase consisted of the following constituents in 1 l. of medium. Solution 1, casein hydrolysate (enzymatic), 40 gm., sodium succinate, 1·25 gm.; sodium thioglycollate, 1·0 gm., $Na_2HPO_4.12H_2O$, 5·8 gm., KH_2PO_4, 0·48 gm., gelatin, 2·0 gm. Solution 2 (stock vitamin preparation), calcium pantothenate, 1·0 mgm., pyridoxamine, 2·0 mgm.; riboflavin, 0·1 mgm., nicotinamide, 8·0 mgm., uracil, 15·0 mgm., biotin, 10·0 μgm., vitamin B_{12}, 63·0 μgm. Solution 3, dextrose (50 per cent), 5·0 ml. After preparation of solution 1, the incomplete medium was adjusted to *p*H 7·6 with 5 *N* sodium hydroxide. Solution 2 was then added and the combined medium was then placed in an autoclave for 15 min. at 15 lb./sq. in. After cooling, Seitz-filtered glucose (solution 3) was added aseptically. 9-l. 'Pyrex' carboys containing 6·5 l. of medium were used. The inoculum was prepared in 500 ml. of broth of the same composition as that being used to produce collagenase. The maximum yield of enzyme was obtained after 48 hr. incubation at 24–25° C. The bacterial cells were removed by filtration through 'Hyflo Super Cel' and the *p*H of the filtered broth adjusted to 7·5–7·6 with 5 *N* sodium hydroxide. Collagenase activity was measured by hydrolysis of undenatured Achilles tendon collagen as described in ref. 3. One collagenase unit was defined as the amount of activity catalysing the solubilization of 1 mgm. of 40–60 mesh collagen under standard conditions. It was found that the foregoing relatively simple procedure provided broths with collagenase activity that averaged 107 units of enzyme a ml. The average yield of purified preparations of collagenase expressed as collagenase *A* was 140 mgm./l. of fermentation beer.

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Crystallization of Deuterated Ribonuclease

WE have prepared deuterated bovine pancreatic ribonuclease (Armour, lot 381–059) by the procedures of Hermans and Scheraga[1]. Both RNaseH_{20} (all but 20 exchangeable H's exchanged) and RNase all-deuterated crystallized from 55 per cent (v/v) aqueous 2-methyl-2,4-pentanediol (deuterated solvent) in form II[2]. The intensities of the X-ray reflexions along the principal axes were identical within experimental precision to those of non-deuterated crystals out to $2\theta = 60°$ (1·5 Å resolution), at higher angles RNase gives poor reflexions. The lattice constants were identical to those of undeuterated crystals within the normal variation (± 0·05 Å and ± 0·03°). The small differences between deuterium bonds and hydrogen bonds do not change the structure of RNase within the limits observable by X-ray diffraction. Detectable changes might be produced in the case of a crystallizable protein with a transition temperature near room temperature instead of the high transition temperature (61° C.) of RNase[3].

The deuterated crystals were prepared by Miss Sylvia Scapa and Mrs. H. R. Bello; diffraction patterns were taken by Miss E. De Jarnette.

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Properties of Xanthine Dehydrogenase in the Silkworm, *Bombyx mori* L.

THE enzyme which catalyses the oxidation of purine derivatives xanthine and hypoxanthine to uric acid has been demonstrated in several insects[1–5]. In the silkworm, *Bombyx mori*, it functions as a dehydrogenase. This inference is based on the fact that the formation of uric acid and oxygen consumption of the fat body homogenates show marked increases only when methylene blue is present as a hydrogen carrier[6,7]. The inference seems to be justifiable from the work of Remy *et al.*[8] using chicken liver. To gain further knowledge of the nature of xanthine dehydrogenase in the silkworm, it will be desirable to use the highly purified enzyme as in milk[9,10] and liver[11] oxidase preparations.

The present report deals with preliminary work on the properties of the xanthine dehydrogenase in the silkworm. The enzyme was prepared from the fat body according to the methods of Kielley with a few modifications (Hayashi, unpublished). The enzyme activity was determined from oxygen consumption and uric acid formation by a method similar to that described in the previous paper using crude tissue homogenates of silkworm larvæ, with methylene blue as a hydrogen carrier between xanthine dehydrogenase and molecular oxygen[7].

Purified preparations of the silkworm enzyme are yellowish-brown in colour. The enzyme has two absorption bands with maxima at 360 and 405–410 mμ, the latter disappearing when reduced by hydrosulphite. The absorption spectrum of the silkworm enzyme is similar to that of the chicken liver enzyme[8] and aldehyde oxidase[12]. The difference spectrum was not conspicuously characteristic of flavin and is probably a compound of flavin plus an unknown group.

In recent work it has been reported that xanthine oxidative enzyme from some animal tissues contains iron, molybdenum and flavin as the prosthetic groups. The mammalian liver xanthine oxidase of Kielley[11] has an Fe : Mo : flavin ratio of 4 : 1 : 1 and the chicken liver xanthine dehydrogenase of Remy *et al.*[8] has a ratio of 8 : 1 : 1. From the purified preparation of xanthine dehydrogenase from silkworm fat body, it was determined that the ratio is 9 : 1 : 1, thereby showing closer resemblance to the latter rather than the former prosthetic group.

Milk[10] and chicken liver[8] enzyme are capable of oxidizing a number of oxypurines and aldehydes as well as reduced diphosphopyridine nucleotide (DPNH), but slowly with pterines. The silkworm xanthine dehydrogenase oxidized xanthine, hypoxanthine, guanine, adenine, aldehydes, 2-amino-4-hydroxypteridine and xanthopterin, while the oxidation of DPNH by the enzyme proceeded faster only when cytochrome *c* was added as an electron acceptor. In these substrates 2-amino-4-hydroxypteridine and xanthopterin were oxidized faster than the purines such as hypoxanthine and xanthine.

A wide variety of electron acceptors has been used in the enzyme system. Methylene blue, tetrazolium salts, indophenol, diphosphopyridine nucleotide (DPN), cytochrome *c*, flavin adenine dinucleotide (FAD), and sodium pyruvate have been shown to be acceptors. The enzyme activity of the reaction systems mentioned showed its maximum rate with methylene blue; DPN and FAD showed the same rate.

The activity of the silkworm enzyme has a wide *p*H range of 6–11 with a maximum velocity at *p*H 8·4. A number of substances have been found to be possible inhibitors of silkworm enzyme. Among them the more effective inhibitors are potassium cyanide and *o*-iodobenzoic acid, whereas xanthine oxidase inhibitors such as ascorbic acid, *p*-aminophenol, hydroxylamine, quinine, and antimycin *A* were not recognized as inhibitors.

As shown from the results stated above, the property of the xanthine enzyme system in silkworm fat body is not similar to that of mammalian liver enzyme but bears a striking likeness to chicken liver enzyme except in the case of substrate specificity. From this, it is inferred that the properties of xanthine oxidative enzymes vary from source to source.

I thank Prof. K. Hasegawa and Dr. K. Muramatsu for valuable advice and help throughout this work.

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Conversion of 2-^{14}C-Pteroyl-L-Monoglutamic Acid into Isoxanthopterin in *Drosophila melanogaster*

2-^{14}C-Pteroyl-L-monoglutamic acid (0·5 mc. per millimole, 1 mgm.) was incorporated into banana food medium (100 gm.), and adult flies of *Drosophila melanogaster* (Oregon, wild-type) were allowed to feed and lay eggs on this medium. Larvæ (six days old) were removed from this medium, transferred to moist filter paper and allowed to pupate. Pupæ (100; 86 mgm.; not less than 48 hr., not more than 56 hr. pupation) were crushed with sand and 0·5 *N* aqueous ammonia solution (0·5 ml.) and heated on a steam-bath (10 min.). Solids were centrifuged off and supernatant liquid spotted on Whatman 3 *MM* paper. The solids were washed again with 0·5 *N* aqueous ammonia solution (0·4 ml.), centrifuged off and supernatant liquid added to the first spot. The paper area was cut out and radioactivity measured by immersion in a solution of 0·3 per cent diphenyloxazole in toluene (2 ml.) and counting with a 'Panax' scintillation counter (background, 1·096 counts/sec.; background + sample, 1·357 counts/sec.; sample, 0·26 count/sec.; standard error, ± 0·035 count/sec.). The radioactive area of the paper was chromatographed on 3 *MM* paper with propanol/5 per cent aqueous acetic acid (2 : 1 vol. by vol.) as developing solvent. To improve spot resolution, the paper was developed three times. On viewing in 365-mμ light, a bright purple fluorescent area was seen. This was cut out and chromatographed on 3 *MM* paper with butanol/acetic acid/water (4 : 1 : 5 vol. by vol.) (triple development). After air-drying, the paper was viewed in 365 mμ light and the fluorescent spots of isoxanthopterin (purple) and 2-amino-4-hydroxypteridine-6-carboxylic acid (bright blue) cut out. The acid spot was measured for radioactivity (background, 0·65 count/sec.; background + sample, 1·10 counts/sec.; sample, 0·45 count/sec.; standard error, 0·033 count/sec.). The isoxanthopterin spot was further purified by chromatography on 3 *MM* paper with *tert.*-butanol/pyridine/water (50 : 15 : 35 vol. by vol.). The isoxanthopterin spot was measured for radioactivity (background, 0·65 count/sec.; background + sample, 0·76 count/sec.; sample, 0·11 count/sec.: standard error, 0·030 count/sec.).

The counting efficiencies of the isoxanthopterin spot and the original paper area (after development) were measured by adding known weights of 2-^{14}C-pteroyl-L-monoglutamic acid and determining the radioactivity of the paper areas. The counting efficiency of the isoxanthopterin spot was about 12·1 per cent and that of the original paper area about 6·7 per cent. The isoxanthopterin therefore represented about 20 per cent of the radioactivity present in the sample, the remainder being accounted for by the 2-amino-4-hydroxypteridine-6-carboxylic acid.

The pterin isoxanthopterin is widely distributed in Nature. It has been suggested that it is the end-product of the catabolism of folic acid (pteroyl-L-monoglutamic acid), being formed by the following steps[1,2]:

$$\text{pteroyl-L-monoglutamic acid} \xrightarrow[\text{(2) hydrolysis}]{\text{(1) dehydrogenation}}$$

$$\text{2-amino-4-hydroxypteridine-6-aldehyde} \xrightarrow[\text{oxidase}]{\text{xanthine}}$$

$$\text{2-amino-4-hydroxypteridine-6-carboxylic acid}$$

$$\xrightarrow{\text{decarboxylation}} \text{2-amino-4-hydroxypteridine}$$

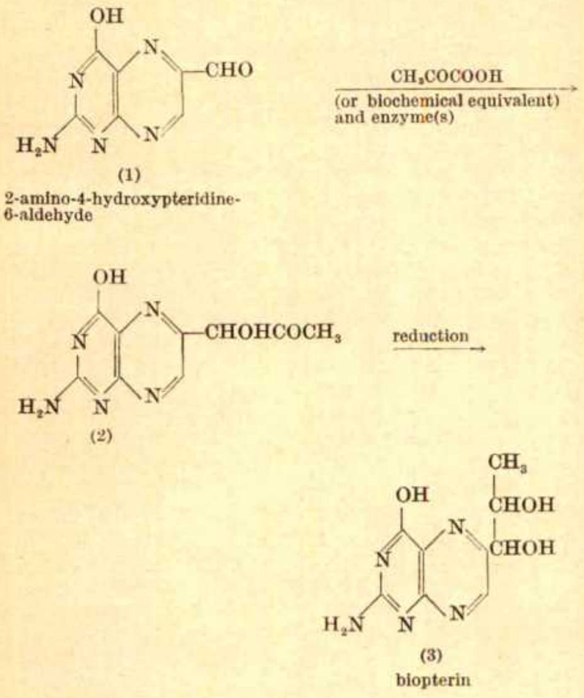
$$\xrightarrow[\text{oxidase}]{\text{xanthine}} \text{isoxanthopterin}$$

Isoxanthopterin can be readily isolated from the pupæ of *D. melanogaster*, and it has been shown that 6,7-^{14}C-2-amino-4-hydroxypteridine is converted only into radioactive isoxanthopterin in pupæ[3]. Work with mutants of *D. melanogaster*, which do not produce isoxanthopterin but instead accumulate large amounts of 2-amino-4-hydroxypteridine and biopterin, shows that this failure to produce isoxanthopterin is due to deficiency of the enzyme xanthine oxidase[3].

D. melanogaster larvæ and pupæ can synthesize folic acid in limited amount but require to be supplied with folic acid for their normal growth[4].

The experimental results establish that some of this supplied folic acid is converted into isoxanthopterin by the pupæ.

D. melanogaster mutants that do not form isoxanthopterin accumulate 2-amino-4-hydroxypteridine and biopterin. The latter is not formed from the former[3]. If the biogenetic scheme suggested is correct, then if xanthine oxidase is lacking, large amounts of 2-amino-4-hydroxypteridine-6-aldehyde should be formed. This could be converted to biopterin by the following steps:


OH
N
N
CHO
H₂N
N
N
(1)
2-amino-4-hydroxypteridine-6-aldehyde
CH₃COCOOH
(or biochemical equivalent) and enzyme(s)
OH
CHOHCOCH₃
H₂N
(2)
reduction
CH₃
CHOH
CHOH
(3)
biopterin

Analogies for the first step have been recorded. The enzymatic condensation of pyruvic acid with acetaldehyde and propionaldehyde yields acetoin and methyl acetoin[5]. Ultra-violet irradiation of pyruvic acid and benzaldehyde gives benzacetoin[6]. Benzacetoin is formed from benzaldehyde in alcoholic fermentation[7].

In accord with this prediction, mutants of *D. melanogaster* lacking xanthine oxidase accumulate biopterin as well as 2-amino-4-hydroxypteridine.

I am grateful to Prof. F. Weygand for a gift of 2-^{14}C-pteroyl-L-monoglutamic acid, to Dr. A. H. Ward for assistance with the radioactivity measurements and to Dr. M. Hollingsworth for the *Drosophila* stock.

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PHYSIOLOGY

Soluble Collagen in Bone

CONSIDERABLE efforts have been made, so far, to investigate the physico-chemical as well as the biochemical nature of soft tissue collagen such as skin and tendon, especially. In refs. 1–3 it is suggested that the soluble collagen is the precursor of collagen. However, only scanty information has been provided in regard to the important role of bone collagen in the formation of bone[4]. Trials in refs. 5 and 6 have not been successful in proving the existence of soluble collagen.

To clarify the mechanism of ossification, we carried out experiments on the extraction and refinement of bone collagen. The first problem of the extraction of bone collagen is to eliminate the bone mineral which occupies the major part of bone. Some attempts have been made to solve this problem and de-calcification by dilute acids has been found to be most suitable. We took special care to prevent a loss of soluble collagen, since it might be missed in the process of demineralization; this enabled us to prove that the soluble collagen was obtainable also from bone.

Cortical bone of fresh bovine femur was pulverized with dry ice to obtain 100–200 mesh powder after complete removal of attached soft tissues and de-fatting with ether, then freed from remaining soft tissues by flotation[7]. This sample was stored at − 20° C. As for de-calcification, bone powder thus prepared was suspended in 0·1 *N* hydrochloric acid and dialysed at 4° C. in cellulose tubing, average pore radius 24 Å. (Visking Co.), against a flow of 0·1 *N* hydro-

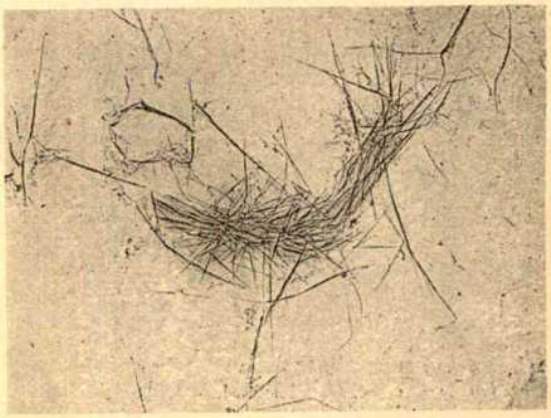

Fig. 1. Reconstituted fibres obtained from the soluble fraction of cortical bone (bovine femur) show a needle-like shape under the microscope. (× 300)

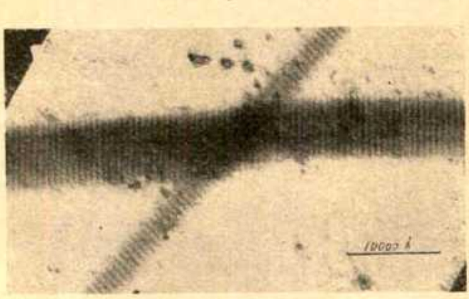

Fig. 2. Electron-micrograph of the reconstituted fibrils (*p*H 5·5, McIlvaine buffer) obtained from the soluble fraction of cortical bone (bovine femur). This shows the characteristic periods of 640 Å.; fixed with phosphotungstic acid, shadowed with palladium–platinum

chloric acid (10 l./day). After completion of demineralization (about 2 weeks), the non-dialysable portion of the extract in the tubes was separated from the residue by centrifugation (3,600g for 10 min.), sealed in the tubes and dialysed again at 4° C. by renewing the distilled water (10 l./day for 4 days).

Precipitate which appeared during dialysis was dissolved in McIlvaine buffer (7.1 vol. of 0.2 M sodium phosphate aq. : 12.9 vol. of 0.1 M citric acid aq., pH 3.8) after centrifugation (3,600g for 10 min.) and the supernatant was dialysed at 4° C. against McIlvaine buffer (11.6 vol. of 0.2 M sodium phosphate aq. : 8.4 vol. of 0.1 M citric acid aq., pH 5.5–5.6) containing 2 per cent sodium chloride followed by concentration with 50 per cent gum arabic.

A crystal of sodium chloride was added occasionally to promote the formation of fibre. As shown in Fig. 1, fibres showed a needle-shape under the microscope. They were rinsed with water repeatedly before fixation with 1 per cent phosphotungstate, washed again and suspended in water, then placed on grids and desiccated with calcium chloride. Thus, the electron-microscopical observation revealed the characteristic pattern of collagen with a 640-Å periodicity. This collagen fibre subjected to a repetition of this process gave the typical pattern of reconstituted collagen fibre with 640-Å periodicity (Fig. 2).

In addition, the amino-acid content of this reconstituted collagen was similar to that of so-called bone collagen, and it seems probable that the collagen fibre described is identical with collagen also from its contents of proline and glycine.

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Flow of Blood in the Forearm of Persons acclimatized to Heat

IT is known, from experiments performed in climatic rooms in temperate countries, that the flow of blood in the forearm increases when people are exposed to heat, and that the magnitude of this increase diminishes progressively if the exposure to heat is repeated on successive days[1,2]. At the end of these experiments, however, the flow of blood in the forearm was always higher during the exposure to heat than it was before the experimental subjects were first exposed to heat. It cannot, therefore, be decided from these observations whether the flow of blood in the forearms of people who are fully acclimatized to heat, by long residence in a hot environment, are higher or lower than those of people who are not acclimatized to heat. An investigation into the flow of blood in the forearms of indigenous peoples of a hot, humid climate would provide information on the effects of long-term acclimatization to heat, but no measurements of blood-flow in tropical subjects appear to have been made until now.

In order to supply these data, the flow of blood in the forearms has been measured, by venous occlusion plethysmography in twenty-one male Asian subjects living in Singapore. The subjects were fifteen Chinese, four Indians and two Malays; their average age was 23.1 yr. (*S.D.* = ±3.8) and their mean body-weight was 57.7 kgm. (*S.D.* = ±7.9). All subjects had been born in Singapore, and they were therefore 'naturally' acclimatized to heat[3]. They were tested at the environmental temperatures of Singapore (mean dry-bulb temperature, 28.3 ± 1.1° C., mean wet-bulb temperature, 25.2 ± 0.7° C.) and they wore their normal clothing[4]. Each subject lay on his back for 90 min. before any measurements were made. Blood-flows were measured in four sets of five flows; each set lasted 2.5 min. and was separated from the next set of readings by a time interval of 15 min. The mean blood-flow for any one subject therefore represented the average of twenty flows taken over a period of approximately 1 hr.

The mean blood-flow in the forearms of the twenty-one subjects was 2.70 ml./100 ml. forearm/min., with a standard deviation of ±0.88 ml. There was some evidence that the flows of blood in the forearms of these subjects were not greatly altered by enclosure of the arm in a water-filled plethysmograph. Thus, during the hour in which blood-flows were measured, there were only random variations in the flow readings. Progressive cooling or warming of the forearm might have been expected to result in trends in the blood-flows over a period of 1 hr. The temperature of the water in the plethysmograph was 35.0° C.; the skin temperature of the forearm when it was in the plethysmograph must have been close to 35° C. In a similar group of subjects to that taking part in the present investigation, at a room temperature of 29.6° C., the mean skin temperature of the forearm when it was exposed to air was 34.88° C. (ref. 4). The skin temperature of the forearm while measurements of blood-flow were being made could not therefore have been very different from that before the arm was inserted into the plethysmograph.

From these considerations it seems reasonable to assume that the figure of 2.70 ml./100 ml./min. may be accepted as an estimate of the rate of blood-flow through the forearms of young men, at rest, in a hot, humid climate. This figure is of the same order of magnitude as flow-rates of 2.6–3.1 ml./100 ml./min., which have been reported for the clothed forearms of people at normal room temperatures (18.5°–20.0° C.) in England[5] and in North America[6]. The forearms of young men in Singapore are habitually bare; the skin temperatures of their forearms are, however, higher than those of the clothed forearms of people in England and North America[4]. For this reason, it might be expected that the flows of blood in the forearms of the subjects in Singapore would be greater than those of people in temperate countries. The experiments reported here have not provided any evidence to support this. It is possible that the relatively low values for blood-flow in the forearms of the Singapore subjects may be related to the fact that people acclimatized to heat in Singapore have low mean arterial blood pressures[7].

It is important to stress that these conclusions apply only to men who are at rest and who are not visibly sweating. Increased activity or the onset of sweating might result in a greatly increased bloodflow through the forearms of these subjects but evidence for this is, at present, lacking

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New Indirect Fick Procedure for the Determination of Cardiac Output

THE indirect Fick method for determining cardiac output, which requires neither catheterization nor arterial puncture, would be the method of choice but for one serious drawback, namely, the difficulty of obtaining a reliable value for the carbon dioxide tension in (oxygenated) venous blood It is now generally recognized that, when the breath is held, no equilibrium between alveolar and mixed venous P_{CO_2} is attained in the 15–20 sec. before recirculation intervenes It has been proposed to plot the increase in pressure of alveolar carbon dioxide, P_{CO_2}, while the breath is held, and to compute the mean value of venous P_{CO_2} by means of extrapolation[1] Since alveolar P_{CO_2} cannot be measured while the breath is held, this method requires the determination of alveolar P_{CO_2} at the end of a number of successive periods of holding the breath of different lengths. This results in considerable scatter of the experimental points and, thus, in an appreciable degree of uncertainty regarding the value for alveolar P_{CO_2}

It was shown earlier[2] that, in the case of re-breathing in a bag initially filled with pure oxygen or with an oxygen – carbon dioxide mixture, the alveolar carbon dioxide tension after the *n*th breathing cycle, p_n, is given by an inhomogeneous linear second-order difference equation with constant coefficients

$$p_n = a + bp_{n-1} + cp_{n-2}$$

and that the second exponential, which is a function of the tidal volume/dead space ratio, becomes negligible after the first or second breathing cycle From then on, the process is a simple exponential one This means that, after the first few breaths, the p_n vs. p_{n-1} plot is a straight line

$$p_n = a + bp_{n-1}$$

or, if p_1 be the initial value of p

$$p_n = a(1 + b + b^2 - - - - - - b^{n-2}) + b^{n-1}p_1$$

The sum of the series in brackets being $(b^{n-1}-1)/(b-1)$, we have

$$p_n = \frac{ab^{n-1} - 1}{b - 1} + b^{n-1}p_1$$

For $n \rightarrow \infty$, a positive finite end value (p_∞) will be approached if $0 < b < 1$

This value is given by:

$$p_\infty = a\frac{0 - 1}{b - 1} = \frac{a}{1 - b}$$

Whether the asymptotic value is higher or lower than the initial value depends on the numerical values of a, b, and p_1

For p_2 to be higher than p_1 (also $p_3 > p_2$, etc.) we have

$$p_2 > p_1 \rightarrow a + bp_1 > p_1$$

or, since $bp_1 + (1-b)p_1 = p_1$, the condition can be written as:

$$a > p_1(1 - b)$$

Similarly, $p_n < p_{n-1}$ if $a < p_1(1-b)$

The first case corresponds to that in which the initial partial pressure of carbon dioxide in the bag is less than the true mean value of venous P_{CO_2}, and the second to that in which it exceeds this value The procedure is extremely simple At the end of a normal expiration, the subject is connected, by means of a three-way tap, to a 1-litre bag filled with an oxygen-rich oxygen–carbon dioxide mixture and re-breathes for a period not exceeding 15–16 sec At the end of each expiration, the concentration of carbon dioxide (per cent) in the bag is measured with the aid of a fast carbon dioxide analyser Scatter is minimized by having the subject re-breathe at a fixed tidal volume and at a fixed frequency (checked by metronome)

The p_n versus p_{n-1} plot is drawn, and if, after the first few points, a straight line is obtained, its slope and intercept of y (that is, b and a, respectively) are determined by the method of least squares, and p_∞ is calculated

Under certain conditions, such as unequal ventilation, etc, no straight line is obtained. In those cases the method is not applicable. The procedure is illustrated by Figs 1, 2 and 3. Fig 1 shows the record obtained with an infra-red analyser; the percentages of carbon dioxide after successive breathing cycles are plotted against time in Fig 2; the corresponding p_n versus p_{n-1} plot is shown in Fig 3 From the values for a and b (0 856 and 1·071 (760−47/100) = 7 636, respectively), p is found to be 52 30 mm mercury.

Values obtained with this method possess a high degree of reproducibility, as has been found in our laboratory and at the Lovelace Foundation Cardiac

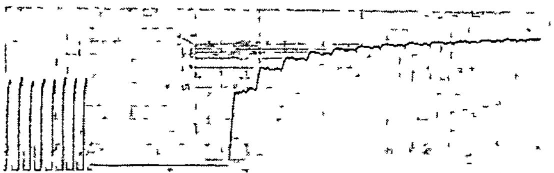

Fig 1 Record obtained from an infra-red analyser Concentration of carbon dioxide in bag at the end of successive expirations

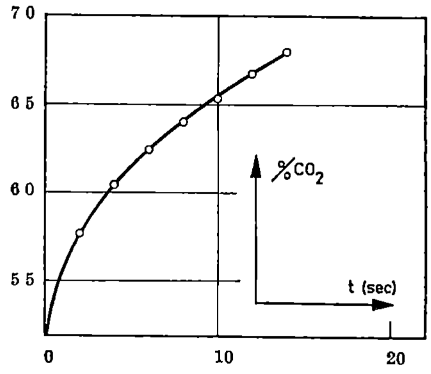

Fig 2

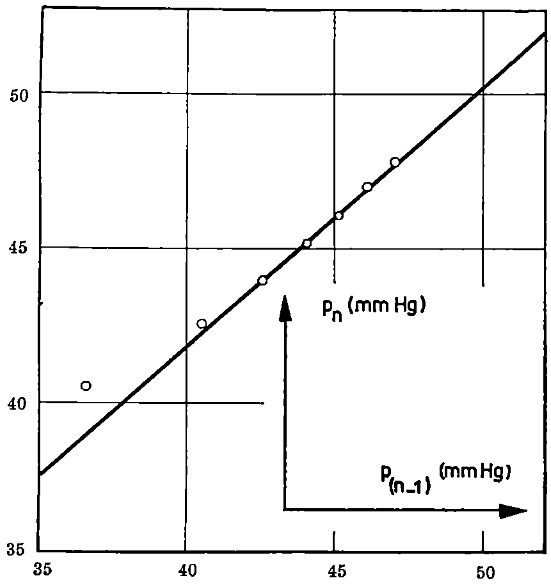

Fig 3

output values of resting normal subjects lie, as a rule, between 5 and 6 litres/min. At the time of writing, Dr. Mostyn (University of Alberta, Canada) is engaged in a series of experiments where the values for cardiac output obtained by the method described are compared with those found by the direct Fick method

A full report, including discussion of errors, will be published shortly in the *Acta physiol et pharmacol neerlandica*

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Œstrus-inducing Activity of Testosterone in the Ewe

On the basis of results obtained with testosterone administered, together with pregnant mare serum gonadotrophin, to anœstrous ewes Cole[1] suggested that androgen may be responsible for the induction of œstrous behaviour in the sheep Robinson[2], however, has shown that œstrogen given following a period of progesterone priming will induce both behavioural and vaginal œstrus in spayed ewes, and it is probable that normal cyclic œstrous behaviour is due to an interaction of these two hormones In the course of work on the interaction of several steroid hormones on behavioural and vaginal œstrus in spayed ewes, a preliminary trial showed that a single dose of testosterone or of testosterone propionate resulted in mating behaviour in a number of animals Consequently, a factorial design test, using 72 spayed ewes, was conducted to examine this phenomenon and to define the nature of the apparent œstrous response The variables introduced (with $n = 12$) were priming treatment (no progesterone or 10 mgm per day for 12 days)[3], and dose of androgen (1 0, 3 2 or 10 0 mgm). The androgen was testosterone propionate Sexual receptivity of the ewes was determined using 8 intact rams fitted with 'Sire-sine' harnesses and crayons[4] and the vaginal response was determined by smears taken for four days following injection of androgen[5] Results are presented in Table 1.

Table 1 BEHAVIOURAL AND VAGINAL RESPONSE TO TESTOSTERONE PROPIONATE ADMINISTERED TO 72 EWES WITH OR WITHOUT PROGESTERONE PRETREATMENT

	Dose of testosterone propionate (mgm)	Treated with progesterone	Not treated with progesterone	Total
Behavioural response (n = 12)	1 0	6	4	10
	3 2	10	11	21
	10 0	12	12	24
	Total	28	27	55
Vaginal response (n = 12)	1 0	1	—	1
	3 2	1	1	2
	10 0	—	—	—
	Total	2	1	3

Full sexual receptivity was induced by testosterone propionate and the response appeared unaffected by previous administration of progesterone, and so differed markedly from that to œstrogen[3]. The dose response effect ($P < 0\ 001$) had the following form

$$y = 42\ 8\,x + 41\ 6$$

where $\sin^2 y$ = proportion of ewes responding, and $x = \log_{10}$ dose androgen, expressed as free testosterone (mgm). The median effective dose was 1 20 mgm , which suggests an œstrus-inducing activity comparable to that of œstriol[6]

Vaginal smears from only 3 ewes showed evidence of epithelial cornification and desquamation characteristic of œstrogen activity Only one of these ewes demonstrated behavioural œstrus, suggesting that these apparent responses were artefacts On the day of service, vaginal smears consisted of thin watery mucus with leucocytes and spermatozoa On the 3 succeeding days there was massive leucocytic invasion, and many smears consisted entirely of thin mucus and leucocytes with some cellular debris This is in marked contrast to the picture when behavioural œstrus is induced with œstrogen, where smears 2 and 3 days after service consist almost entirely of desquamated cornified cells, and leucocytes are almost absent[3,5]

The effect of testosterone in inducing behavioural œstrus in this test appears to have been one direct on the central nervous system It does not appear to have been a precursor for œstrogen, in which case the vaginal smears would have presented evidence of œstrogen activity It is well established that libido in women is due largely to androgen, presumably of adrenal origin[7], and the presence and production of androgens in ovarian tissue have been demonstrated[8] Cole, Hart and Miller[1] observed that some ewes treated with pregnant mare serum gonadotrophin and 100 mgm of testosterone exhibited homosexual activity The median effective dose of testosterone, as assessed from our test, is only about 1 per cent of the dose administered by these workers No abnormal behaviour was observed at our dose-rates

Œstrus has two distinct components—anatomical and psychic—and these can now be differentiated in the ewe Œstrogen, alone, commonly will induce vaginal œstrus without behavioural œstrus[3,5] Œstrogen, following a period of progesterone influence, will induce both vaginal and behavioural œstrus[2,3]. Testosterone will induce behavioural œstrus minus vaginal œstrus

Three conclusions may be drawn

(1) Œstrous behaviour may be attributed to direct action on the central nervous system of an appropriate hormone, and not to the conditioning of peripheral receptors in the uterus[2] or in the vagina

(2) The pattern of the behavioural response to gonadal hormone is determined by the sex of the recipient rather than by the nature of the hormone administered

(3) It is not suggested that androgens normally are responsible for cyclic œstrous behaviour in the sheep It is well established that œstrogen, in association with progesterone, has that role It is clear, however, that the terms 'œstrogenic' and 'androgenic', in their commonly accepted context, are largely meaningless when applied to behavioural responses

This project forms part of a programme on the physiology of reproduction in the sheep financed by the Australian Sheep and Wool Research Committee Testosterone propionate was kindly donated by G D. Searle and Co , Chicago, and progesterone by Fawns and McAllan Pty , Ltd , Melbourne

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IMMUNOLOGY

Transfer of Immediate (Anaphylactic) Hypersensitivity by Homologous Bone Marrow or Spleen Cells

It has been repeatedly shown that lymph node cells from immunized donors transferred to irradiated recipients can transfer a specific antibody production and immunity state[1-3] We postulated that by implanting cells from a hyper-sensitized donor into a recipient made tolerant by whole-body irradiation, it would be possible to transfer the immediate type of hypersensitive state The present experiments demonstrate that the anaphylactic state can be obtained by transplantation of homologous bone marrow or spleen cells from sensitized donor guinea pigs into whole-body irradiated homologous recipients

Guinea pigs of a heterozygous randomly bred colony were used They were between 300 and 400 gm body-weight Donor animals were injected beneath the skin with 2 × 0·05 ml of horse serum emulsified in Freund's adjuvant mixture Six days later, bone marrow cells obtained from the femurs, tibiæ and humeri were suspended in cold Tyrode's solution The suspension of spleen cells was prepared by squeezing the mashed tissue through gauze; finally, cell suspensions were filtered through a loose cotton pellet In a group of sensitized animals injected intravenously six days later with 0·15 ml of horse serum no signs of anaphylactic reaction were observed (four animals) Animals challenged on the ninth day (two animals) manifested moderate symptoms, and on the fifteenth day three tested animals exhibited fatal anaphylactic reaction

In several experiments, previously irradiated animals and twenty-two unirradiated ones received cell suspensions intravenously Irradiated animals were exposed to 300, 400 or 700 r whole-body X-irradiation under the following conditions. 220 kV., 15 m amp , 0·5 mm of copper and 1 mm of aluminium, 40 cm from target to animal skin, 83 r / min According to the cells which they received the recipients were divided into three sub-groups The first obtained a mixture of about 11 × 10^7 viable nucleated bone marrow cells and 10 × 10^7 viable spleen cells The second sub-group received 11 × 10^7 bone marrow cells only and the third 10 × 10^7 spleen cells only Viability was estimated by the number of stained (dead) cells in a 0·2 per cent solution of eosin in Tyrode's solution. Cell suspensions were injected into the jugular vein within 3–5 hr after irradiation under light local anæsthesia (2 per cent novocain)

Nine days and in some cases 16 days after the cell transfer animals were challenged by intravenous injection of 0·15 ml horse serum (Not all the irradiated animals had survived up to that time) The ratio of the number of reacted to the number of tested animals as well as anaphylactic index for all experimental groups are shown in Table 1

As seen in Table 1, all the irradiated animals showed anaphylactic responses (in a few minutes after challenge). The degree and duration of the reaction were not the same in all animals but always pronounced, three animals died of shock. Only some of the unirradiated animals showed very weak symptoms which could be attributed to anaphylactic response

From these results it seems possible to conclude that:

(1) By injection of bone marrow or spleen cells it is possible to transfer the immediate type of hypersensitivity into recipient animals. The intensity of the

Table 1

Recipient animals group	Day of challenge (post-transfer)	Bone marrow + spleen cells		Bone marrow cells		Spleen cells		Total	
		Reacted to test	Anaphylactic index*	Reacted to test	Anaphylactic index*	Reacted to test	Anaphylactic index*	Reacted to test	Anaphylactic index*
Unirradiated	9	0/3	0	2/12	0·1	2/5	0·7	4/20	0·2
	16	0/2	0	—	—	—	—	0/2	0
300r	9	—	—	8/8	2·0	3/3	2·0	11/11	2·0
400r	9	6/6	2·8	4/4	3·0	6/6	3·5	16/16	3·1
	16	4/4	3·8	—	—	—	—	4/4	3·8
700r	9	4/4	3·8	5/5	2·4	2/2	2·0	11/11	2·7

* Anaphylactic index expresses the relative degree of anaphylactic state It is calculated by multiplying the number of animals showing fatal symptoms by 5, severe symptoms by 4, moderate symptoms by 3, mild symptoms by 2, very weak or uncertain symptoms by 1, without symptoms by 0, and dividing the sum of the products by the total number of animals challenged This is a slightly different system from that described in ref 5

anaphylactic response showed that the ability of spleen and bone marrow cells to induce anaphylactic response by transfer was about the same.

(2) Passive transfer of antibodies as a reason for reaction is not probable because (*a*) sensitized animals challenged on the sixth day did not show anaphylactic symptoms; (*b*) in this case unirradiated animals should have reacted also. This second reason seems to exclude direct antigenic stimuli of recipient animals if antigen were transferred together with cells from sensitized donor guinea pigs.

(3) The success of transfer seems to depend mainly on the possibility that the recipient animal rejects transferred cells. Failure to induce anaphylactic reaction is probably due to rejection of the sensitized cells before they can produce enough anti-horse serum antibodies. If the transferred cells have a better opportunity to colonize the recipient tissues and mature some days in them, the production of antibodies may be sufficient and an injection of horse serum can elicit an anaphylactic reaction. In this experiment the necessary conditions for successful cell transfer were obtained by irradiation.

(4) The number of cells necessary to achieve the anaphylactic state in irradiated recipient animals was evidently much smaller than in the case of normal recipient animals[4].

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Agarophoresis and Immuno-electrophoresis of the Proteins of Gastric Juice

A NUMBER of authors have used paper and freely moving boundary electrophoresis for investigating proteins of gastric juice. We have tried to add to the results obtained by these methods by using electrophoresis in agar[1] and immuno-electrophoresis[2]. With these methods, especially in the case of immuno-electrophoresis, we found that there exists a fundamental difference in the behaviour of acid and anacid gastric juices.

Agarophoresis. The electrophoretograms of fifteen anacid gastric juices (*p*H 4–7) of patients with histamine-resistant achlorhydria showed five protein fractions, the mobility of which corresponded to that of serum proteins (Table 1, Fig. 1*a*). In three anacid gastric juices where all subsequent portions contained free hydrochloric acid after stimulation by histamine, two additional zones faster than albumin were observed. The electrophoretic mobility of this pre-albumin is identical with that of crystalline pepsin. In ten acid gastric juices seven fractions including two pre-albumin zones of pepsin were noted (Table 1, Fig. 1*c*).

Table 1. PROTEIN FRACTIONS OF GASTRIC JUICE AFTER ELECTROPHORESIS SEPARATION IN AGAR

Samples	Pepsin 2 ×	Albumin	Globulins				
			α_1	α_2	β_1	β_2	γ
15 anacid gastric juice	—	15	—	7	15	15	12
3 anacid gastric juice	3	3	1	3	3	3	3
10 anacid gastric juice	10	10	2	8	6	10	—

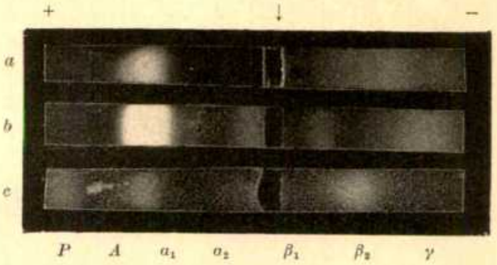

Fig. 1. Electrophoresis in agar: *a*, anacid gastric juice; *b*, serum; *c*, acid gastric juice

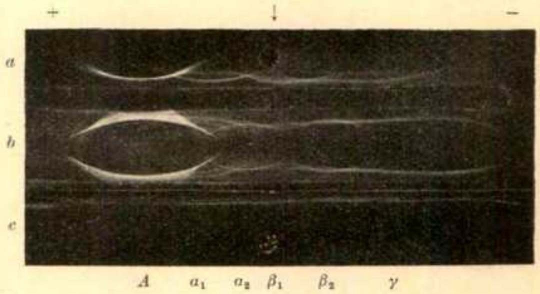

Fig. 2. Immuno-electrophoresis: *a*, anacid gastric juice; *b*, serum; *c*, acid gastric juice

Immuno-electrophoresis. The proteins of 22 anacid gastric juices precipitated on immuno-electrophoretic plates by rabbit antihuman serum developed 3–10 precipitation lines (Fig. 2). Their location both in the direction of electrophoresis and of diffusion corresponded to the precipitation lines of blood serum. In the double diffusion test[3] the albumin of the gastric juice gave the identity reaction with serum albumin. γ-Globulin reacted likewise with γ-globulin antiserum. The precipitation line of β_2-globulin was only about 30 per cent determined, although a zone of strong density was observed in all gastric juices by simple agarophoresis. This indicates that in all anacid gastric juices besides β_2-globulin, which is not precipitated by antihuman serum and thus differs immunologically from serum proteins, an inconstant serum β_2-globulin in lower concentration is present.

The proteins of ten acid gastric juices were not precipitated with antihuman rabbit serum. It follows from our investigation on the influence of single components of gastric juice on serum proteins that the protein fractions lose their immunochemical properties by the action of the activity of pepsin and the acid medium in dependence on time.

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Attachment of Antibody to Influenza Virus

A KINETIC analysis of the neutralization of influenza virus by rabbit antibody indicated that the combination proceeds in two stages[1]. The first stage is the formation of a freely dissociable union between the virus and antibody. This is followed by a second stage which leads to the stabilization of the complex between the virus and antibody. The reaction kinetics also indicated that a single antibody molecule could form a stable combination with the virus particle. In an attempt to elucidate the nature of the stage of stabilization, the interaction between influenza virus and the univalent antibody fragments, produced by papain digestion[2], was investigated[3]. This investigation demonstrated that although the monovalent fragments could combine reversibly with the virus and neutralize its infectivity, they were unable to form a stable combination with the virus. It could be concluded that the stabilization of the combination between the virus and antibody depended on the divalent nature of the antibody molecule. On the basis of the foregoing conclusions and observations the following hypothesis emerged. The first stage in neutralization would be the attachment of the antibody molecule to the surface of the virus through one combining site and the second and stabilizing stage would be brought about by the attachment of the second combining site on the divalent antibody molecule to the same virus particle.

This hypothesis was supported by the finding that the fraction of virus particles which could undergo the stabilization reaction declined sharply when the reaction was studied in the region of extreme antibody excess[3]. This would be expected for the following reason. The stabilization stage proceeds at a much slower rate than the first stage of the interaction between virus and antibody. Thus, when the virus is mixed with high concentrations of antibody, antigenic sites on the surface of the virus particle will be rapidly occupied by antibody molecules attached through only one combining site. The combining sites of the virus will be saturated with antibody and the neighbouring molecules may sterically hinder the attachment of any antibody molecule to one and the same virus by both its combining sites. Indeed, work on the interaction between tobacco mosaic virus and antibody, carried out with the aid of the electron microscope, demonstrates that in the region of antibody excess the antibody molecules are attached to the virus surface through one combining site, with their long axes radiating out from the surface of the particle[4,5]. A similar type of combination has been observed in these Laboratories in work on the interaction between influenza virus and a large excess of antibody.

The kinetic evidence demonstrated the occurrence of a reaction of stabilization, but could not provide detailed information on the mechanism of the stabilization reaction. The following experiment was therefore conducted in an attempt to obtain more direct information on this point. Purified influenza A virus (*MEL*) was mixed with a solution of the globulins obtained from an *MEL* virus rabbit antiserum and held at room temperature for 4 hr. The concentration of the virus in the mixture was 4×10^4 hæmagglutination units/ml. and the concentration of antibody was 300 hæmagglutination inhibition units/ml. Virus and antibody titrations were carried out as described by Fazekas de St. Groth *et al.*[6,7]. The complexes of virus and antibody were sedimented in the ultracentrifuge, washed with saline and finally resuspended in distilled water. As a control, a preparation of *MEL* virus that had not been mixed with antibody was treated in the same way as the mixture of virus and antibody.

Specimens of the two suspensions were prepared for electron microscopy by mixing equal volumes of the suspensions and a 1 per cent solution of sodium phosphotungstate[8]. A drop of this suspension was allowed to dry on carbon-coated 'Formvar' membranes and the specimens were observed in the electron microscope (Siemens Elmiskop I). Fig. 1*a* shows the morphology of the virus particle and Fig. 1*b* the type of complex formed when the virus reacts with antibody. It can be seen that the antibody molecules become attached to the terminal ends of the sub-units which constitute the surface of the virus. The antibody molecules appear to be attached to the individual virus particle through both sites situated at the extremities of the antibody molecule, thus forming a loop on the surface of the virus particle.

It is known that the two combining sites on an antibody molecule have identical specificities[9,10]. This may well imply that the combining strength at each site is very similar. If this were so, the combination of the antibody with a virus particle through both combining sites of the antibody molecule would be much more stable than the combination through only one site, provided the strain introduced by the bending of the molecule did not significantly reduce the strength of the bonds formed through each combining site. The fact that a two-site attachment rather than a single-site attachment is produced when the virus and antibody react under conditions where the antibody is not in very large excess indicates that the former type of attachment is more stable than the latter.

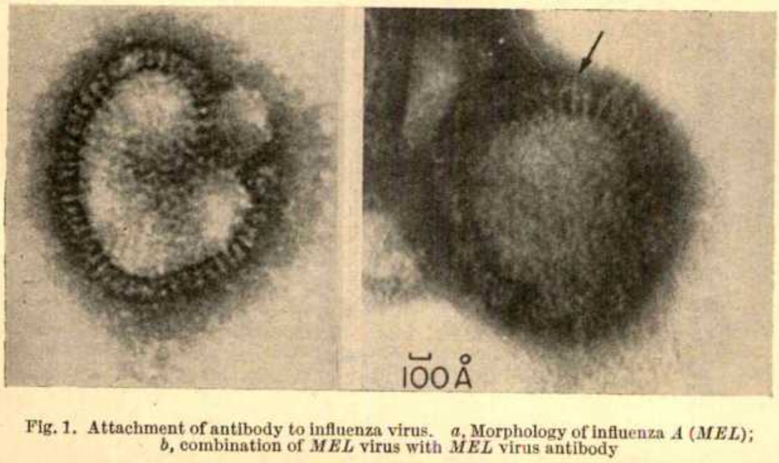

Fig. 1. Attachment of antibody to influenza virus. *a*, Morphology of influenza *A* (*MEL*); *b*, combination of *MEL* virus with *MEL* virus antibody

Thus, the observation of two-site combination between virus and antibody provides a physical mechanism which would account for the stabilization reaction.

A more detailed publication demonstrating the effect of the concentration of the reactants on the type of combination formed is in preparation

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HÆMATOLOGY

Absence of Red Cell Enzyme Deficiency in Australian Aborigines

The geographical distribution of erythrocyte glucose-6-phosphate dehydrogenase deficiency is now known to be widespread Thus, this genetically determined enzyme defect has been shown to be present in parts of Africa, Asia and Europe, and in some Indian groups in South America[1]. Marked variations in the incidence of the defect have been observed between different populations and in some instances between different groups in the same population. In south-east Asia recent work has shown the trait to be present in Thais[2], Malays[2a], Indonesians (Lie-Injo, L E , personal communication), Filipinos[3] and Melanesians[4]

In the present work, using the rapid screening test described by Motulsky[5], a total of 722 Australian Aborigines were examined for evidence of glucose-6-phosphate dehydrogenase deficiency The subjects tested live in scattered areas of northern, central and western Australia All have tribal affiliations The groups studied are shown in Table 1

Table 1

Area	Males	Females	Total
Cape York Peninsula			
Edward River	43	45	88
Mitchell River	84	62	146
Lockhart River	27	—	27
Aurukun	39	37	76
Weipa	12	29	41
Wrotham Park	12	11	23
Central Australia			
Alice Springs	140	—	140
Western Australia			
Port Hedland	40	59	99
Marble Bar	11	13	24
Nullagine	27	31	58
Total	435	287	722

No cases of enzyme deficiency were found in this large population sample, indicating that the gene frequency is extremely low or non-existent. This finding is of interest in several respects Except for occasional cases of malaria introduced in recent times from surrounding areas Australia is malaria-free. The incidence of red cell enzyme deficiency has been shown, in Africa[3,6], New Guinea[4,7] and Sardinia[8], to be related to that of falciparum malaria, probably representing a selective advantage by way of increased resistance to malarial mortality Absence of the deficiency in Australian Aborigines supports this concept, as there would be no such advantage enhancing the survival of affected people in Australia

Glucose-6-phosphate dehydrogenase deficiency has an incidence as high as 20 per cent in males of coastal New Guinea[7] The penetration of the blood group *B* gene in Aborigines of the Cape York Peninsula groups is accepted as representing admixture from New Guinea[9] It is evident, however, that this penetration has not resulted in the introduction of the enzyme deficiency, or, if the gene has been introduced in the past, it has not survived That the well-known Malay contact with Aborigines in several areas of the north and north-west coast has not resulted in the introduction of the trait is not surprising, since its incidence in Malays is low, probably less than 1 per cent

Absence of this deficiency of red cell enzyme, then, adds important evidence to that already accumulated, including the absence of thalassæmia and the abnormal hæmoglobins, which demonstrates the degree of isolation of the Aborigines from surrounding peoples This trait will serve as an additional link in the chain that may eventually disclose the origin of the Australian Aborigines

Gratitude is expressed to Dr P Dawes and Miss M. Byrum. of the Alice Springs Hospital, to Dr. R Doherty, of the Queensland Institute of Medical Research, and to Dr. R L Kirk, of the University of Western Australia, for their kind assistance in collection of blood samples This work was supported in part by the National Health and Medical Research Council

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Distribution of Haptoglobin Types in Israel

The interest in the patterns of haptoglobin types has been furthered during the past several years due to the introduction of starch-gel electrophoresis for their demonstration[1] Different populations have been investigated up to now[2,3].

Since differences between Jewish ethnic groups in Israel were described in relation to blood group patterns[4], incidence of erythrocyte glucose-6-phosphate dehydrogenase deficiency[5], and taste sensitivity to thiourea (Sheba, Ch , Ashkenazi, I , and Szeinberg, A., personal communication), it was of interest to investigate the haptoglobin patterns among them

Blood specimens from 526 blood donors from the blood bank of Tel Hashomer Hospital, and from patients without any serious illness, were examined. The material consisted of 170 Ashkenasi Jews (East European origin), 118 Iraqi Jews (Babylonians), 104 North African Jews (Morocco, Tunisia, Algeria and

Lybia) and 134 mixed population. The method used was vertical starch-gel electrophoresis as described by Smithies[6]. The starch was sliced longitudinally into two parts, one was stained with benzidine, the other with brom-phenol blue or amido-black 10 *B*. To the sera 120–150 mgm. per cent of oxyhæmoglobin was added to saturate the haptoglobins completely. The results are presented in Table 1.

Table 1. HAPTOGLOBIN TYPES AND FREQUENCY OF ALLELE Hp^1 AMONG DIFFERENT ETHNIC GROUPS IN ISRAEL

Group	No. tested	*Hp* 1–1		*Hp* 2–1		*Hp* 2–1 *M*		*Hp* 2–2		Gene frequency Hp^1
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	
Ashkenazi	170	13	7.65	88	51.76	—	—	69	40.59	0.34
Iraqi	118	9	7.63	50	42.37	—	—	59	50.00	0.29
North African	104	8	7.69	43	41.35	1	0.96	52	50.00	0.29
Rest of population	134	13	9.70	51	38.06	—	—	70	52.24	0.29

No cases of ahaptoglobinæmia were found except in hæmolytic anæmias, which are not included in this work. Only one case of 2–1 *M* was found, that being among the North African Jews. The gene frequency of Hp^1 in Ashkenazi Jews was found to be 0.34, while that found among the Iraqi and the North African Jews was 0.29. This difference is not statistically significant. The expected values of phenotype were calculated on the basis of the Hardy–Weinberg equilibrium and were found to correspond closely to the observed numbers.

Our results show that the frequency of Hp^1 among the Ashkenazi Jews is similar to that found in European populations[2], while the Iraqi and North African Jews have a lower frequency of Hp^1 similar to that found in Asian populations[3]. Other Jewish and Arab populations are under investigation.

This work was supported by Rockefeller Foundation grant *RE. GA. BMR.* 5936. We thank Dr. Eloise Giblett for her valuable suggestions.

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Distribution of Group-specific Components (*Gc*) in the Sera of Native Africans

THE group-specific components of sera consist of electrophoretically variable α_2-globulins demonstrable by means of an immuno-electrophoretic technique[1]. This system allows a division of human sera into three qualitatively different patterns characterized by the presence of a fast-moving group-specific component (*Gc* 1–1), a slow-moving component (*Gc* 2–2), which in immuno-electrophoresis both give normally arc-shaped precipitates, or the simultaneous presence of both fast- and slow-moving components (*Gc* 2–1), which in immuno-electrophoresis give an extended two-peaked precipitate due to a reaction of immunological identity between these electrophoretically different although immunologically identical fast- and slow-moving components[1]. Investigations on sera from 42 families with 137 children and more than 600 mother–child combinations have shown that these serum patterns are genetically determined by two autosomal alleles (Gc^1 and Gc^2) without dominance where the serum types *Gc* 1–1 and *Gc* 2–2 are the homozygotes and *Gc* 2–1 the heterozygote. Up to now, no exceptions to this genetical theory or unresolved typing difficulties has been found in the material investigated[2,3].

Table 1. FREQUENCIES OF THE Gc^1-GENE IN VARIOUS POPULATIONS

Population	No. tested	Gc^1	*Gc*-determinations carried out by
Sweden	1,744	0.74	Hirschfeld[3]
Swedish Lapps	79	0.87	Hirschfeld*
New York Whites	86	0.74	Bearn and Cleve†
New York Negroes	120	0.90	Bearn and Cleve†
Navajo Indians	197	0.98	Bearn and Cleve†

* Hirschfeld, J., and Beckman, L., *Acta Genet.*, **11**, 185 (1961).
† Personal communication.

Table 2. DISTRIBUTION OF *Gc*-TYPES IN SERA FROM 100 AFRICANS

	Gc 1–1	*Gc* 2–1	*Gc* 2–2	Gc^1
obs.	82	17	1	0.91
exp.	81.9	17.2	0.9	

The frequencies of the Gc^1-gene in populations hitherto investigated are given in Table 1. In Table 2, the distribution of *Gc* in a serum sample consisting of sera from 100 native Africans collected in Léopoldville, Congo, are given. The results seem to show that the system might be of value in connexion with anthropological investigations, and further work concerning the distribution of *Gc*-types in different populations is in progress.

One of us (J. H.) gratefully acknowledges a grant from Lennanders Fond, Uppsala, Sweden.

Note added in proof. A more detailed survey of the distribution and inheritance of the *Gc*-system will be given elsewhere[3].

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RADIOBIOLOGY

Autoradiographic Study of Cell Migration during Histogenesis of Cerebral Cortex in the Mouse

AUTORADIOGRAPHY with thymidine labelled with tritium has proved useful for the study of histogenesis in the mammalian brain[1–6]. Labelled thymidine injected into animals becomes incorporated almost exclusively into deoxyribonucleic acid of cells preparing to divide and remains in their nuclei as a permanent label, the radioactivity is diluted to half with each cell division[7]. When the autoradiographic method is applied to tissues of animals killed serially, one can determine the time and place of origin of cells, their route and rate of migration, and their ultimate fate.

The question has been raised as to whether cells which appear to change position during development are truly migrating or are merely being displaced by cells which form at subsequent stages. The present work demonstrates that migrations of cells do indeed take place in the developing brain.

Tritiated thymidine (5 μc./gm. of body-weight) was injected once intravenously into pregnant mice. The animals were injected in pairs on the 11th, 13th, 15th, or 17th days of gestation. One animal of each pair was killed 1 hr. after injection, and offspring of the other injected animal were killed on the 10th postnatal day. Brains were processed for autoradiography as described elsewhere[1].

In an embryo killed 1 hr. after injection on the 11th day, the telencephalic vesicle consists only of a primitive ependymal zone; most of its cells are labelled, as has been observed previously[1]. Many of the primitive ependymal cells later migrate outward to form the anlage of the cortical plate. In embryos injected on the 13th, 15th, or 17th day of gestation and killed 1 hr. later, the cerebrum shows labelled cells almost exclusively in the primitive ependyma and relatively few in the developing cortical plate. Thus, the source of most cortical cells is the primitive ependyma of the lateral ventricles. After mitosis in the primitive ependyma, cells migrate outward to form the cortical plate and differentiate into its constituent neurons.

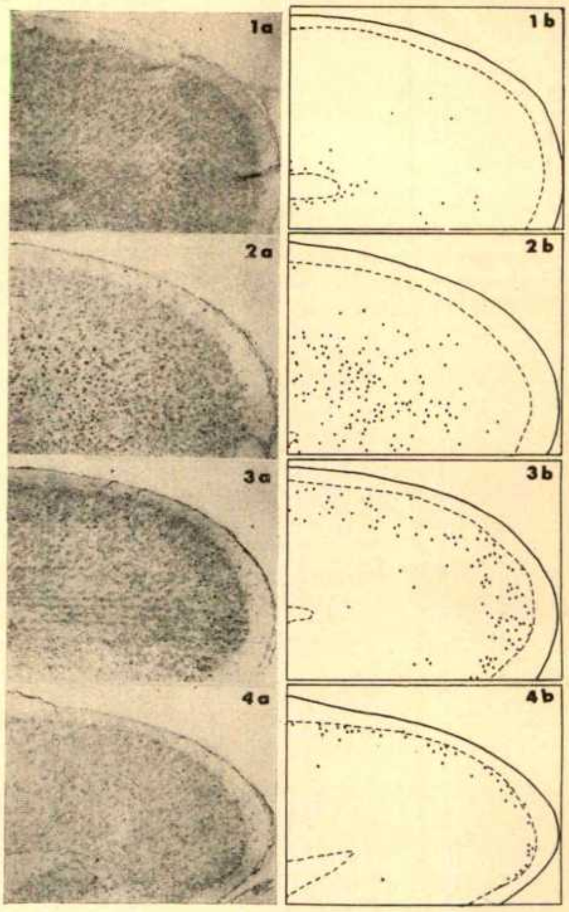
To study the postnatal disposition of cortical cells arising in the 11-, 13-, 15-, and 17-day embryos, we photographed the entire anteroposterior extent of the cerebral cortex of the corresponding four 10-day old mice. For each brain a series of overlapping photomicrographs was made at a magnification of 150 diameters on 4 × 5 in. sheet film, and prints were assembled as a montage to show the distribution of labelled cells. Comparable sections of occipital cortex from the 4 mice are illustrated in Figs. 1–4 and the following description refers to this region of cortex.

Many cells labelled on the 11th day of gestation undergo only 1 or 2 further divisions and are recognized in the brain of the 10-day old animal as heavily labelled cells. These cells are almost exclusively in the deepest part of the cortex adjacent to the white matter (Fig. 1). Other cells labelled on embryonic day 11 divided on subsequent embryonic days and appear in the 10-day old mouse as weakly labelled cells scattered through all cortical layers.

The mouse labelled on the 13th day of gestation and killed 10 days after birth shows a greater number of heavily labelled cells; these are located predominantly in the middle third of the cortex (Fig. 2). These cells originated later than the cells which arose on the 11th embryonic day and migrated past their predecessors upon entering the cortical grey matter.

The same pattern of origin and migration obtains for cells forming later in gestation. Cells which arise on the 15th embryonic day migrate past cells which had formed earlier and come to lie in the outermost third of the cortex (Fig. 3). Relatively few cells arising on the 17th embryonic day are destined for the occipital cortex; these migrate to the most superficial cell layer, the presumptive lamina ii (Fig. 4).

As Figs. 1–4 show, cells originating on any particular embryonic day come predominantly but not exclusively into a given cortical level. Cells forming successively later in embryonic life migrate outward past their predecessors and come to lie at more and more superficial levels. The correlation between these levels and cortical laminæ as conventionally designated will be considered in a more complete account of cortical histogenesis now in preparation.



Figs. 1–4. Photomicrographs (*a*) and matching drawings (*b*) of comparable parasagittal sections of occipital cortex from four 10-day-old mice injected with thymidine labelled with tritium on the eleventh, thirteenth, fifteenth and seventeenth days of gestation respectively. Dots in drawings indicate heavily labelled cells. Intensity of labelling in photomicrographs varies because tissues were exposed to the emulsion for different durations prior to development of the autoradiograms. Outer broken line represents inner boundary of molecular layer of cortex and inner broken line the boundary between cortex and white matter. (× *c*. 38)

Cortical zones other than occipital cortex display a similar pattern of histogenesis. The times of origin and migration of cells into other cortical regions differ from the times described for occipital cortex.

If cells do not migrate outward, but are merely displaced peripherally by new cells which arise afterwards in the primitive ependyma, one would expect the more superficial layers of cortex to form first and the deep layers last, as has been suggested[8]. Our autoradiographic results indicate just the reverse, and show that cells do migrate extensively in the developing mammalian cerebrum.

This curious reverse order of formation of cortical layers may have significance for the attainment of intracortical synaptic connexions. It is recognized that the axons of most cortical neurones are directed inward toward the white matter and that their dendrites are directed outward[9]. The orientation of the circuits established by synaptic contact of these processes is predominantly perpendicular to the plane of the cortex[10]. These synaptic contacts might be attained during the histogenetic period, as cells migrate past those neurones that have arrived previously in the developing cortex. The perikaryon of a cell migrating outward may perhaps establish contact with another cell already in position in the cortex and may then migrate farther while trailing its axon behind, still in contact with the perikaryon or the developing dendritic tree of the cell that had arrived previously. The cell contacts established during histogenetic migration may also be relevant for the formation of particular patterns of synaptic connexions in other parts of the nervous system, as discussed elsewhere[3,4].

This work was supported in part by research Grants *B* 2853 and *B* 1938 from the National Institutes of Health, U.S. Public Health Service.

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Uptake and Incorporation of Tritiated Thymidine in *in vitro* Culture

THE duration of the 'synthesis phase' of deoxyribonucleic acid (DNA) of the mitotic cycle (phase *S* of Howard 1956[1] and Lajtha *et al.* 1954[2]) has been determined for several cell types. As yet no distinction has been made between the uptake of labelled thymidine and its incorporation by the cell into a more complex structure, presumably DNA.

Sagittal slices of the upper tibial epiphysis of the rat, rabbit and guinea pig were cultured *in vitro*, in the presence of tritiated thymidine (0·5–1 μc. tritiated thymidine, specific activity 0·360 c./m.mol., to 5–6 ml. medium), in an attempt to elucidate the growth pattern of the epiphyseal growth cartilage and the articular cartilage. The explants were fixed in 10 per cent formalin at *p*H 7·4–7·6. Some were embedded in butyl and isobutyl methacrylate and sectioned without decalcification; others were first decalcified with 10 per cent formic acid for 12 hr. before being embedded in paraffin wax. Sections 4–7μ thick were cut, the embedding medium removed, and autoradiographs made according to the Pelc stripping film technique[3], and exposed for 3–6 weeks. Grain counts were made on cells in the proliferative zone (flattened cells) of the epiphyseal growth cartilage.

If the tissue was sectioned without previous decalcification, the grain counts attained nearly the maximum value after ½ hr. in culture. By 1 hr. the maximum value was found; this persisted for the duration of the experiment (Fig. 1).

On the other hand, if the tissue was first decalcified with formic acid, maximum counts were only attained on explants that had been cultured for 6–9 hr. (Fig. 2, rat). Similar results were obtained by giving the tritiated thymidine to rats *in vivo*.

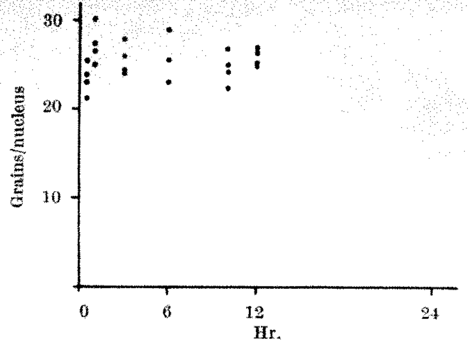

Fig. 1

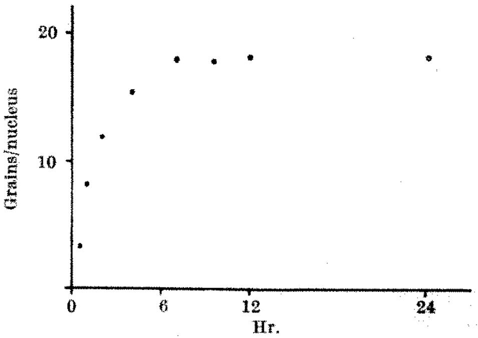

Fig. 2

These results suggest that a maximum load of tritiated thymidine is taken into the cell nucleus very rapidly. In fact, this occurs so rapidly, in less than 1 hr., that a carrier mechanism might have to be invoked to explain it. But once in the nucleus, it takes a finite time for the tritium-labelled substance to be incorporated into a large and more complex molecule capable of resisting removal by 10 per cent formic acid. This process of incorporation would appear to take between 6 and 9 hr. to be completed in the cells of the proliferative zone of the epiphyseal growth cartilage.

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BIOLOGY

Fluctuations in Marine Populations in Icelandic Waters

IT is well known that populations of many animals living at high latitudes are subject to considerable fluctuations. Fairly regular periodic variations are reported in a number of cases. The 10-yr. cycle of the rock ptarmigan in Iceland reported by Gudmundsson[1] furnishes a striking example. The cause of this cycle is unknown.

Marine populations are also subject to large fluctuations but there are few reports on regular or periodic variations. This may in many cases be due to lack of observations.

There are available fairly reliable data on the catching of cod in the waters south-west of Iceland since 1900. In particular, there is available a set of data of a rather uniform quality for the winter

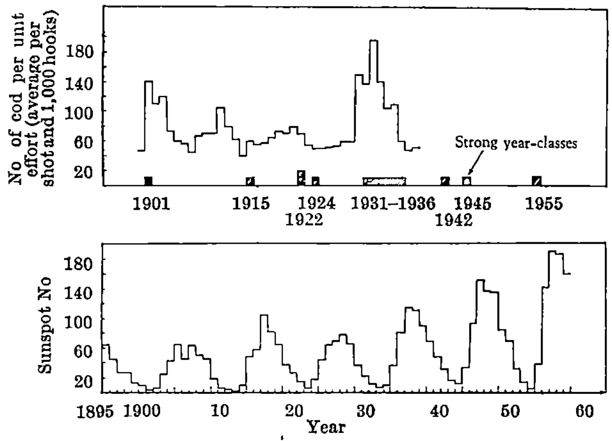

Fig. 1. Catch of cod per unit effort in the Vestmanna Islands during 1900–40, and its relation to observed strong year-classes and the sunspot number

seasons during 1900–40. Data on the catch per unit effort have been collected in the Vestmanna Islands and are given as the yearly average number of cod caught per shot and 1,000 hooks. The data are given in Fig. 1. Unfortunately these data have not been collected for the period since 1940.

The catch per unit effort given in Fig. 1 is subject to a rather regular variation with a period of approximately 10 yr. This happens to be the same period as that of the rock ptarmigan. There is little doubt that the catch per unit effort depends largely on the number of individuals per unit volume, and the data therefore give a certain measure of the variation in the size of the spawning stock.

The fluctuations in the strength of the spawning stock of cod are due to pronounced differences in the strength of the individual year-classes. Certain year-classes prevail in the commercial catch. The recruitment age for more than 80 per cent of the stock is 7–10 yr.

Reliable data are available on the strength of individual year-classes since 1920. Moreover, it is known that the 1901 and 1915 year-classes were relatively strong although very little is known about other year-classes during 1900–20.

The prevailing year-classes are given in Fig. 1. There appears to be a tendency for an 11-yr period in the emergence of strong year-classes. The class of 1915 is somewhat anomalous, and the period 1931–1936 is characterized by a series of medium-size classes. This, however, does not interfere with the rather regular pattern.

For several decades it has been repeatedly pointed out that certain biological phenomena appear to exhibit periodicities related to the 11-yr solar cycle. In most cases, however, the evidence has been very weak and there have been no reliable indications as to the relation between the solar and the biological phenomena.

Quite recently, however, it has been pointed out by MacDonald and Roberts[3] that solar corpuscular emission may affect the surface weather in a significant way. Also, Landsberg *et al.*[4] have been able to detect a weak tendency for a sunspot rhythm in the temperature of the United States.

In view of these circumstances we have compared the emergence of strong year-classes of the Icelandic cod with the sunspot rhythm. The Zurich sunspot number has been plotted in Fig. 1. The results at hand indicate quite definitely that the strong year-classes emerge preferably during periods of low solar activity.

In order to extend these observations we have studied the available reports on the catch of other species in Icelandic waters. Unfortunately these reports are much less reliable than the above data on the catches of cod. However, the results are not altogether negative.

The catch of herring during the summer season in the waters north of Iceland, measured in volume per boat, exhibits relative maxima in 1923, 1933 and 1944. These are years of low solar activity. Moreover, the total catch of salmon in the rivers of Iceland, measured in number of salmon per annum, exhibits pronounced maxima in 1913, 1922 and 1933, which are years of low solar activity. On the other hand, the available data on the total catch of lumpfish in Icelandic waters exhibits maxima in the periods of high solar activity. Data on lumpfish are at hand for 1915–50. The maxima correlate moderately well with the solar maxima of 1917, 1928 and 1938.

A discussion of possible causes of the more or less apparent correlation between the solar rhythm and biological phenomena is beyond the scope of this communication. We realize, of course, that the fluctuations of the populations may be due to some local or internal cause and the relation to the solar rhythm only accidental.

In order to look for a climatic factor we have made a preliminary investigation of the solar-weather relationship for Iceland during 1900–60. The fluctuations in the annual and the monthly means of the temperature and the precipitation in Reykjavik have been investigated. The results are negative so far. No relation to the solar rhythm has been detected. However, we wish to emphasize the preliminary nature of this work.

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Colour and Density of Light and Broiler Performance

It has been shown that some form of artificial lighting is necessary to achieve the most rapid gains of weight in broilers[1]. With controlled environments, light intensities of 0.1–6.0 ft.-c. gave equally good growth-rates in broiler chicks during the first eighteen days, and 12.0–24.0 ft.-c. produced slightly depressed growth-rates during this period[2]. A field scale survey has noted that in many broiler houses in Britain the light intensity is maintained at 0.05–0.20 ft.-c. as a control measure for the prevention of cannibalism[3]. An experiment is being conducted which has already compared three types of continuous lighting in relation to broiler performance.

Six groups, each of 111 Rhode Island Red × Light Sussex cockerels hatched on August 27, 1960, were reared under identical conditions except for variations of light in environmental chambers 14 × 8 ft. Two groups served as controls and their chambers were each lighted by two 15-W white incandescent

Table 1. WATER CONSUMPTION, MORTALITY AND GROWTH TO 7 DAYS OF AGE

Lighting treatment	Mean water consumption (pints) per pen First 3 days	Mean water consumption (pints) per pen First 7 days	Mean water consumption (pints) per survivor First 7 days	No. mortalities per pen during first 7 days	Weight increases per chick during first 7 days (oz.)
Controls	11.2	35.5	0.323	1.0	2.47
Dull red	9.2*	30.0	0.284	5.5*	2.12*
Dull white	10.5	34.0	0.313	2.5	2.26
Least difference to be significant between two means, $P < 0.05$	1.0	4.3	N.S.	1.9	0.15

N.S., no significant difference.

* Significantly different from the control, $P < 0.01$.

Table 2. AVERAGE RESULTS TO 72 DAYS OF AGE

Lighting treatment	Mean No. mortalities per pen	Live-weight (lb.) Total per pen	Live-weight (lb.) Average per broiler	Food consumption (lb.) Total per pen	Food consumption (lb.) Average per broiler	Food efficiency (food consumed per unit of live-weight gain)
Controls	1.0	402.7	3.661	1085.7	9.87	2.696
Dull red	7.5*	367.6*	3.552	976.0*	9.43*	2.655
Dull white	3.5†	387.8†	3.607	1009.5*†	9.41*	2.603*
Least difference to be significant between two means $P < 0.05$	1.9	10.6	0.101	3.3	0.21	0.043

* Significantly different from the control, $P < 0.01$,

† Significantly different from the dull red treatment, $P < 0.01$.

bulbs. Two further pens each had two 40-W. red bulbs and the remaining two had two 5–8-W. white economy bulbs. Lighting was continuous, and the intensities recorded were 0.20–0.50 ft.-c. in the control pens, and 0.05–0.10 ft.-c. in the other four pens which were lighted by dull white and dull red bulbs.

During the first few days the form of lighting affected the amount of water consumed. This is shown in Table 1. Differences in mortality and growth-rates to one week of age were also apparent. The use of dull red lighting was associated with increased mortality compared with the control ($P < 0.05$). At the end of the first week the controls were heavier per survivor than the birds of either of the two experimental groups ($P < 0.05$ dull white lighting, $P < 0.01$ dull red lighting).

Table 2 shows the results to 72 days of age. The control chicks ate significantly more per pen and more per bird than did either of the two experimental treatments. Although the total live-weight of the controls was greater than that of either of the other two experimental groups ($P < 0.05$ dull white lighting, $P < 0.01$ dull red lighting), the average live-weight of the controls was only significantly heavier than the broilers reared under the dull red lighting. These variations were partly due to differences in mortality. The birds receiving dull white light had an improved food conversion factor compared with the control ($P < 0.01$) and compared with those receiving the dull red treatment ($P < 0.05$). No cannibalism was observed under any treatment, and there was no downgrading because of stubby, undeveloped feathers when the broilers were sold.

This experiment, which is being extended, is conducted with the aid of grants and equipment from the Agricultural Research Council, Calor Gas, Ltd., Electrical Development Association, Fenton Byrn, Ltd., Maywick Appliances, Ltd., and Wernick Construction Co., Ltd., in the Department of Animal Husbandry at the Royal Veterinary College, London.

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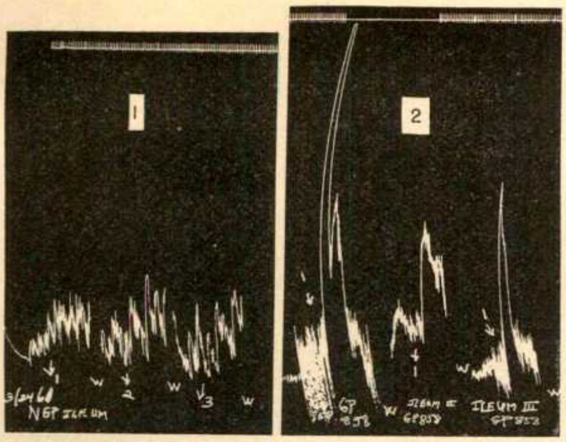
Antigenicity of Guinea Pig Testicular Cells grown in Cell Culture as determined by the Isolated Ileum Technique

I HAVE previously recorded experiments relating to the antigenicity of testicular tissue and of sperm as well as extracts thereof[1]. For many reasons, for example to facilitate identification of spermatogenic tissue antigens and to elucidate the mechanism(s) of cell destruction occurring in immunologically induced aspermatogenesis, it was important to have replicating cell cultures of testicular tissue. Replication, rather than mere maintenance, of testicular cells in culture has recently been accomplished, and the results will be published *in extenso* elsewhere. The attainment of this goal imposed the requirement of demonstrating that spermatogenic cells grown in culture continue to synthesize antigens that are present in the germinal epithelium of the testicle *in vivo*. Among such antigens are those capable of (1) eliciting anaphylactic contracture of sensitized ilea, (2) inducing aspermatogenesis, (3) provoking the production of humoral antibody. We have found that replicating guinea pig cells carried in culture for more than 2 months are capable of causing anaphylactic contracture of sensitized ilea and can also be used to sensitize guinea pigs.

Eight adult male guinea pigs (600–700 gm.) were injected with guinea pig testicular homogenate in Freund's adjuvant. The preparation of the adjuvant–antigen mixture and the method of injection have already been described[1]. Briefly, a weighed amount of testicular tissue is homogenized in an equal volume of 0.9 per cent sodium chloride solution and the homogenate is then incorporated into an equal volume of the adjuvant so as to form a stiff gel. This preparation is injected intracutaneously in 6–8 sites in the nuchal region of the male guinea pigs. Three weeks after injection, 4–5-cm. segments of the ileum are removed from these animals and suspended in an oxygenated bath of Locke–Ringer's solution. When challenged with specific antigen (homologous sperm or purified extracts thereof), these segments contract anaphylactically. This test has been found to be a consistently reliable indicator of the presence of antibody to spermatogenic antigen(s). In the present work, therefore, the presence of spermatogenic antigen(s) in the cell cultures of the guinea pig testicular tissue was determined by using the cell



Figs. 1 and 2. (1) An ileal segment of a non-sensitized guinea pig is not caused to contract when challenged with three different cell cultures of (at 1, 2 and 3, respectively) guinea pig testis. (2) Ileal segments from a guinea pig sensitized with homologous testis homogenate contract anaphylactically when challenged with the same three cell cultures noted in Fig. 1

cultures to challenge ileal segments of guinea pigs sensitized with whole testicular homogenate and by using homologous sperm to challenge ileal segments of guinea pigs sensitized with the cell cultures of guinea pig spermatogenic tissue.

Experimental Series I. Ileal segments from non-sensitized guinea pigs were suspended *in vitro* and challenged with three different cultures of guinea pig testicular cells that were collected 2 months following initiation of the cultures. The cells were separated from the culture media by centrifugation and were suspended in Locke–Ringer's solution (1 ml. : 0·5 ml. of packed cells) so that approximately $3 \cdot 3 \times 10^7$ cells were present per ml. of fluid. The cells were then homogenized in a glass hand homogenizer and 1·0 ml. of the homogenate was added to the bath fluid containing the ileum. Responses of the segments of ileum were recorded with the aid of a kymograph.

Experimental Series II. Segments of ileum from guinea pigs sensitized with homologous testicular homogenate were challenged with the following: (1) homologous spermatozoa; (2) the three preparations of cell cultures used in experimental series I (above); (3) a concentrate of the tissue culture media separated from the cultured cells by centrifugation.

Experimental Series III. Eight male guinea pigs were injected with the three cell cultures homogenized in saline and incorporated into Freund's adjuvant. Three weeks later, segments of the ilea of these animals were challenged with homologous sperm.

The homogenized cultured spermatogenic cells did not cause contracture of ileal segments from non-sensitized guinea pigs. This is illustrated in Fig. 1, a kymograph tracing demonstrating that not one of the three preparations (injected into the bath at 1, 2, and 3, respectively) affected the tonus of the segment. Moreover, the media in which the cells were grown did not cause contraction of ileal segments from non-sensitized or sensitized animals.

In sharp contrast are the responses of ileal segments of sensitized animals when challenged with the same three cell culture preparations. In Fig. 2 it is apparent that each of the cell homogenates caused a strong contracture (at 1, 2, and 3, respectively) of an ileal segment from a guinea pig injected with whole testicular homogenate. Similar responses were obtained when ileal segments from animals sensitized with the cell culture homogenates were challenged with homologous sperm.

The results of these experiments indicate that guinea pig testicular cells grown in culture for more than 2 months contain antigens that can sensitize ilea and that elicit contracture of the ileum of sensitized guinea pigs. The antigens were not carried over from the original cells because the final cultures represent seven serial transfers and it is calculated that few, if any, of the original cells could have been present in the final populations employed. Thus, antigen synthesis *in vitro* must have occurred. These observations now make it possible to examine more definitely the problem of elucidating the mechanism(s) of cell destruction occurring in immunologically induced aspermatogenesis.

This work was supported by the Population Council, Inc.

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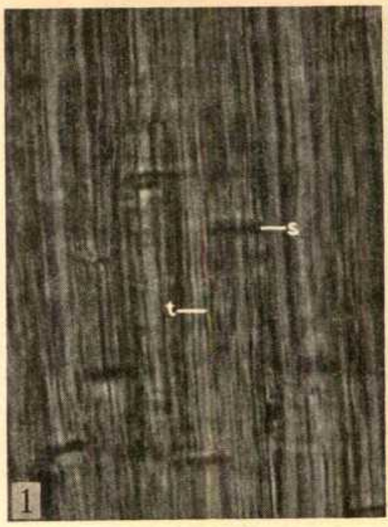
Cell Reduction in the Embryonic Development of the Cestode *Diorchis ransomi* Schultze, 1940

DURING work on the embryonic development of the cestode *Diorchis ransomi* Schultz, 1940, considerable attention was paid to the final phase called the pre-oncosphere. This phase is relatively little known so far. The present communication deals with only one feature of the pre-oncosphere, namely, the reduction in number of embryonic cells. A more detailed account of the work will be published in *Acta Parasitologica Polonica*.

The embryogenesis was investigated in 5μ-thick serial sections of gravid segments. The staining methods used were : Prenant's trichrome, Feulgen technique, Brachet technique and toluidine blue with a control hydrolysed by hydrochloric acid. As a result of the reconstruction of particular embryos from serial sections I was able to establish with reasonable accuracy the number of cells in subsequent developmental phases.

In the early embryos three types of cells were recognized : macromeres, mesomeres and micromeres. They were identified by their dimensions and nuclear structures. The mesomeres formed the outer membrane of the embryo. The other cells multiplied very intensively, the maximum number being 75–80. The majority of them were micromeres. The cytoplasm of early embryo cells was a syncytial one and ribonucleic acid (RNA) was shown to be present after treatment with the technique of Brachet.

The pre-oncosphere phase began after the formation of the outer membrane. Besides the other morphological and cytochemical changes, considerable cell reduction took place at this phase. The 75–80 cells previously observed in the embryo decreased, finally reaching about 26 in the fully formed oncosphere. At the same time the following cytochemical observations were made. After staining with the Feulgen reagent, two types of micromere nuclei were recognized. The first were homogeneous nuclei, which were very numerous, but irregular fragments of decomposed nuclei were also observed. The latter were nuclei with a distinct chromatin network and were relatively small in number. Simultaneously, the Brachet technique showed a considerable decrease in RNA in the syncytial micromere cytoplasm, which remained colourless with all the staining methods used. The mesomere nuclei did not show any particular change in the pre-oncosphere phase. The cytoplasm became compact and formed a clearly outlined layer around each nucleus. The Brachet technique and toluidine blue revealed a large increase of RNA content in the cytoplasm. This mesomere structure showed that these are the plastin cells described by Vogel[1].



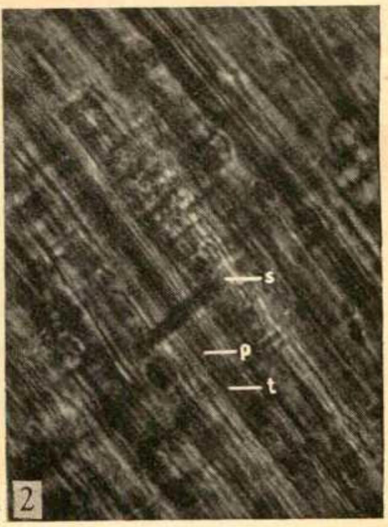


Fig. 1. A fresh hand section of *Primula* petiole phloem showing five sieve plates (*s*) crossed by parallel transcellular strands (*t*). The photomicrograph is an enlargement of a single frame from a 16-mm. ciné film. (× *c*. 1,730)

Fig. 2. A preparation similar to Fig. 1. Transcellular strands (*t*), in which particles (*p*) appear as bright spots and cross a single large sieve plate (*s*). (× *c*. 1,730)

In the fully formed oncosphere two types of cells were visible. There were 9–13 small nuclei with syncytial colourless cytoplasm in the hook region. About 12–16 plastin cells filled the oncosphere almost entirely. Their strongly basophilic cytoplasm was rich in RNA. Observations of the early metacestode of *D. ransomi* in its intermediate host *Cypridopsis vidua* (Mull.) showed the rapid growth and multiplication of plastin cells. The small nuclei of the hook region disappeared completely after the oncosphere reached the hæmocœl of the host.

In recent papers the only observation known to me on cell reduction in cestode embryos was made by Joyeux and Baer[2] in their work on *Catenotaenia pusilla* (Goeze). They did not, however, deal with the problem in detail.

Ogren[3], in his investigations on *Mesocestoides corti*, Hoeppli, observed the decrease of small cells in the final phase of embryonic development. In his opinion this was a result of the transformation of the small cells into compact plastin cells. The results obtained by me on *D. ransomi* do not seem to confirm Ogren's interpretation. The reduction of the total number of embryonic cells proved that some of the small cells had disappeared. The homogeneous nuclei seen in many micromeres supported this observation. According to recent research on the biological role of RNA (ref. 4) the disappearance of RNA from micromere cytoplasm indicates the lack of developmental potential.

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Transcellular Strands and Particle Movement in Mature Sieve Tubes

I HAVE suggested elsewhere[1] that transcellular protoplasmic streaming is the major cause of translocation in vascular plants. The hypothesis depends on the existence of transcellular tubular strands which cross the sieve tube lumina and pass through sieve pores thus providing a structural basis for cytoplasmic communication throughout the vascular system. Within the tubular strands, microscopic structures, comparable with the thread-like constituents of hair cell cytoplasm[1] (and perhaps related to the endoplasmic reticulum seen in electron micrographs of plant cytoplasm[2,3]) are thought to be continuous in the longitudinal direction and to be surrounded by a fluid matrix.

The purpose of this communication is to report the observation of transcellular particle movements along these strands in living mature sieve tubes. Fresh hand sections of *Primula obconica* petiole phloem were mounted in 0·3 *M* sucrose solution and observed in transmitted light with a × 60 apochromatic objective. The observations have been recorded on 200 ft. of 16-mm. ciné film. Figs. 1 and 2 are individual frames taken from the ciné film and show the tissue in which moving particles were seen. Because the particles move within tubular strands they are not sharply defined in such single photomicrographs but they are easily seen in movement in the ciné film and the following statements are based on observations made on the film. Throughout the film, strands (*t*, Figs. 1 and 2) 1·5–2·0μ in diameter are seen in parallel arrangement in sieve tube elements and continuous across sieve plates. Particles (*p*, Fig. 2) can be observed to pass along the lumina of sieve tube elements, and through sieve plates, within transcellular strands and move in opposite directions in single sieve tubes, sometimes within adjacent strands. The particles occur approximately 3μ apart in close linear order in the strands, and their size is comparable with plant mitochondria (diam. 0·5μ). The rate of movement lies within the range 3–5 cm./hr., which is much slower than the translocation-rates recorded in experiments with whole plants, but it is not necessary to assume that particles are the only mobile contents of transcellular strands, and particles may move faster in the stems of plants with long internodes. Moreover, the speed and numbers of moving particles in hand-sections could be affected by slight damage caused in sectioning. The surprising fact is that transcellular particle movement has been observed through as many as ten sieve tube elements (total length 1·0 mm.) in the linear file of a single sieve tube, in spite of the damage done to transcellular strands at the cut ends of the section.

The particles presumably travel long distances through the phloem and could therefore be involved in transporting mobile materials from the leaf to the stem, the apex or the root. Although particles move past one another in adjacent strands they have not been observed changing direction and are probably part of a two-way transport system. Two-way movement without change of direction implies either that particles originate where transcellular movement begins and are assimilated at their destination or that particles are part of a circulation system in the plant. If the first explanation is correct, particles originating in the stem or root are assimilated in the mature leaf. It is difficult to accept this possibility because leaves export the products of photosynthesis in quantity and import only traces of mobile materials[4-6]. On the other hand, if a circulation system exists in the plant, particles could move out of the leaf loaded with mobile materials and then move back to the leaf after discharging their contents. Circulation streaming seems a more plausible explanation of two-way particle movement in the phloem, and it is interesting that particle circulation is known to occur as part of protoplasm streaming in hair cells.

I wish to thank Prof. Preston for his support and advice during this investigation.

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MICROBIOLOGY

Effect of Ultra-Violet Radiation and Desiccation on Different Age-Cultures of *Escherichia coli K12*

DURING the course of an investigation on the effect of monochromatic ultra-violet radiation on *E. coli* some unusual observations were made concerning the sensitivity of different age-cultures to ultra-violet light and desiccation. A survey of literature[1-3] revealed conflicting reports on the subject. This work was undertaken to resolve the problem.

For investigating the effect of ultra-violet light on *E. coli* 0·04 ml. (one drop) of suitably diluted culture was spread on a clean sterile glass slide and exposed to ultra-violet radiation for 30 sec. at a distance of 45 cm from the source. A Hanovia 215-W quartz mercury lamp housed in an asbestos-lined metal box was used as a source. The exposed slide was then pressed on a nutrient agar Petri dish and gently removed after 15 min. The plates were incubated at 37° C. for 24 hr. This entire operation was carried out in very dim light. After the incubation period the developed colonies were counted. The controls were similarly and simultaneously prepared.

For desiccation work, the glass slides spread with a suitably diluted culture were placed in a metal box and dried at 37° C. in an incubator with the lid slightly open for 3 hr. After this period, the slides were treated as before. The control slides were similarly prepared, but immediately pressed on agar plates without allowing them to dry. These experiments were repeated several times with both glass and stainless steel slides which gave identical results. This eliminates the possibility of attributing the lethal effects to free alkali on the glass surface.

Table 1. EFFECT OF ULTRA-VIOLET RADIATION AND DESICCATION ON *E. coli*

Age of the *E. coli* culture (hr.)	Percentage viable colonies after drying	Percentage viable colonies after exposure to ultra-violet radiation
0·75	20·8	7·2
1·5	18·5	6·0
3	6·0	2·3
4·5	2·0	1·2
6	4·0	1·5
8	10·0	2·6
12	18·5	3·7
16	20·2	4·0
18	20·1	4·1
24	20·3	4·1

It will be seen from Table 1 that the 0·75-hr.-old culture (in the lag phase of growth) is most resistant to both ultra-violet radiation and desiccation, whereas the 4·5-hr.-old culture (in the logarithmic phase of growth) is least resistant to these agents. The 24-hr.-old culture (in the maximum stationary phase) is fairly resistant to both the ultra-violet light and desiccation.

The resistance of *E. coli* to ultra-violet light and desiccation seems to be a maximum in the lag phase and starts diminishing during early logarithmic phase and is regained as the culture reaches the maximum stationary phase. The resistance to desiccation seems to be completely regained after 24 hr., but the resistance to ultra-violet radiation is not completely regained even after 24 hr.

It has been observed (ref. 4) that during the later part of the lag phase the nucleic acid content, protein content and the cell size are at their maximum levels and start diminishing in logarithmic phase. It is also well known that the bacterial cells are not dividing in the lag phase and their cell walls and membranes are unbroken. It therefore appears that the intact cell membranes, the large amount of nucleic acids, etc., and the large cell size may be responsible for the resistance of the cells in this phase to ultra-violet radiation and desiccation.

It is interesting to note that work conducted on the effect of X-rays, heating, cooling, freezing and freeze-drying, seems to suggest that bacteria are less resistant to these agents in the logarithmic phase than in the stationary phase. It would be extremely informative to investigate the effects of these agents on the bacteria in the lag phase.

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Spheroplasts of Marine Bacteria induced by the Action of Penicillin

SEVERAL species of marine bacteria which are characterized by osmotic dependence have been induced to form spheroplasts by the action of penicillin added to cultures growing optimally in a nutrient broth prepared with sea water. Two factors indicated the likelihood of obtaining this result. First, recent papers[1-3] have contained results indicating that the internal osmotic pressure of marine bacteria and halophiles was equal to, or less than, the external environment; consequently, spheroplasts of these bacteria should be stable in the growth medium Second, the media, in particular those containing sea water, are characterized not only by a high osmotic pressure but also by a high content of magnesium ions, a characteristic of solutions used to stabilize penicillin-induced spheroplasts of Gram-negative bacteria[4] The techniques for inducing spheroplasts of marine bacteria and a comparison of the osmotic fragilities of cells and spheroplasts of one species are described briefly in this communication.

The five types of bacteria used have been described recently[5], all were Gram-negative rods characterized by osmotic fragility and salt requirements for growth. The medium consisted of a tryptic digest of casein, trypticase (Baltimore Biological Laboratory, Inc), 1 per cent, dissolved in artificial sea water of the following composition sodium chloride, 0 4 *M*, magnesium chloride, 0 05 *M*; sodium sulphate, 0 01 *M*; and potassium chloride, 0·01 *M*. The experimental cultures were grown in 20-ml amounts of medium in 250-ml Erlenmeyer flasks each fitted with a side-arm tube to allow periodic measurement of turbidity. Subsequent to inoculation, as increasing turbidity indicated growth, penicillin *G* was added to the flasks. Three amounts of penicillin, to give final concentrations of 1·0, 100 and 1,000 units per ml , were tested Wet mounts of cultures were prepared hourly and examined by phase microscopy

The osmotic fragilities of cells and spheroplasts of one species (*MB* 22) were compared by determining the turbidity[6] and indole formation[7] of preparations washed in sea water and suspended in graded concentrations of sodium chloride The turbidity was measured photometrically with a Spectronic 20 The indole formed from L-tryptophan was determined by the method of Wood, Gunsalus and Umbreit[8]

Morphological alteration was observed in all species after 1 hr cultivation in the medium with 100 units of penicillin per ml , after 2 hr all cells of four of the five species had been converted to spheroplasts. The sequence of spheroplast formation was typical[4], from the initial 'rabbit ear' structure to the formation of spherical bodies having the crescent-shaped refractile area One organism, *MB* 2, did not form spheroplasts, but showed greatly enlarged and swollen 'rabbit ear' structures After 5 hr in 1 0 unit of penicillin per ml , *MB* 14 had formed spheroplasts, *MB* 22 had formed serpentine cells about ten times normal length, and the other species showed no apparent change One organism (*MB* 21), a peritrichous Gram-negative rod, was converted to spheres after 2 hr incubation with 100 units per ml. of penicillin, but examination after 5 hr. showed the culture to have returned to its rod form, possibly by virtue of an active penicillinase Motility persisted in the cultures treated with penicillin, in some cases even after the formation of spheres, but was lost in all species, with the exception of *MB* 21, after several hours.

Table 1. COMPARISON OF OSMOTIC FRAGILITIES OF CELLS AND SPHEROPLASTS OF *MB* 22

Molarity of sodium chloride in suspending fluid	Residual turbidity (per cent)*		Residual indole formation (per cent)†	
	Cells	Spheroplasts	Cells	Spheroplasts
0 0	73	17	0	0
0 1	86	83	60	40
0 2	95	95	93	47
0 3	95	95	100	93
Artificial sea water	100	100	100	100

* Percentage of optical density as compared with optical density in artificial sea water Optical density of cells in artificial sea water, 0 56, optical density of spheroplasts in artificial sea water, 0 59

† Percentage of indole formed as compared to indole produced in artificial sea water (10 6 μgm /10 min)

Comparison of the osmotic fragilities of cells and spheroplasts of *MB* 22 (Table 1) indicates that tryptophanase activity and turbidity are both dependent on the osmotic pressure of the fluid in which the cells are suspended. However, it cannot be said with certainty that the spheroplasts are more susceptible to lysis than the cells The low residual turbidity of the spheroplasts in distilled water may indicate the lack of cell wall material Microscopic observation of the distilled water lysates of spheroplasts showed only disorganized debris while cell wall ghosts were seen in similar lysates of the cells

The formation of stable spheroplasts from marine bacteria in a culture medium having an osmotic pressure equivalent to that of sea water suggests that the marine bacterium may not require the rigid cell wall to maintain its cellular integrity in the natural environment Possibly the rigid cell wall indicates the evolution of these forms from organisms adapted to more hypotonic environments, since it is in the latter circumstances that the rigid cell wall of bacteria has a selective advantage making the organism osmotically independent of its environment

This work was supported, in part, by the National Science Foundation grant 7075

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Flagellar Changes in *Escherichia coli* induced by Temperature of the Environment

THE effect of change of environmental temperature on the motility of a strain of *Escherichia coli*, type I (N C T C 8196) has already been described[1] This strain, which appeared to be non-motile when examined after 18-hr growth at 37° C in meat extract broth or in the same medium solidified with agar, was found to be motile in the early stages of exponential growth (5 hr at 37° C.) and after growth for 18 hr. or longer at 20° C Storage of non-motile cells grown at 37° C at a lower temperature (20° C) for 1–2 days led to the recovery of motility by most of the cells in the culture. Cells grown at 20° C. retained motility for several weeks, but lost it rapidly when transferred to the incubator at 37° C

The effect of temperature of growth and storage on a further fifteen strains of *E coli* has since been investigated, and seven of these showed rapid loss of motility in cells grown at 37° C and long-lasting motility in cells grown at 20° C. Of the remaining eight strains some were non-motile at all growth temperatures and others developed and retained motility at growth temperatures of 15–37° C. In these seven strains, showing transient motility when grown at 37° C and long-lasting motility at lower growth temperatures, there was some variation in the recovery of motility when non-motile, flagellate cells

Table 1 EFFECT OF GROWTH TEMPERATURE ON FLAGELLA, MOTILITY AND CELL YIELD IN CULTURES OF THREE STRAINS OF *E coli* TYPE 1 GROWN FOR 24 HR IN MEAT EXTRACT BROTH

Growth temperature (° C)	Strain *W I G*			Strain *J*			Strain 1093		
	Cell No (percentage of light absorbed)	Flagella (percentage of cells flagellate)	Motility (percentage of cells motile)	Cell No (percentage of light absorbed)	Flagella (percentage of cells flagellate)	Motility (percentage of cells motile)	Cell No (percentage of light absorbed)	Flagella (percentage of cells flagellate)	Motility (percentage of cells motile)
15	13	65	1–5	32	0	0	30	50	40–50
20	28	85	80–90	38	65	60–70	41	100	20–30
23	48	90	<1	60	65	60–70	72	75	5–10
25	67	95	0	68	65	60–70	77	75	1–5
30	79*	85	0	79*	55	10–20	80*	75	<1
36	77	60	0	78	25	0	74	40	0
40	41	25	0	68	<1	0	65	30	0
44	29	0	0	49	0	0	53	0	0

* Optimum temperature for cell growth

were stored at 20° C. The recovery of motility varied from a minimum of 1–5 per cent of the cell population to a maximum of about 80 per cent, according to the strain. This recovery of motility at lower temperatures could not be wholly accounted for by the growth of new cells, since in some strains the number of cells with recovered motility greatly exceeded the increase of population. It should be emphasized that the loss of motility at 37° C was not, in most cases, due to a loss of flagella since in six of these seven strains cells grown or stored at 37° C retained their flagella. The flagella had lost the power to propel the cell—they had become paralysed.

The influence of temperature on motility in a number of bacterial species has been described[2-5], but in the examples reported loss of motility would seem to be due to loss of flagella in cells grown at a high temperature or loss of function in already-formed flagella in cells exposed to a high temperature. Thus the rapid loss of function by flagella during growth at the optimal temperature (30–37° C) and the prolonged maintenance of function by flagella formed at lower temperatures seemed to merit further investigation.

As mentioned earlier, a total of sixteen strains of *E coli* type I were examined. They consisted of four laboratory stock cultures and twelve strains of *E coli* type I consecutively isolated in the bacteriological laboratory of the Western Infirmary, Glasgow. They possessed the following characters: lactose fermented with acid and gas, indole formed, methyl red test positive, Voges–Proskauer test negative and no growth in Koser's citrate. Motility was determined in wet films from meat extract broth cultures or from the condensate water of meat extract agar slopes. Rough estimates were made of the proportion of motile cells, the estimates being checked by a second observer. Flagella were demonstrated and counted in films stained by Leifson's stain[6] or Kirkpatrick's silver stain[7].

Out of the seven strains showing motility after 5-hr. growth at 30–37° C and loss of motility after 18-hr growth at this temperature, the first three examined were subjected to more detailed investigation. Each strain was grown in meat extract broth at temperatures ranging from 15 to 44° C. At the end of 24-hr incubation the proportion of cells showing flagella and the proportion of motile cells were determined. The amount of growth was measured turbidimetrically in the Spekker absorptiometer (previous measurements showed that the percentage of light absorbed was closely related to cell numbers). The results are shown in Table 1. The finding that the optimum temperature for demonstration of motility in 24-hr cultures may be considerably lower than the optimum temperature for cell yield confirms what most bacteriologists find. The three strains show an essentially similar pattern although they differ in regard to the optimum temperature for growth, flagella, and motility. Thus it is possible to distinguish three types of cell in each strain (*a*) non-motile, non-flagellate cells grown at elevated temperatures (above 40° C. for strain *W I G*), (*b*) non-motile, flagellate cells grown over a middle range of temperatures (23–40° C for strain *W I G*), (*c*) motile, flagellate cells grown at low temperatures (below 23° C for strain *W I G*). Another way of putting it would be to say that in the upper range of growth temperatures flagella are not produced, in the middle range they are formed, but lose activity rapidly—they become paralysed; and in the lower range flagella are formed which retain their function for 24 hr or more (strain *W I G* is still motile after storage for 3 weeks at 20° C).

An inevitable question is whether there are any differences in structure or composition between active and paralysed flagella, and the simplest approach, perhaps, is to look for antigenic differences. This was done and differences were found. Rabbits were immunized with formolized cells of the three types of strain *W I G*—non-flagellate cells grown at 43° C., flagellate, non-motile cells grown at 36° C, and flagellate, motile cells grown at 20° C. The resulting antisera showed, as one might expect, considerable cross-reactions. But, after a number of absorptions with cells heterologous in regard to growth temperature, sera specific for the homologous cells were obtained. Table 2 shows the results of experiments in which absorbed antiserum prepared against cells grown at 36° C (flagellate, non-motile) and absorbed antiserum prepared against cells grown at 20° C (flagellate and motile) were titrated against formolized '*H*' suspensions of the three types of cell listed here. That these results are due to the presence of antibodies to specific flagellar antigens is shown by our finding that the absorbed antisera did not agglutinate '*O*' suspensions or cells which had been stripped of their flagella by high-speed agitation in the M S E blender[8]. Clearly, therefore, there are antigenic differences between paralysed flagella produced by growth at elevated temperatures and active flagella formed in growth at lower temperatures.

Table 2 AGGLUTINATION OF CELLS OF STRAIN *W I G* GROWN AT 43° C, 36° C and 20° C AGAINST ANTISERA PREPARED AGAINST CELLS GROWN AT 36° C AND 20° C AND LATER ABSORBED WITH NON-HOMOLOGOUS CELLS

Agglutination tubes incubated for 4 hr. at 56° C. and 20 hr at 20° C

Cell suspension of cells grown at temperature shown (° C)	Agglutination titre as reciprocal of dilution of antiserum	
	Antiserum to 36° C. cells (absorbed by cells grown at 20° C and 43° C)	Antiserum to 20° C cells (absorbed by cells grown at 36° C and 43° C)
43	Nil	Nil
36	1,280	Nil
20	Nil	640

Summarizing, one might say that in the sixteen strains of *E coli* type I examined, seven showed the possible existence of two forms of flagella—a functional or active form produced in growth at low temperatures and a non-functional or paralysed form produced by growth at higher temperatures Conversion from one form to the other could be obtained in varying degree by switching the incubation temperature For the one strain examined serologically, these two forms differed antigenically

No simple explanation of the phenomenon occurs to us One can scarcely account for it by postulating exhaustion of nutrients or accumulation of toxic metabolites at the higher temperature, since cells which had lost motility could regain it on transfer to a lower temperature In some strains the number of cells recovering motility exceeds the number of new cells formed. Loss of motility through oxygen deficiency would seem to be ruled out by the occurrence of the phenomenon in actively aerated cultures Nor is it enough to say that high temperatures favour a flagellar structure inimical to motility since young cells are motile at elevated temperatures. One might, of course, postulate the accumulation of toxic products at higher temperatures which are subjected to further change in reactions favoured by lower temperatures, but this ignores the finding of antigenic differences A satisfactory explanation still eludes us

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Free-Radical Formation during Storage of Freeze-dried *Serratia marcescens*

BACTERIA which have survived freeze-drying retain the capacity to regenerate colonies after prolonged storage under vacuum, in contrast to their rapid loss of this capacity when stored in air The question of whether bacteria stored under such conditions are dormant or whether some metabolic activity continues to maintain the bacilli in a living state is of fundamental importance to a further understanding of the physiology of life in low-moisture environments Heckly[1] has reviewed many of the problems associated with freeze-drying Results of preliminary work on freeze-dried cells had indicated a slow gaseous uptake when the material was stored in dry air and a greater uptake if stored under moistened air Because the results were not reproducible, other techniques were examined, specifically that of electron paramagnetic resonance, in the hope of finding an indirect measurement of respiration The fundamental theory and practice of electron paramagnetic resonance has been reviewed by Ingram[2], while a review of biological applications of the phenomenon has been given by Sands[3]

Serratia marcescens was grown in Bunting's[4] medium (modified, except in one instance, by withholding the iron citrate) in shake flasks at 34° C for 24 hr Cells were collected by centrifuging and resuspended in 0 066 *M*, *p*H 7 0, potassium phosphate buffer by mixing with a pipette for about 5 min The suspension was recentrifuged and again the cells were suspended in buffer fortified, except in one instance, by the addition of lactose[5] Suspensions were divided into two portions, one portion was either placed in an autoclave or treated with mercuric chloride to kill the bacilli, the other portion was untreated

Preparations were distributed, 16 ml per 60 ml Army-style vaccine bottle, shell frozen, and the bottles were placed under vacuum (ambient temperature, 24 hr , 2–5μ mercury pressure) All preparations in a given experiment were frozen and dried simultaneously The bottles were then removed to a dry-box and the dried material was transferred to either weighed glass vials or into quartz tubes suitable for electron paramagnetic resonance work Vials and tubes were then flame-sealed either under atmospheric pressure or at a pressure of less than 10μ mercury In one instance the air was replaced with water-pumped nitrogen Reconstitution of the dried material was accomplished by adding an appropriate volume of sterile distilled water to the bottles Viable cell numbers were determined by serial decimal dilution followed by surface colony counts on Bunting agar plates Colonies were counted after 24 hr incubation of the plates A Varian *V*–4500 electron paramagnetic resonance spectrometer with 100-kc /s field modulation and a 6-in magnet were used to obtain the spectra of the dried samples

Viable dried bacilli were found to exhibit a weak, single-line resonance spectrum having a *g*-value near 2, and of line-width approximately 11 gauss (peak-to-peak on derivative curves) A similar phenomenon has been reported for other dried biological samples[6] Samples sealed in air, however, showed a marked increase in the intensity of their resonance spectra with time A sample stored 90 days under vacuum produced no augmented signal, but the signal immediately showed a change in intensity when the vial was opened to the atmosphere

In Fig 1 the reciprocal of the relative intensity, as the fraction of the original signal intensity, is plotted against time and the loss of colony-forming units is plotted on the same scale The results show that the most rapid increase of the signal occurred during the first week of storage, after which the rate decreased The concentration of radicals in these samples, determined by comparison with a standard sample of known concentration, was approximately 10^{12}/mgm of sample, whereas the initial number of viable bacilli varied between 4 and 8 × 10^{9}/mgm , there were about 200 radicals formed per living bacillus An outline of experimental details and results is shown in Table 1

The vial associated with experiment 1 *b* (Table 1) was re-evacuated overnight after a fairly high number of radicals had formed, but the signal did not decrease The vial was then opened and distilled water was added to the contents Subsequent analysis failed to show the presence of a measurable free-radical content

These results may be interpreted in three ways (*a*) An easily oxidized molecule, perhaps a ring structure, may have reacted, non-enzymatically, with oxygen to form a free-radical which was stable under the low moisture conditions Scholes *et al* [7] have discussed reactions of this nature Such substances could cause the death of a cell at the moment of

Table 1. SUMMARY OF THE PREPARATION AND STORAGE OF FREEZE-DRIED *Serratia marcescens* AND THE ELECTRON PARAMAGNETIC RESONANCE SIGNALS NOTED THEREAFTER

Exp. No.	Variation of storage medium	Pre-treatment	Storage atmosphere	Percentage of immediate recovery	Electron paramagnetic resonance spectra
1*a*	Iron + lactose	autoclaved	air	zero	constant medium strength
b	Iron + lactose	none	vacuum	100	weak*
c	Iron + lactose	none	air	100	small, changing to large
2*a*	No iron, no lactose	$HgCl_2$	air	zero	weak
b	No iron, no lactose	none	vacuum	2·0	weak
c	No iron, no lactose	none	air	2·0	weak, but slightly increased after 40 days
3*a*	No iron, lactose	none	vacuum	100	weak
b	No iron, lactose	none	air	100	small, changing to large
4*a*	No iron, lactose	none	nitrogen	80	faint, no appreciable immediate change
b	No iron, lactose	none	air	80	small, changing to large

* The vial was opened to the atmosphere after 6 weeks and re-sealed. A signal then appeared.

reconstitution. (*b*) An enzyme system may have been injured or inhibited, resulting in the accumulation of a free-radical intermediate within the cell. Presumably, the injury might have caused the death of the cell, though not necessarily, because the accumulated radical could act as suggested in (*a*). In support of this suggestion, Yamazaki *et al.*[8] have shown unequivocally that radical intermediates are formed in typical enzymatic oxidations. (*c*) The relatively low number of radicals per bacillus indicates that a combined form of a transition element present in trace amounts could have been responsible for the signal analogous to (*a*).

A strong similarity appears to exist between the loss of viability and the growth of the free-radical concentration in the sense that bacilli stored under vacuum conditions, which favour high retention of viability, showed no change in radical concentration, whereas those stored under atmospheric conditions, an adverse environment, exhibited a consistent rapid increase of free-radical concentration. Furthermore, a marked change occurred in both the rate of loss of viability and the rate of increase of the radical concentration after about one week's storage (Fig. 1). Thus, a correlation is indicated between the death process and the growth of the free-radical concentration, although present results preclude the assignment of a direct cause-and-effect relationship.

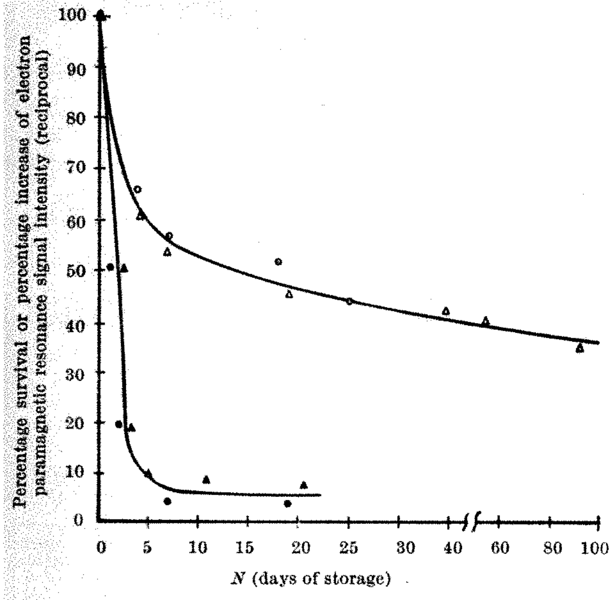

Fig. 1. Comparison of the change in electron paramagnetic resonance signal intensity with the loss of viability of *Serratia marcescens* stored in air. The points ○ and ● are for a single preparation; △ and ▲ are from different preparations.

●▲, percentage survival, $100 \times \frac{\text{No. of viable cells at } N \text{ day.}}{\text{No. of viable cells at 0 day.}}$

○△, percentage electron paramagnetic resonance intensity increase, $100 \times \frac{\text{intensity at 0 day}}{\text{intensity at } N \text{ day}}$

Both oxygen and moisture are involved in the loss of colony-forming units. Monk *et al.*[9] have shown that viability is lost most rapidly when the bacteria contain about 30 per cent moisture. Definitive data concerning this point might be forthcoming if one compared the production of free radicals to the loss of viability under varied moisture conditions, or determined whether a reduction of the free-radical content increased the apparent number of colony-forming units. The evidence suggests that some changes, perhaps metabolic in nature, occur in the dried state.

This work was sponsored by the Office of Naval Research under a contract with the University of California.

After this communication was submitted, our attention was directed to the finding by Lion *et al.*[10] that free radicals are formed during storage of freeze-dried *Escherichia coli*. This, together with our subsequent finding that similar phenomena occur when freeze-dried *Sarcina lutea* or *Micrococcus radiodurans* are tested, indicates that free-radical formation associated with the death of dried micro-organisms is generalized in nature. We take exception, however, to the suggestion that normal metabolic reactions might not be involved, because the evidence points strongly toward the oxygen-transport system as being that portion of the metabolic make-up of living structures most likely unbalanced by oxygen under dry conditions.

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VIROLOGY

Serial Propagation of Types 1, 2, 3 and 4 Dengue Virus in HeLa Cells with Concomitant Cytopathic Effect

ATTEMPTS to obtain cellular destruction with dengue in stable human cell lines have thus far been unsuccessful. Banta[1] was unable to produce a specific cytopathic effect with type 1 dengue virus in HeLa cells. Beasley *et al.*[2] demonstrated replication of type 2 dengue virus on primary infection of *KB* cells, but concomitant cell damage was not observed.

It has been pointed out by Dubos and Schaedler[3], and is indeed an established clinical fact, that virus diseases often run a milder course in malnourished than in well-fed persons. There is evidence also that *in vitro* processes necessary for viral multiplication are influenced by the state of health of the cell cultures. Thus, Morgan *et al.*[4] have shown that in tissue culture, viruses remain dormant so long as the infected cells are maintained in deficient culture media, while Enders[5] has emphasized that any apparent effect of a given substance on viral multiplication may be quite indirect and related only to preservation of cell viability. Cell viability, especially in work carried out with stable cell lines, depends both on nutrients available during the growth period and on substances used in the maintenance medium following infection of cells. The latter conditions have been studied more extensively. Thus, Ginsberg *et al.*[6] introduced tryptose phosphate broth (Difco) as a supplementary beneficial factor for maintenance of HeLa cells.

I, conversely, have been interested in variation of the growth-promoting medium and the effects of this variation on subsequent viral synthesis, in particular on induction of a clear-cut, reproducible cytopathic effect. During the past five years, a standard HeLa (Gey) cell line, obtained in 1954 from the Tuskegee Institute, Alabama, has undergone more than 335 regular transfers in a growth-promoting medium containing among other ingredients 15 per cent tryptose phosphate broth. Monolayers of HeLa cells cultivated over long periods in this medium are less easily broken up by 0·25 per cent trypsin (Difco) than are those of HeLa cells grown conventionally in adult human serum and Eagle's basal medium. It is assumed, although experiments pertaining to this question have not yet been terminated conclusively, that this difference is due primarily to the ability of tryptose phosphate broth to produce a high degree of cohesion between the individual cells. Whereas non-infected HeLa tryptose phosphate broth cells show good cell viability during experimental stress periods and do not become detached easily from the tube wall, infected HeLa tryptose phosphate broth cells round up and become detached readily from glass, thus exhibiting a clear-cut cytopathic effect which is easily distinguishable at an early stage. The HeLa tryptose phosphate broth cell line has been tested experimentally since 1957 and has responded with regular cytopathic effect to the action of approximately 40 arbor viruses[7-9], including St. Louis encephalitis and Japanese *B* encephalitis, both of which gave irregular results in the hands of Scherer and Syverton[10].

In the present work, HeLa tryptose phosphate broth cells, grown in slant-type tubes in 30 per cent human serum, 10 per cent fœtal bovine serum, 45 per cent Hanks's balanced salt solution and 15 per cent tryptose phosphate broth, were washed once with Hanks's balanced salt solution, and were then inoculated with ten-fold dilutions of 10 per cent suckling mouse brain suspensions of each dengue virus: type 1 (ref. 11), Hawaiian strain, seventy-eighth passage; type 2 (ref. 12), New Guinea strain, forty-fifth mouse passage; type 3 (ref. 13), twenty-sixth mouse passage; and type 4 (ref. 13), twelfth mouse passage. Cell cultures were maintained in 97 per cent Eagle's medium and 3 per cent fœtal bovine serum, and were examined at intervals for 21 days. Careful attention was given to the *p*H of inoculated cultures, which was regulated at 7·2±0·2. Fluids were changed daily during the first week following cellular infection and thereafter three times weekly.

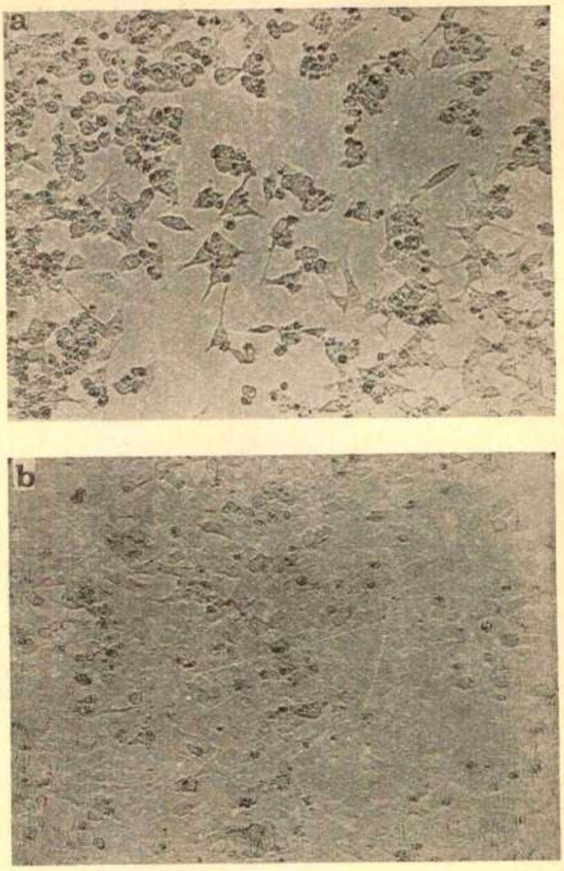

Fig. 1. Unstained HeLa cell cultures in slant-type tubes inoculated with (*a*) normal rabbit serum and type 4 dengue virus, (*b*) with anti-type 4 rabbit serum and type 4 dengue virus. (× *c*. 133)

The results (Table 1) show that the four immunologically different types of dengue virus can be propagated with concomitant cytopathic effect in HeLa tryptose phosphate broth cells. During serial tissue culture passages, carried out at 7-, 10- or 14-day intervals, TCD_{50} titres per 1 ml. ranged from 10^{-3}–10^{-6} for type 1, 10^{-4}–$10^{-6.5}$ for type 2, 10^{-3}–$10^{-6.0}$ for type 3 and $10^{-2.5}$–10^{-5} for type 4. The cytopathic effect was similar for all virus types (Fig. 1*a*) except that it was induced more rapidly by types 2 and 3. Five to seven days following inoculation of 1,000–10,000 TCD_{50}, focal lesions made up of rounded and granulated cells appeared throughout the cell sheet. These micro-plaques became more numerous and confluent during the second week post-inoculation. During the third week 50–70 per cent

Table 1. SERIAL PASSAGE OF TYPES 1, 2, 3 AND 4 DENGUE VIRUS IN HELA TRYPTOSE PHOSPHATE BROTH CELLS

Virus type	No. of *in vitro* passages	TCD_{50} (neg. $\log_{10}$ per 1 ml.)	Cumulative $\log_{10}$ of dilution of original inoculum
1	1	3·0	1
	7	6·0	15
	11	4·0	20
	14	5·5	27
	21	4·8	41
2	1	5·5	3
	6	4·0	15
	9	6·5	22
	14	5·0	44
	27	6·5	75
3	1	5·5	5
	3	3·0	7
	7	5·5	15
	10	4·5	27
	18	6·0	53
4	1	4·5	1
	2	2·5	2
	5	4·5	11
	8	5·0	19
	16	4·0	37

of the cells had become detached from glass and disappeared into the maintenance medium, and the cells still attached to the tube wall were rounded and granulated. Only a few healthy cells, from which regeneration occurred during observation of cell cultures during 1–2 months, were present. When inoculated into 2-day-old mice by the intracerebral route, fluids of cultures exhibiting cytopathic effect proved fatal within 6–10 days. The development of cytopathic effect, similar to that described by Hotta and Evans[14,15] for types 1 and 2 dengue virus propagated in monkey kidney cell cultures, was inhibited by type-specific immune serum (Fig. 1*b*). In the *in vitro* neutralization tests, undiluted, freshly drawn[16] serum samples from immunized and normal rabbits were used.

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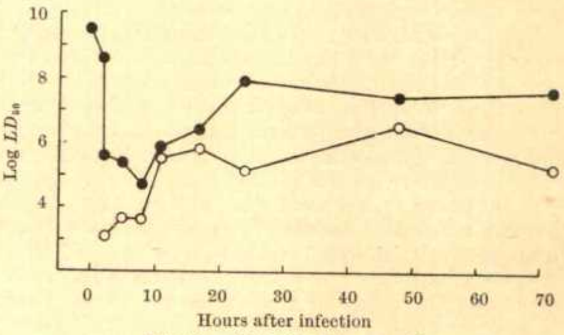
Reproduction of Tick-borne Encephalitis Virus in HeLa Cells

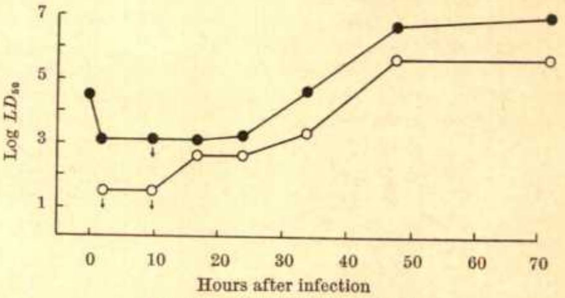
To examine the exact mechanism of reproduction of the tick-borne encephalitis virus in susceptible host cells *in vitro*, the intracellular proliferation and release of the virus into the medium have been compared with the sequence of morphological changes studied by different methods. HeLa cell monolayer cultures were used, infected with a large or a small dose of a selected variant of the Czechoslovak strain *Hypr* of tick-borne encephalitis virus[1], which is cytopathic for HeLa cells.

To ensure that materials were prepared under identical conditions for all the methods used, the contents of one Roux flask were collected for each time period, and infected cells were obtained from a homogeneous cell sheet, covering the bottom and cover-slips stuck to it by chicken plasma. Results obtained by the following methods were compared: (i) virus assessment in the cultural fluid and in cell crushes by repeated freezing and thawing; (ii) intracellular localization of virus by the fluorescent antibody method, using high-titre anti-*Hypr* horse γ-globulin conjugated with fluorescein isothiocyanate[2]; (iii) phase-contrast microscopy of living cells for cytopathic changes.

After an infection with 3,200 LD_{50} per cell, virus proliferation began between the eighth and eleventh hour after infection and reached its maximum in 24 hr. (Fig. 1). Corresponding with these results, specific fluorescence, indicating intracellular virus



Infection with 3,200 LD_{50} per cell



Infection with 1 LD_{50} per 33 cells

Fig. 1. Reproduction of a selected cytopathic variant of tick-borne encephalitis virus (strain *Hypr*) in HeLa cells. ●, Free virus in the whole medium (40 ml.); ○, total intracellular virus

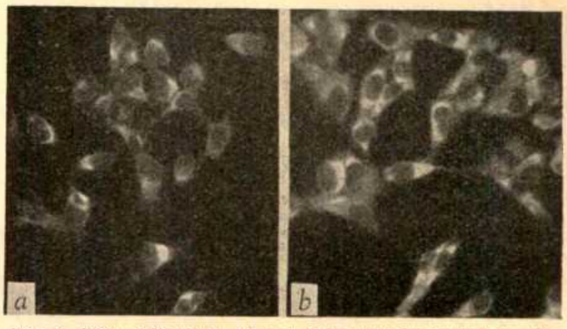


Fig. 2. HeLa cells 12 hr. (*a*) and 24 hr. (*b*) after infection with tick-borne encephalitis virus. Virus localization by fluorescent antibodies

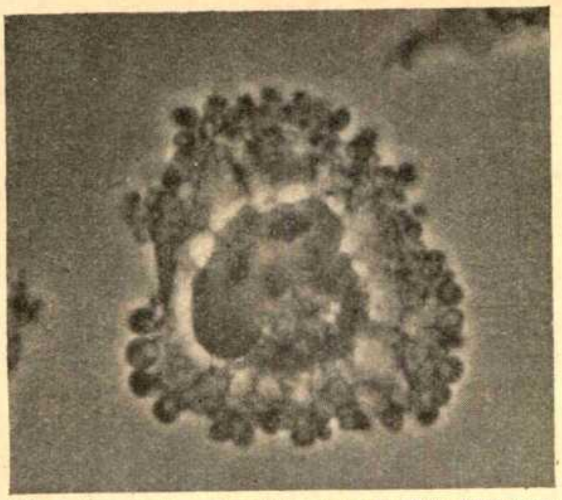


Fig. 3. Vacuolization and protrusions on cell surface of HeLa cell 24 hr. after infection with tick-borne encephalitis virus

material, was present in the majority of cells beginning with the eleventh hour after infection and intensified up to the twenty-fourth hour, starting in the paranuclear area (Fig. 2*a*) and extending later to the rest of the cytoplasm (Fig. 2*b*). At the end of the twenty-four hour period, well-marked cytopathic changes appeared in some cells, with vacuolization and protrusions on the cell surface (Fig. 3).

An infection with a small dose of virus (1 LD_{50} per 33 cells) led to the first slight increase of intracellular virus between the tenth and seventeenth hour, followed by a further increase which started with the twenty-fourth hour and reached a maximum 48 hr. after infection. The first fluorescence was rather delayed in appearance and was a maximum in not more than 50 per cent of the cells of the tissue culture. The initial cytopathic changes appeared approximately at the same time as the fluorescence.

Reproduction of other viruses of the tick-borne encephalitis complex in different cell cultures by these methods is under investigation.

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Accumulation of Asparagine in Maize Plants infected by Maize Rough Dwarf Virus and its Significance in Plant Virology

It has already been shown that the rough dwarf disease of maize, so far known only from Italy, Spain and Israel, is caused by a virus[1]. Since this virus has a pronounced dwarfing effect on maize seedlings, which in most cases proves lethal, it would be expected that certain physiological abnormalities could be determined at a rather early stage of infection. The effect of this virus on the balance of free amino-acids in infected maize plants is being studied in our laboratory. During the course of this work an abnormal accumulation of asparagine was noticed in all plants bearing the virus infection symptoms, whereas in all healthy plants of the same age this amide was at the most only faintly detectable by the method of two-dimensional paper chromatography which we used[2]. Moreover, when *l*-asparagine was added to the sap extracted from diseased plants and chromatographed, its spot coincided with the one suspected to be that amide.

Later, a quick method was worked out for determining the presence of asparagine in sap of plants infected by this virus. The test is based on the distinctive orange-brown colour which asparagine gives with ninhydrin[3]. Fresh maize stalks were ground in a meat mincer; the sap squeezed through cheese cloth and then centrifuged for 10 min. at 4,000 r.p.m. For the ninhydrin test 1 ml. of the supernatant was heated for 3 min. in a boiling water-bath, diluted 10-fold in water and then filtered through Whatman No. 1 filter paper. The reaction mixture includes 0·3 ml. diluted sap solution and 1 ml. of 0·25 per cent of ninhydrin in absolute ethanol. Colour was developed by boiling in a water-bath for 2 min. followed immediately by adding 2 drops of sulphuric acid (1 : 6) and agitating the test-tube. The blue coloration disappeared in a very short time and the orange-brown coloration formed by asparagine turned yellow. The latter colour is sufficiently stable as to allow a qualitative test, although it, too, fades away slowly and therefore cannot serve as a quantitative method. It should, however, be mentioned that proline and hydroxyproline give a different heat-stable coloration on boiling with ninhydrin, but these two amino-acids did not show up on our chromatograms and therefore do not interfere in this case.

It should also be pointed out that the relative amount of sap obtained from diseased plants was noticeably higher than that from non-infected ones. This rules out the possibility of attributing the high asparagine content merely to an alleged lower water content of infected plants.

The ninhydrin test of sap was always strongly positive for all maize seedlings showing rough dwarf symptoms, whereas for healthy plants it was negative, or very seldom, weakly positive. The positive reaction, however, should not be attributed wholly to asparagine, since glycine, tyrosine, phenylalanine, histidine and tryptophan all give an orange-brown coloration with ninhydrin[3]. Part of the yellow colour obtained may perhaps be due to these and other reactions; but it is mainly due to asparagine, as was shown on our chromatograms.

The changes in free amino-acids and amides of the host plant resulting from virus infection were recently reviewed by Porter[4], who mentions at least three instances where virus infections in plants were followed by a net increase in asparagine, as compared with healthy plants. Further evidence is now available concerning at least eight more virus diseases, in which specifically asparagine is reported to accumulate in the host plant following infection. These are tobacco leaf curl virus in *Nicotiana* leaves[5], papaw mosaic and papaw leaf curl viruses in papaw leaves[6], *Ricinus* mosaic virus in castor bean leaves[7], *Luffa* yellow mosaic virus in *Luffa* leaves[7], potato spindle tuber virus[8] and potato leaf roll virus[9] in potato tubers, and finally the present record on maize rough dwarf virus. The only contradicting evidence is by Commoner and Nehari[10], who observed a deficiency in asparagine content in disks of tobacco leaves infected with tobacco mosaic virus. Since these measurements were made on leaf disks, even though floated in nutrient solution, their results cannot be safely applied to the situation in the whole plant. In fact, when the same experiments were repeated on intact plants[4], a net increase in asparagine content was evident as early as during the first 120 hr following infection

Asparagine, as well as glutamine, are known to accumulate in tissues of plants growing under unfavourable conditions, whereby proteolysis and subsequent deamination bring about an increased release of ammonia The presence of these compounds in higher concentrations in virus-infected plants should not be regarded as merely resulting from some secondary effects on the protein metabolism of the diseased plant It has already been indicated[11] that these amides are implicated—whether directly or indirectly—in the biosynthesis of purine, pyrimidine, nucleosides and nucleotides, thus bearing great significance in relation to nucleic acid metabolism, leading to the biosynthesis of the virus material itself[12] This may also offer some explanation for the specificity of the occurrence of asparagine in tissues of plants infected by viruses and for its conspicuous rareness in cases of plant diseases caused by other pathogens

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GENETICS

Radiosensitive Stages in the Spermatogenesis of *Drosophila melanogaster*

THE existence of differential radiosensitivity during certain stages of spermatogenesis in *Drosophila melanogaster* was first proposed by Luning[1] Further evidence provided by Auerbach's[2] three-day brood technique suggested that the highest mutation frequency observed in the 3–6-day period following treatment indicated peak sensitivity in immediately post-meiotic stages Additional support for this view that spermatids were particularly susceptible to the mutagenic action of X-rays was derived principally from the results of irradiating immature sperm in the pupal testis[3,4] More definitive daily brood analysis[5], however, established a marked dominant lethal frequency ranging over the sixth, seventh and eighth days following the irradiation of newly emerged males, but from these results Bateman was able to draw no firm conclusion other than that this probably represented sensitive meiotic and post-meiotic stages Using the same technique, Ives[6] ascribed the first appearance of induced cross-overs on the seventh day after irradiation to the utilization in this brood of sperm treated in early meiotic and pre-meiotic stages In a similar experiment, he obtained a maximum frequency of both autosomal recessive and sex-linked recessive lethal mutations on this same day Savhagen[7] considered sex chromosome loss and non-disjunction also in parallel investigations Here again, the highest frequencies of $X0$ males were observed on those days which, by comparison with the non-disjunction experiment, probably corresponded to stages treated before anaphase I of meiosis.

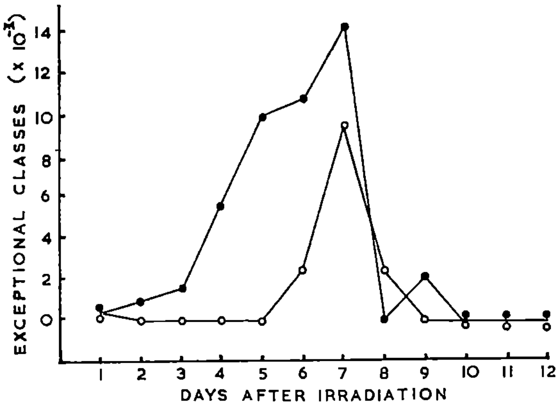

Fig 1 Brood patterns for the 12 days following irradiation ○, XXY females produced by non-disjunction in immature sperm, ●, $X0$ males produced by loss of X or Y chromosome

This communication is a preliminary account of experiments designed to detect simultaneously complete loss of X or Y chromosomes, deletion in the Y chromosome and non-disjunction between X and Y chromosomes This is achieved by means of a new, doubly marked Y chromosome stock kindly provided by Dr. D L Lindsley of Oak Ridge This Y chromosome, designated sc^8 YB^s, has the normal allele of the X chromosome marker yellow (y^+) inserted into the short arm and the dominant Bar-Stone marker (B^s) into the long arm After X-irradiation with 1,000 r , males with a wild-type X chromosome and this doubly marked Y are mated daily to three females homozygous for the X chromosome markers yellow, apricot, echinus (*y apr ec*) The normal offspring from this

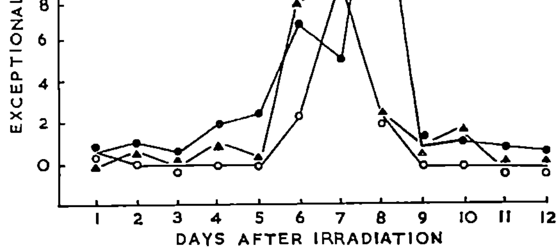

Fig 2 Brood patterns for the 12 days following irradiation ○, XXY females produced by non-disjunction in immature sperm, ●, males with B^s marker deleted, ▲, males with y^+ marker deleted

cross are wild-type (heterozygous) females and non-yellow, apricot, echinus, Bar males Complete loss of X or Y chromosomes will yield sterile $X0$ males phenotypically yellow, apricot, echinus, non-Bar Deletion of either y^+ or B^S marker from the Y chromosome will yield yellow, apricot, echinus, Bar males (abbreviated to yellow males) or non-yellow, apricot, echinus, non-Bar males (or simply non-Bar males) respectively Non-disjunction of X and Y will lead to the recovery of Bar females with XXY constitution Where possible, all exceptional flies were analysed genetically to confirm this classification

Fig 1 shows the comparative yields of $X0$ males and of non-disjunctional females while the frequencies of each deleted Y chromosome class and of non-disjunctional females are given in Fig 2 On the assumption that the determination of non-disjunction will occur only at or before anaphase in the first meiotic division, then it seems likely that the seventh brood in particular represents treated pre-meiotic stages These results also confirm the high sensitivity of meiotic and immediately pre-meiotic stages—presumably primary spermatocytes—to the mutagenic effect of X-rays

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A Gene synthesizing Protease and a Pre-Viral Genome in Silkworms

ALMOST twenty years ago[1], we put forward a working hypothesis that viruses may originate in nucleoproteins in cells, and in 1943 [2] we discovered that the viral nuclear polyhedrosis of larvæ of *Bombyx mori* can be induced by a metabolite The later development of work on induction has suggested[3] that the polyhedral virus would be derived from the chromosomal segment containing the genes controlling the synthesis of protease and deoxyribonuclease We have now demonstrated that the pre-viral genome contains a gene synthesizing an alkaline protease and is located in the chromosome of healthy silkworms

Our previous investigation[4] showed that the pupa of *B mori* has no alkaline protease and never produces polyhedra without infection About 200 pupæ of the Zelem × plem strain were each injected with 0 02 ml of polyhedral solution (0 1 mgm /ml), and polyhedra were collected in the cold by our method[5] from infected individuals before death Viral particles were then isolated from the polyhedral crystals by the procedure previously described[6] and polyhedral protein from the crystals was also prepared at the same time However, the first centrifugation was carried out immediately after dissolving polyhedra in a mixture of sodium carbonate and sodium chloride, without standing overnight The proteolytic action of viral particles and polyhedral protein obtained was measured by the method previously reported[6] The results are given in Table 1, in which the protease activity is expressed in µgm tyrosine per hr produced by 10 mgm of the particles or protein

Table 1 PROTEOLYTIC ACTION OF POLYHEDRAL VIRUS

pH of reaction mixture	8 2	9 0	9 8	10 5	11 0	11 5
µgm of tyrosine: Viral particles	4	10	17	28	31	29
µgm of tyrosine: Polyhedral protein	1	3	7	13	14	12

It is thus evident that the polyhedrosis virus forms a protease which shows the highest activity at pH 10 5–11 0 As the polyhedral virus is of deoxyribonucleic acid nature and produced only in the nucleus, it can be concluded that there exists in the virus a gene which possesses the ability to synthesize the alkaline protease

Our previous experiments[5,7] have further shown that the *B mori* larva has an alkaline protease and produces polyhedra containing a protease of the same nature The white cuticle of the p_{44} strain and the black cuticle of the p^s strain respectively were taken in the midst of the final diapause, namely just at the end of the formation of new cuticle during the third ecdysis, and 0 1 gm. of the cuticle consisting of epi-, exo- and endo-cuticle was ground with 10 ml of 0 02 N sodium carbonate

These two strains were then crossed and the cuticle from the descendants in the F_1- and F_2-generations was treated with the sodium carbonate solution The proteolytic action of the suspensions obtained was immediately measured at pH 10 5 in the same way as before[6] The results are given in Table 2, in which the protease activity is expressed in µgm tyrosine produced by 10 mgm of cuticle per hr

Table 2 PROTEOLYTIC ACTION OF LARVAL CUTICLE

Generation	p_{44}	p^s	$p_{44} \times p^s - F_1$	$p_{44} \times p^s - F_2$	
Colour of cuticle	White	Black	Black	White	Black
µgm of tyrosine	81	161	183	71	181

It is thus obvious that there exists a correlation between the proteolytic activity and colour of the skin, one of the tissues in which polyhedral virus is produced As it has already been established[8] that the plain white of the p_{44}-cuticle and the striped black of the p^s-cuticle are governed by the genes in the second chromosome in the larval cell, and that the latter is the dominant, we may conclude that the gene synthesizing the alkaline protease is located in the chromosomes, possibly in the second chromosome Actually in an experiment the number of white and black larvæ in one group of the F_2-generation was 138 and 429, respectively Similar results concerning the activity of cuticular protease and the ratio of F_2 larvæ were also obtained in the case of the reciprocal cross

In view of these observations, it seems reasonable to assume that, by the inducing process, this special pre-viral genome in larval chromosomes can be activated to form the polyhedral virus

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk * are open to the public*)

Monday, November 27

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W 12), at 4 p m — Prof A G MacGregor "Endocrine Interdependence" *

University of London (at King's College, Strand, London, W C 2), at 5 30 p m —Dr F A E Pirani and Dr G F Elliott "Ballistic Missiles and Nuclear Weapons—The Development of Weapons Technology" * (Last of seven lectures in War Studies)

University of London (in the Gustave Tuck Theatre, University College, Gower Street, London, W C 1), at 5 30 p m —Dr D Müller (Copenhagen) "Microbiological Formation of Hydrocyanic Acid" *

Tuesday, November 28

University of London (in the Anatomy Theatre University College, Gower Street, London, W C 1), at 1 15 p m —Dr G B Brown "What is Wrong with Relativity ?" *

Royal Institution of Naval Architects (at the Institute of Marine Engineers, 76 Mark Lane, London, E C 3), at 5 30 p m — Dr J E Richards "High Temperature Gas-Cooled Reactors for Marine Propulsion"

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Dr E G Gray "Electron Microscopy of C N S" * (Thirteenth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 5 30 p m —Prof K R Popper "Science and Growth of Knowledge" * Third of four Shearman Memorial Lectures)

University of London (in the Eugenics Lecture Theatre, University College, Gower Street, London, W C 1), at 5 30 p m —Prof G Pontecorvo, F R S "Genetic Analysis via Mitotic Segregation" *

University of London (at the Institute of Archæology, 31–34 Gordon Square, London, W C 1), at 5 45 p m —Mr Emmanuel Anati "The Rock Engravings of the Negev Desert, Israel" *

Society of Chemical Industry, Plastics and Polymer Group at 14 Belgrave Square, London, S W 1), at 6 30 p m —Dr C A Finch "Polyolefin Fibres and Films"

Society of Instrument Technology (at Manson House, 26 Portland Place, London, W 1), at 7 p m —Mr R F Rodger ' Automatic Plant Analysis by Electrochemical Methods"

Wednesday, November 29

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W C 2), at 5 30 p m — Mr E Wolfendale "Recent Developments in Semiconductor Devices and their Applications"

University of London (in the Eugenics Lecture Theatre, University College, Gower Street, London, W C 1), at 5 30 p m —Prof G Pontecorvo, F R S "Prospects for Genetic Analysis in Man" *

British Institution of Radio Engineers, Electro-Acoustics Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Mr R Yorke "An Experimental Assessment of Loudspeaker Performance".

Royal Society of Arts (at John Adam Street, Adelphi, London, W C 2), at 6 p m —Mr Paul Adorian "Television in Schools"

Society of Environmental Engineers (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S W 7), at 6 p m —Mr B H Venning "The Response of Dynamic Systems to Shock Excitation"

Institute of Information Scientists (at A E I House, 33 Grosvenor Place, London, S W 1), at 6 30 p m —Dr A R Michaelis "Films for the Communication of Science"

Royal Institute of Chemistry (at Shell-Mex House, Strand, London, W C 2), at 6 30 p m —Annual General Meeting

Wednesday, November 29—Thursday, November 30

Institution of Mechanical Engineers, Hydraulic Plant and Machinery Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 10 45 a m on Wednesday and 10 30 a m Thursday—Conference on "Oil Hydraulic Power Transmission and Control"

Wednesday, November 29—Friday, December 1

Iron and Steel Institute (at the Federation of British Industries, Tothill Street, London, S W 1, and at the Iron and Steel Institute, 4 Grosvenor Gardens, London, S W 1)—Autumn General Meeting, and Forty-third Meeting of the Iron and Steel Engineers Group

Thursday, November 30

Royal Society (at Burlington House, Piccadilly, London, W 1), at 2 30 p m —Anniversary Meeting

University of London (at the Wright–Fleming Institute of Microbiology, St Mary's Hospital Medical School, Norfolk Place, London, W 2), at 5 p m —Prof R Hare ' Sir Almroth Wright'' (Commemorative Lecture) *

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Dr I Friedmann "Electron Microscopy in Pathology of the Ear, Nose and Throat" * (Fourteenth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 5 30 p m —Prof K R Popper "Science and the Growth of Knowledge" * (Last of four Shearman Memorial Lectures)

Royal Institute of Chemistry, London Section (joint meeting with the Battersea College of Technology Chemical Society, at the Battersea College of Technology, Battersea Park Road, London, S W 11), at 7 p m —Dr C C Hall "Chemical Aspects of the Work of the Warren Spring Laboratory"

Thursday, November 30—Friday, December 1

Institution of Electrical Engineers, Measurement and Control Section (in conjunction with the British Nuclear Energy Conference, at Savoy Place, London, W C 2)—Symposium on "Nuclear Electronics"

Friday, December 1

Royal Institution (at 21 Albemarle Street, London, W 1), at 9 p m —Mr E B Worthington "The Science of Nature Conservation"

Saturday, December 2

Association of Agriculture, and the British Agricultural History Society (at the London School of Economics, Houghton Street, Aldwych, London, W C 2), at 11 a m —Agricultural History Conference

British Mycological Society (at the Linnean Society of London, Burlington House, Piccadilly, London, W 1), at 2 p m —Annual General Meeting, followed by Miss G M Waterhouse "The Zoospore" (Presidential Address)

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 3 30 p m —Mr W K Ford "Tropical Insect Life" *

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Research Assistant (male, with a degree in chemistry and interests in biochemistry), to assist in a Medical Research Council supported programme of research into dental disease—The Secretary, London Hospital Medical College, Turner Street, London, E 1 (December 2)

Assistant Lecturers or Lecturers in Experimental Psychology, Applied Mathematics, Pure Mathematics, Inorganic Chemistry, Physical Chemistry, Applied Mathematics or Statistics Mechanical Engineering, Civil Engineering, and Production Engineering—The Registrar, The University, Nottingham (December 9)

Lecturers in Materials in the Department of Engineering, to be responsible for equipping a new materials laboratory and teaching modern physical theories of material behaviour—The Registrar, The University, Leicester (December 9)

Lecturer in Social and Developmental Psychology—The Registrar, The University, Leicester (December 9)

Senior Lecturer in the Department of Biochemistry—The Registrar, The University, Leicester (December 9)

Assistant Experimental Officer (with a pass degree or equivalent qualification) in the Department of Physiology and Plant Culture, to assist in physiological studies in controlled environments —The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (December 10)

Senior Lecturer and a Lecturer in the Department of Mathematics, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (December 20)

Chair of Applied Physics—The Registrar, The University, Hull (December 31)

Chair of Sociology—The Registrar, The University, Hull (December 31)

Lecturer or Senior Lecturer in Genetics at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, December 31)

Lecturer in Bacteriology in the School of Veterinary Medicine—The Registrar, Trinity College, Dublin 2 (January 1)

Chair of Theoretical and Inorganic Chemistry in Victoria University of Wellington, New Zealand—The Secretary Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, January 10)

Lecturers in Physics—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne 1 (January 15)

Lecturer or Assistant Lecturer in Zoology at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 15)

Research Officer (preferably with an honours degree in science, agriculture or forestry, and experience in research) of the Tussock Grasslands and Mountain Lands Institute—The Registrar, Lincoln College, Lincoln, New Zealand (January 15)

Chair of Engineering in the University of Malaya, Kuala Lumpur —The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Kuala Lumpur and London, January 31)

LECTURERS OR ASSISTANT LECTURERS (3) (with a special interest in organic or inorganic or physical chemistry) IN CHEMISTRY at the University College of The West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 31).

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree or equivalent and experience in biological work), for work on the flow and coagulation of blood—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting Ref 61/19

ASSISTANT LECTURER, Grade B (with a good honours degree in mathematics and preferably some teaching or research experience) IN MATHEMATICS—Clerk to the Governors, South-East Essex Technical College, Longbridge Road, Dagenham, Essex

ASSISTANT PROFESSOR IN THE DEPARTMENT OF PHYSICS—Head, Department of Physics, Dalhousie University, Halifax, Canada

ASSISTANT PROFESSOR OF PHYSICS—Prof C E Challice, Department of Physics, University of Alberta, Calgary, Alberta, Canada

BIOLOGY GRADUATE (preferably with a knowledge of immunological technique) IN THE ENDOCRINE DEPARTMENT, to do research on gonadotrophins—The Secretary, University College Hospital Medical School, University Street, London, W C 1

LABORATORY TECHNICIAN, for a research laboratory specializing in steroid biochemistry—Dr Ludwig Kornel, Division of Endocrinology University of Alabama Medical Center, Birmingham 3, Alabama, U S A

MEDICAL ASSISTANT EDITOR (registered medical practitioner not older than 45 years) to the *British Medical Journal*—The Editor, *British Medical Journal*, B M A House, Tavistock Square, London, W C 1, marking envelope "*British Medical Journal*—Assistant Editor"

POSTDOCTORAL FELLOW IN THE DEPARTMENT OF BIOCHEMISTRY—C S Hanes, Department of Biochemistry, The University, Toronto

POSTGRADUATE RESEARCH ASSISTANT (with an honours degree or equivalent) IN PHYSICAL-ORGANIC CHEMISTRY—The Head, Chemistry Department, University of Saskatchewan, Saskatoon, Canada

RESEARCH ASSISTANT, for work on the clathration and occlusion of rare gases—Prof R M Barrer, Chemistry Department, Imperial College of Science and Technology, London, S W 7

RESEARCH ASSISTANT IN THE DEPARTMENT OF PATHOLOGY, for tissue culture work on the nervous system—The Professor of Pathology, School of Medicine, The University, Leeds 2

RESEARCH ASSISTANT (with a degree or H N C in electrical engineering or applied physics, or an equivalent qualification, and preferably some experience in electronics and/or vacuum techniques), for work on the High Voltage Electron Microscopic project—The Secretary, Department of Physics, Cavendish Laboratory, Free School Lane, Cambridge

SENIOR LECTURER (graduate of a British university) IN BIOCHEMISTRY—Clerk to the Governing Body, Battersea College of Technology, London, S W 11

TECHNICIAN IN BIOCHEMISTRY (A I M L T , B Sc , or A R I C)—The Director, Department of Pathology, Paddington General Hospital, London, W 9

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CO-OPERATION IN RESEARCH

IT is something more than mere coincidence that Dr B V Bowden, in a discourse at the British Association at Norwich last September on the impact of automation, should have urged not simply the wider and more imaginative use of the electronic computer but also pointed to the implications of such developments for university and technological education In fact, the position of the universities in this field has been the subject of special comment in the general survey from the University Grants Committee which this year accompanies the usual returns from universities and university colleges in receipt of a Treasury grant Furthermore, in the June 1961 issue of *Vestes*, the Australian universities review, J M Bennett describes the university computing centre in Australia, where three such centres have been operating since 1956 Again, the current issue of *Impact* includes a paper by Prof R Courant which, while discussing general problems confronting computing centres, emphasizes particularly the major effort required, especially in universities, to train a generation of scientists eager and competent to make full use of computers as scientific tools—an effort which requires the breaking down of departmental boundary lines and as such may involve changes in departmental organization, as well as co-operation on a broad scale with industrial research organizations A recent article by Dr R C Campbell in *Chemistry and Industry* (p 1786, November 4), on the administrative uses of a computer in a university, points to some interesting possibilities in the use of computers and indicates an encouraging prospect for such developments

Mr Bennett, who notes that more than 3,000 automatic digital computers are now in service throughout the world and that more than 100 are in service in universities in the United States, while nine British universities have computing centres, considers not merely the man-power required to operate computers and to utilize them, but equally the problems of finance and administration The university computing centres in the United Kingdom are financed largely from public funds through the University Grants Committee as a result of an agreement reached with the Treasury in 1954 Grants amounting to £381,000, out of the total university expenditure of £417,000, were eventually made for the purchase of computers and ancillary equipment on the recommendation of the University Grants Committee, advised by the Advisory Committee on High-Speed Calculating Machines of the Department of Scientific and Industrial Research The initial allocation was primarily on a regional basis At that time, Cambridge already possessed a computer and Sheffield was able to make very favourable arrangements for hiring computer time Accordingly, Durham, Leeds, London, Manchester, Oxford, Southampton and Glasgow received computers, and eventually Liverpool purchased a computer, partly from industrial contributions and partly from its own resources

In 1957, an Inter-University Committee on Computing was established, under the chairmanship first of Prof D R Hartree and later of Sir Willis Jackson to advise the Committee of Vice-Chancellors and Principals on policy questions and to promote co-operation in this field between the user universities and between universities and other bodies In 1959 this Committee, in its first report, emphasized the rapid expansion of university work, which had been under-estimated and was steadily squeezing out extra-mural work, and recommended further extension of computing facilities in the universities As a result, the University Grants Committee agreed, in October 1959, to consider a further allocation of computers to the universities in Britain When approached, twenty-two universities, including all but one of the universities already possessing a computer, replied that they wished to apply for a new machine

Two universities applied for *Atlas* computers and the University Grants Committee took the view that provision of machines costing £1 5–£3 million each was a question to be handled on a national basis, and accordingly the claims of these two universities and of other institutions for *Atlas* facilities have been assessed by a working party under the Minister for Science As a result of this survey, the Government has placed an order for such a computer to be installed at Harwell under the auspices of the National Institute for Research in Nuclear Science, and it is assumed that these facilities will be available to those universities which need them The University of Manchester, by its own special arrangement with the makers, will possess an *Atlas* computer which will be available to certain other universities

As regards the medium-sized computers, the University Grants Committee appointed a small sub-committee, under the chairmanship of Sir Willis Jackson, to give detailed consideration of the types of machine required by the universities, to questions of timing and priorities, and to the use that might be made of the older displaced machines This sub-committee included representatives of the Department of Scientific and Industrial Research and the Ministry of Education, and had the assistance of Dr Howlett, of the Computing Department of the Atomic Energy Research Establishment, its advice was accepted by the University Grants Committee, which in March 1961 sought authority from the Chancellor of the Exchequer for a programme to provide medium-sized computers, involving expenditure of about £2 million, between 1961 and 1965 This authority has been given, and accordingly twenty-one universities are expected to have their own computer facilities, while the remaining universities have access to others

This, however, is only a particular aspect of the relations between the universities and the Government in Britain, or indeed of university expenditure on research, but it is of special interest in view of Lord Hailsham's remarks about those relations in his recent addresses on science and government in a free society (see *Nature*, November 4, p. 393, and p. 787 of this issue). Lord Hailsham did not suggest that the British system, which, in effect, allows Government funds to be administered by the universities themselves, is the only possible way of ensuring adequate Government support for university research without infringing academic freedom, but he did claim that the system works, and his addresses make very plain the dangers which attend the creation of separate Government-sponsored institutes and generally the separation of research and teaching. He was emphatic that the most advanced scientific work should be located in or near the universities, where this is possible, or directed by university personnel.

In his earlier address, Lord Hailsham was concerned with maintaining a healthy balance between research and teaching, between Government research and that proceeding in the universities and in industry, between pure and applied research and development work, and he was no less conscious of the growing opportunities for international co-operation in research. It is with the balance between research and teaching that the University Grants Committee is concerned, and at the request of the Advisory Council on Scientific Policy it has made a further estimate of the resources in the universities which are devoted to research in the pure and applied sciences, medicine, dentistry, agriculture, forestry and veterinary science. Although it wishes to have further information of the numbers of technicians engaged in the various departments in the universities, since this matter, which has an important bearing on the effective use of research workers, is one to which the Committee is paying special attention in assessing the financial needs of the universities for the next quinquennium, the results of this survey are given in the Committee's general survey attached to the latest *Returns**.

The survey covered 16,882 university staff and research students, of whom 53 per cent were in the pure sciences (mainly physical) and 19 per cent in the applied sciences. Medicine (3,324), chemistry (3,013), engineering (2,445) and physics (1,909) claimed the largest numbers. Of the total, 7,522 were research students, and the proportion of research students to university staff varied from 2·0 : 1 in chemistry to 0·2 : 1 in medicine and dentistry. About 49 per cent of the time of staff and research students is estimated as devoted to research, varying from as little as 21 per cent in dentistry to 57 per cent in zoology. The total of 8,534 technicians included 563 chief technicians, 1,365 senior technicians, and 2,475 technicians, and on an average, there was one technician per member of staff and one to 1·8 members of staff and research students, the ratio varying greatly between subjects. Many of the technicians, moreover, had duties connected with teaching as well as with research, and a comparison with the figure for research laboratories in other institutions suggests that the ratio of technicians to research workers in the universities in Britain is below that necessary to ensure that the time of research workers is used most economically.

The University Grants Committee may well be understating the need, but it is satisfactory to note that it proposes to give special attention to this factor in its current assessment of the needs of the universities. On the expenditure side, the survey estimates that of departmental expenditure totalling £28·3 million, and including academic salaries, wages, departmental and laboratory maintenance and other like items, £14·1 million is attributable to research. If, further, it is assumed that some two-thirds of such 'overhead' expenditure as administration, libraries, maintenance of premises and the like applies to the sciences and applied sciences, and half of this two-thirds is attributed to research, the total rises to £18·3 million. To make this figure comparable with the total of £22 million quoted for the year 1958–59 by the Advisory Council on Scientific Policy in its report for 1959–60, there must be added a proportion for non-recurrent grants for building and equipment. For 1959–60 these grants amounted to £4·2 million, and if account is taken also of awards to research students which do not pass through the university accounts—and these are assumed to be worth on average £470 a year each—a further £3·4 million should be added, bringing the total to £25·9 million, which would represent some £2,140 for each research worker per annum, departmental expenditure alone amounting to £1,666, of which about £865 represents salaries of university staff. It is not clear, however, whether the figure of £25·9 million includes all the sums for research at universities which are made available through the Council for Scientific and Industrial Research and the Research Councils, but the figure compares with £23·3 million for research and development carried out by the universities quoted by the Advisory Council on Scientific Policy in the current report. Sir Harry Melville put the contribution from the Department of Scientific and Industrial Research in research grants and post-graduate studentships at more than £2·5 million in the past financial year.

The distribution of this research effort in the different groups of sciences varies from 38·3 per cent of man-power and 32·8 per cent of expenditure in the physical sciences to 1·8 per cent of man-power and 1·7 per cent of expenditure in dentistry; for the biological sciences the corresponding figures are 9·8 and 10·0, for the applied sciences, 19·1 and 18·0, for medicine, 19·7 and 27·2, and for agriculture, forestry and veterinary science, 5·9 and 6·6.

The University Grants Committee quite rightly once again urges that too great a significance should not be attached to many of the differences in research effort between subjects. Obviously, some regard must be had to personal idiosyncrasies and abilities, and the research effort made in any one subject will undoubtedly vary from time to time, depending on such factors as the presence of an outstanding worker in a subject and the level of interest In fact, the interest now being taken in the balance of research effort in Britain arises essentially from two roots the need to see that in the universities and elsewhere the resources at the disposal of the research worker are such that he can make effective use of his time and of the costly equipment that is now so often required, and secondly, to ensure that there is a reasonable balance of research effort between the universities and other research institutions, Government or independent, between pure and applied research and development, as well as between subjects It is only on such a basis that it is possible to plan a reasonable allocation of the limited resources in Great Britain and to press the claims of a particular research effort in the face of conflicting claims

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* University Grants Committee. Returns from Universities and University Colleges in receipt of Treasury Grant, Academic Year 1959–60. Pp. 76. (Cmnd. 1489.) (London: H.M.S.O., 1961.) 4s. 6d. net.

Lord Hailsham's recent addresses show that he is well aware of the problems involved. and alive to the dangers either of parsimony or of interfering with the universities' choice of projects or disposition of their resources Inevitably, co-operation involves compromise and adjustment as between universities and other institutions, or between Government departments, industry and the universities, and the development of university co-operation in the use of computers, radio telescopes and cyclotrons, quite apart from the growth of international co-operation in research, will accentuate the need for further collaboration. At the present moment, when the University Grants Committee is formulating its proposals for the next quinquennium under the shadow of the Chancellor of the Exchequer's call for economy and reduction in public expenditure, a heavy responsibility rests on the Minister for Science to see that there is no retraction of the universities' research effort, either in respect of its contribution to the advancement of knowledge or to the training of man-power for research The University Grants Committee, from its general review, appears to be taking the right measures to ensure the elimination of waste and the effective disposition of whatever resources may be made available It is for the Minister for Science to satisfy himself that the resources put at the disposal of the universities are indeed adequate for the purpose, having regard to the nation's need for trained man-power, its total research effort in industry and Government institutions as well as in the independent research institutions, and to the possibilities of co-operation both on the national and on the international scale In that task, moreover, he should derive powerful support from the recent report of Lord Plowden's committee in opposing any policy that would tend to apply 'start and stop' to research projects Lastly, in a time of financial stringency, the Minister for Science might be specially watchful to see that no measures are introduced which hinder that invaluable movement and interchange of personnel between institutions or the regular exchange of ideas on which he himself has rightly placed much stress

RESEARCH IN THE UNIVERSITIES*

By The Right Hon. VISCOUNT HAILSHAM, P.C.

Lord President of Council and Minister for Science

THE Rector of Imperial College has reminded me that it is a hundred years since the death of its founder, the Prince Consort, a man of vision and prescience who saw clearly, in an age only partially awakened to such things, the revolutionary changes for good which might be made in the conditions of human life by an unfolding scientific technology, imaginatively applied in a spirit of serious good will In the bright light of mid-Victorian optimism, of which Prince Albert was in part an example and in part an initiator, much that has happened since would have seemed a perversion of those eager hopes But enough has taken place to make sure that they were not altogether misplaced and to give us confidence that in their future realization the College which he founded will continue to play a noble part

When writing of the first professorial unit in the College, Prince Albert described this part as having raised the new College to "a high pitch of usefulness and popularity". Usefulness and popularity are not always co-existent, but they are no bad things, provided they are kept in just the right balance with one another, and this, I believe, is what Imperial College has done.

The Rector also suggested to me, as a main topic for discussion, the subject of research in the universities—a theme particularly suitable in view of the strong tradition of research in Imperial College, and the important part which this tradition plays not only within the College but also in the scientific, technological and industrial life of the country as a whole

This subject contains a clue to the whole development of an integrated modern culture on one hand, and of an adequate technology on the other, at the same time it is, as I shall seek to show, a matter of extreme complexity on which we have not yet discovered all the answers.

Can we begin without some idea of the kind of thing we want our universities to be, and the kind of purposes we want them to serve ? For myself I cannot, and at the risk of exceeding my mandate I must deliver my testament of faith on this topic

The eternal struggle of humanity is to reconcile freedom with authority, individual self-expression with the requirements of society There have been many definitions of freedom, ranging from national independence to freedom of worship, from universal suffrage to full employment, from social security to private enterprise As a lawyer, as a politician, as a moralist, I have discussed them all, and admit that all have a certain contingent value As a philosopher I have found them all inadequate None of them by itself is intellectually satisfying

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* Substance of the Commemoration Day Address at the Imperial College of Science and Technology, delivered in the Royal Albert Hall, London, on October 26

I suspect that one reason for this is that, whatever it means, freedom cannot be seen in isolation from the search for the just and the true, the beautiful and the good—the ultimate values which men place on their existence In their origins in the ancient world, the universities were the places where this search was correlated and discussed, and the academic freedom of the universities themselves, their intellectual stature, their moral quality, seem to me to be matters of immense importance, theoretical not less than practical, in the discussion of the relative values of tyranny and freedom, of democracy and authority, of law and morality But if this is so, it is at least surprising that this is a matter which is almost never mentioned by the political theorists and lawyers who indulge in most of the arguments

But if a unity of purpose, an integrated understanding of man and his environment is not to be found in the universities, it will not be found in the nation, or in the world This is dimly apprehended even by the multitude, even by the popular press when they pay sometimes disproportionate attention to the strange actions of undergraduates—or to the even stranger opinions of professors But in terms it is almost never said Yet the university world is the true melting-pot of society, the one great mixer where class distinctions are being abolished before our eyes, the one aristocracy of merit divorced from privilege, the one democracy where equality of opportunity is not confused with equality of achievement, reward or esteem. In the humanities it is generally accepted that, at this level, teaching and research cannot wholly be separated My thesis to-day is that teaching and research in science must similarly be allied

Yet side by side with their function as the teachers of human society as a whole, the universities have also the task of providing vocational training It is idle to disregard or minimize this aspect of their activities —although many academics are far too fastidious to speak of it openly, and sometimes write letters to the papers decrying its discussion The ugly truth is that if it had not been for this function, the universities would have neither been brought into being nor survived If the Church had not needed educated priests, if the State had not needed educated administrators, if industry had not needed educated executives and technologists, there would have been no universities, and although medicine and law and the engineering professions, at least in Great Britain have contrived partly independent systems, I doubt myself whether even these would survive—or maintain adequate standards—without a parallel system of academic courses to give them intellectual body

But the truth is that the two functions are not independent of one another Even on the vocational plane, teaching of the order we require for our administrative, our scientific and industrial leaders cannot be wholly divorced from research—the true pioneering of knowledge of all kinds Research will tend to become barren and research workers to die out if attempts are made to divorce it from teaching

But how is research to be financed? Who is to call the tune? And if the tune is to be called by the spirit of research itself, the λόγος as Plato puts it, carrying us along like the wind, What conceivable motive will be his who pays the piper ?

The issue of academic freedom has never been very far below the surface of political life At the time of the Reformation, and the Civil War, the questions were obvious But in recent years, and certainly until 1939, they were scarcely asked No one grudged the Prime Minister the right to nominate Regius professors, and academic resources depended as much on fees, endowments and donations as on public funds

But by 1946 it was apparent that the scale of expansion forced on the universities by the range and rapid development of modern knowledge, and the insatiable appetite of industrial society for trained graduates, made it necessary that from henceforth the main patron of the universities would be the State Happily to hand was the appointed instrument of State and university—the University Grants Committee, brought into being in 1919 I would think that on the whole this has managed to the general satisfaction to administer public generosity without corrupting academic freedom

In one direction, however, the University Grants Committee has required supplementation Built into university life is an innate conservatism—no bad quality, this, but one that needs to be balanced From their start, the Research Councils—by which I mean the Department of Scientific and Industrial Research, the Medical Research Council, the Agricultural Research Council, and the Nature Conservancy —have encouraged the initiation of new projects of research, and particularly in relation to medical research have founded whole teams of research workers within, but not necessarily as integral parts of, the universities and in the past four years the total value of the research grants given to universities each year by the largest of these patrons—the Department of Scientific and Industrial Research—has increased three-fold I am rather proud that this has happened entirely within my own periods of office, since I regard the development as wholly to the good

For a second function of the universities—to initiate new studies, to fill in gaps in the old, needs (as no one knew better than Prince Albert) a living patron, something more adventurous, and perhaps a little more selective, than in the nature of things the University Grants Committee can be expected to be The present size of the Government-sponsored research in the universities is, however, such as to give rise to new questions—both for the Research Councils and I would say, also for the universities—which it will be my business and that of my successors in office to investigate in the coming months and years Speaking broadly, it will be necessary to keep in mind the respective functions of the University Grants Committee and the Research Councils But, at this stage, I would only mention, in passing, two small points

First, the permanent structure of the universities is clearly a matter for the universities themselves, and the University Grants Committee is the constitutional channel for Government funds It is vital to remember that the Research Councils' function is not to finance science in the universities as a whole, but to provide funds for new developments in research which cannot be adequately dealt with within the framework of the quinquennial system But at what stage, and by what mechanism, can work originally fostered by the Research Councils be handed over—and how is the hand-over to be reconciled with the academic law of the Medes and Persians against ear-marked grants ?

Secondly, a similar question, though not entirely limited to research, arises in relation to laboratory technicians and assistants—whose activities form an indispensable part of virtually all scientific work in universities It is not my function, or that of the Research Councils, to provide part of the permanent staff of universities The University Grants Committee rightly adheres to its policy of refusing ear-marked grants This is a minor problem and can be solved within the university framework

Since I first assumed Ministerial responsibility for the Research Councils, university research has been affected by a number of changes—changes both in scale and in character—in the field of scientific research generally For one thing, in an increasingly wide range of disciplines the tools of research have become machines—and machines each of which is a major engineering project constituting a significant item in the capital investment programme Apart from the big machines, the accelerators, the Jodrell Banks, the gas-bubble chambers, there is the problem of the new institutions, sometimes clustering around the facilities of a parent body, like the National Institute for Research in Nuclear Science round Harwell, yet half resenting and half resented by the parent organization which gave it birth

Closely related to the machines, but potentially larger still, are newly emerging fields of investigation, too big, almost from the first, for association with teaching universities, too big, almost from the first, for purely national budgets, often, like space research or the *Mohole*, requiring immense vehicles and immense organization for their initiation; sometimes like CERN at Geneva, or *Dragon* at Winfrith Heath, involving international funds and wide implications in the field of foreign policy Already a large number of international organizations, some bilateral, some regional, occasionally world-wide, some for research, others involving development, have sprung into being and require careful finance and clear thinking if they are to develop on the right lines Clearly all these changes raise important implications for the relationship of universities to research, of universities to Government, and of universities to one another

One such implication that is gradually becoming clear is that in certain branches of science it is impossible for every university to have equal treatment from the Government in all spheres Ten linear accelerators each costing £1,000,000 will not make up for the absence of one linear accelerator costing £10,000,000—if one is needed, and it is quite clear that not every university can hope to have a £10-million accelerator In Britain, at any rate for the time being, there can only be one Jodrell Bank, one *Nimrod*, there can only be a few gas-bubble chambers, and only a few research reactors Where are these to be placed ? Who is to run them ? Who is to arrange their research programmes ?

When a number of such various projects are proposed by scientific enthusiasts, How are we to judge which to accept, particularly where these belong to different fields ? How are we to correlate the work of the National Institute for Research in Nuclear Science with the applications to the Department of Scientific and Industrial Research in the field of nuclear and high-energy physics—or with the budget of CERN in which we have only a minority vote ? How are the differing projects for radio astronomy to rank between one another or with the applications for big machines in other fields? How is either to rank with space research—another subject in which other countries are involved ? Can individual universities form partnerships or consortia ? Or should they all be provided for through a national extramural institution like the National Institute for Research in Nuclear Science ? If the latter, where is the facility to be placed—inconveniently for all universities, or conveniently for only one or two ? If a facility is given to one university, should any—and if so what—conditions be exacted for the benefit of the others ? If a facility depends on a particular man, what happens if he dies or resigns to move elsewhere ?

All these questions have actually arisen in a concrete form since I took office Some I have had to decide Some have been decided, with my approval, by the Research Councils We are getting a certain amount of case-law and empirical wisdom about all this But have we got it right ? This is something we shall have to discuss

But it is not only a question of big expensive machines that can be located in or near only some of the universities Somewhat similar problems arise out of the increasing diversification of subject-matter How far is it necessary or desirable, for example, to preserve the existing situation in which the scope for postgraduate work by students of a given institution is often limited by the extent of the facilities—either natural or artificial—which that institution happens by tradition or by accident to have in its possession or in its close neighbourhood ? Clearly, university statutes were—or should be—made for the needs of learning, and not the needs of learning for the requirements of university statutes Should university research still be limited in range by the migratory habit of medieval monks ? Should the availability of freshwater biology as a subject for research in Cambridge or Leeds be dependent on the contents of the Cam, or of the local Yorkshire factory effluent ? Should the availability of the Ph D to the staff of a Government research station depend on the place where each received his first degree ?

All these questions need public discussion Government may have views, but in many cases it will be the vice-chancellors, the courts and senates, the faculties and the colleges, which supply the answers

What is to be the relationship between courses of study and research in the universities and the known requirements of industry ? Distinguished academics often write to *The Times* expressing disquiet at the tendency of industry to demand courses from universities in fields which are remote from traditional fields and are of deliberate relevance to practical life—for example, management studies Similar questions are asked in relation to industrial research and in some of the applied social sciences Is industrial money corrupting, as the author of one letter appeared to think ? If so, ought we not to think the same of Government money ? With due respect to the University Grants Committee, and to the Research Councils, the Government is not fundamentally so disinterested as it pretends Nor perhaps are even academics quite so pure Nearly three-quarters of Government money going into scientific research goes on the defence budget More of this than many people think is spent in the universities—and none of it could be spent at all without research workers, such as those who are trained in the Imperial College of Science and Technology

My own view is that in Great Britain we should welcome and encourage industrial money, if only as a desirable and necessary counterbalance to Government money At least it is not motivated by war or the fear of it. Moreover, there is no academic doctrine more powerful than the motto of the Royal Society "*Nullius in Verba*". When I am asked, as I sometimes am, whether it is not degrading that people should be asking for private donations towards cancer research, because the Government ought to bear the cost, I reply that I would not sleep easy in my bed if I thought that either I or the Medical Research Council were the only source of wisdom to decide which men and what ideas were worthy of support The Americans may go a great deal too far academically in their dependence on private munificence, educationally they suffer enormously from the fact that their schools and universities are in no sense a Federal Government concern But I think we may go too far the other way From my point of view the degree courses available in British universities are too narrow in scope, and some of them are orientated too academically I believe the same to be true to some extent of research work

There is another important aspect to this. One of the most important subjects with which I am called on to deal is the relative failure of British industry to make use of science and the scientist I will not bore you with figures, they are easily available. But, so far as I am concerned, the broad truth is that, outside a few main industries, far too few scientists are employed, and if more were employed some directors might not—in their present frame of mind—know what use to make of them. Not unnaturally, members of Parliament and others ask me what can be done to improve the situation Whatever the immediate answer, I am quite certain that the only radical cure lies in the universities, in a closer contact with industry, both education-wise and research-wise Contact between intelligent and right-minded people is not corrupting; almost invariably it is stimulating, and in my view at any rate academics have almost as much to learn from contact with industry as industry has to learn from the academics Certainly, contact is the only way in which either will learn anything from the other There is a great contrast between the extent of mutual regard and contact between academics and industrialists in Germany, which I have recently visited, and Britain In my view, neither gains here by the degree of apartheid it practices and, incidentally, if that apartheid were broken down, both in teaching and research, my own opinion is that the human sciences, which are excessively neglected in Britain, would also come into their own—both in the field of postgraduate research and in the field of new schools and faculties

I realize that in making these remarks in the Imperial College I am rather preaching to the converted The College's annual reports provide plenty of evidence of strong ties with industry, and of wise generosity by industry in financing work here on a variety of research projects, and there are also such things as the vacation training schemes by which students can gain valuable industrial experience

But is this enough ? Certainly in Germany you get co-operation between universities and industry to a much greater extent To take only two examples in Germany there are far fewer obstacles than exist in Britain to the combining of teaching with management or research in industry, and indeed, you get industrial concerns operating research institutes inside technological universities I know, of course, that much can be said in support of the British attitude, the ideological division between teaching and production, but need we take it quite as far as we do ? I shall not be so rash as to attempt an answer, but I feel it my duty to pose the question When we see the close contact, amounting almost to organic unity, which exists between industry and universities on the Continent, and when we see some of the results in terms of design, production, and sales on one hand, and vigorous high-grade academic development on the other, we must surely ask ourselves whether the system adopted on the Continent does not possess certain competitive advantages which Britain lacks I have spoken of the apartheid which seems to exist in Britain between universities and large sections of industry, may this not be due largely to the mere accident that many of our industries grew up outside the ambit of scientific and technological studies, and should we not see whether ways cannot now be found of bringing them together ?

There can be no easy or single way of doing this But one important need is the creation of the kind of climate of opinion in which industry and the universities turn towards each other quite naturally, and cross the gulf which has too often divided them hitherto

Every one who at some stage or other in his career goes into industry, whether in industrial research or in production or general management, and every one who goes into Government scientific establishments or public administration, will not only be helping to bridge the gulf, he will also be able to spread among others the habit and the desire of making and maintaining the closest contacts with university teaching and university research Similarly, those who remain within the university cannot unreasonably be asked to remember always the valuable help that those in the universities and those in industry can give to each other

I must stress that it is not just a matter of doing well the research that comes to be tackled, or making use of the results of research when it is done, it is a question of research-mindedness, at all points· and here we come back to the very nature of the actual courses which the universities provide Do they impart, along with knowledge, and skill in using knowledge, an adequate appreciation of the role of research, of the techniques of doing it, and of the importance of a readiness and a right way to apply its results ? I ask this not only as regards postgraduate courses, where one ought to be able to hope confidently for an affirmative answer, but even more importantly as respects first-degree courses Have the men who go out into industry straight after taking their first degrees—who after all comprise the main army of scientists and technologists—have they acquired, during their undergraduate training, a sufficient understanding—I will not say of research and of development—but even of design ? Are they carrying a lighted flame—a torch with a battery—or just a reflector ?

I do not need to labour the point and let me make clear that in directing attention, as one particular topic that I feel needs special notice, to this question of contact in research between science and industry, I must in no way be taken as ignoring the importance of the more pure, more cloistered, more fundamental research that must also go on continuously if the well-springs of our science and technology are not to dry up These are fundamental to the whole conception of scholarship—whether in the arts, or the natural sciences In the long run it will be found that the whole future of our civilization depends on the insights—I cannot refrain from calling them the artistic and creative imaginations—of a relatively small handful of men of genius, the true frontiersmen of knowledge.

I have posed a number of questions—and attempted few answers Even some of the questions may be wrongly based—or wrongly put. But I hope at least that they will be food for thought—and I hope also that the course of development in our universities and colleges of advanced technology during the coming years will be such that the answers to these questions become increasingly reassuring.

I find myself at the end attempting to formulate a few general truths which seem to me to emerge from this discussion and from the wider experience which I have gained in the past four years

If the general rule must be that research and university teaching cannot be divorced, the wider development of the subject implies inevitably closer contact between universities and Government research installations of one sort and another, between these and industry, between industries and universities, between universities and one another, between universities and places of research outside the general pattern which cannot conveniently be placed inside teaching institutions, between the scientific body within one national group and others of the same group—even between scientists of opposing groups of nations These are not really rival conceptions for the organization of research, their rules are complementary rather than exclusive, and they are in truth part of a single complex, of a constantly developing conception of a scientific life gradually transforming the body of human society as we know it

But, at the end of the day, if I am pressed with the familiar question of the egg and the chicken, if I am asked to define the point at which new growth is to be inserted into the scientific life of which I speak, I look, on one side, to the universities to provide the new ideas, but I accept the responsibility of Government to supply the initiative and the drive to convert these new ideas into actuality. The more I think of the problem confronting me as Minister for Science, the more I am convinced that the clue to the enigma lies in the universities and the colleges of technology, and where better to seek the answer to the riddle than at the Imperial College, which is in a sense both, and at a commemoration festival—an occasion when many are gathered together from both inside and outside the academic circle, many who are, many who have been, and many who will become leaders of thought in State, industry and learning

The founders of the Imperial College, of course, visualized it as a centre of learning for a more or less centralized empire Already that conception has given rise to that of a commonwealth of free and independent nations But, speaking for myself, I see the centres of learning as something more than this These are the true centres of culture in a nation By improving these we take the shortest—indeed the only practical—course towards raising the standard of knowledge and civilization of the world.

MEDICAL RESEARCH AND THE NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND*

By Dr. CARL R BREWER

Chief of the Research Grants Branch, Division of General Medical Sciences

THE National Institutes of Health, which began in 1887 as a one-room laboratory on Staten Island, New York, to-day comprised forty-two buildings occupying ninety acres of land in Bethesda, Maryland, north-west of Washington, D C

To understand the role of the National Institutes in health research, it is necessary to understand that the United States is one of the most health-conscious countries in the world, and that the American people have repeatedly expressed, through their representatives in the Congress, their fervent desire to wipe out disease and the crippling effects of disease and to help themselves and other nations to a new level of healthful well-being, and to longer and more productive lives

That such goals always have been an integral part of the American philosophy is illustrated by the fact that the U S Public Health Service was established in 1798, that is, 163 years ago That such goals run as a theme through all Departments of the U S Federal Government to-day is illustrated by the fact that several of these major Departments themselves also support biomedical research, in addition to the work done at the National Institutes of Health as a part of the Public Health Service and the Department of Health, Education, and Welfare

The object is to give man a better life through research in medicine, by which is meant the medical sciences, the many portions of the biological sciences related to medicine, and those portions of the physical sciences related to medicine

I will not go into the statistics related to the monetary size of these efforts Figures on the numbers of dollars lack meaning in themselves The dollars are simply the means to the ends of action and results in research—and action and results are what we are after To help illustrate the point that the American people whole-heartedly are supporting the drive for better health, the percentage growth in the past twenty years is worth mentioning

Since about 1940, total expenditures for medical research in the United States (that is, including the moneys of private foundations as well as the Federal Government) have increased more than 1,500 times To-day, the Federal Government is supporting roughly 55 per cent of all medical research in the country, and the National Institutes of Health are responsible for about 75 per cent of the Federal Government expenditures

Thus, the National Institutes of Health form the largest biomedical research institution in the United States, and probably the largest in the world There are about 2,000 research scientists and approximately 6,000 administrators, laboratory technicians and other employees working at the various departments in Bethesda, Maryland. The total programmes of the Institutes are divided between the 'intramural' activities carried out at Bethesda and the 'extramural' activities under which the organization provides grants-in-aid support for biomedical research in the nation's schools of medicine, dentistry, public health, osteopathy, nursing and others, universities, and other private research institutions.

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* Substance of an address given before the International Symposium on Microchemical Techniques, at the Pennsylvania State University, University Park, Pennsylvania, on August 16.

During the Fiscal Year 1961 (which ended June 30, 1961) the intramural programmes totalled approximately 85 million dollars.

Principal among the numerous buildings devoted to the intramural programmes is the Clinical Center, a 516-bed research hospital with 1,100 laboratory units and 1,260,400 sq. ft. of space. It employs more than 1,500 persons.

During the same year, the extramural programmes totalled approximately 475 million dollars (the latter having grown from 850,000 dollars in 1946). In this connexion, 13,500 research grants, 3,618 training grants and 4,205 fellowships in the United States and abroad were awarded.

Perhaps it seems an over-simplification, but it is accurate, to say that the National Institutes of Health are supporting and/or carrying out research in every biomedical science and nearly every disease known to man. At one extreme, they are supporting work on the very origins of life—how the amino-acids came together to form a primitive proteinoid, and developmental biology, embryology, genetics, and diseases of childhood. At the other extreme, the biological processes of ageing and the rehabilitation and home-environmental problems of older people are being examined. Literally, we are concerned with life from before conception to death, and all the stresses which assail man in his existence—organic disease, and psychological and environmental stresses.

Until 30–40 years ago the major causes of serious illness and death were the infectious diseases. These diseases received most of the attention of medical research because they were the major killers.

Then penicillin became available, followed by many other antibiotics, and while these certainly did not end the problems of infectious diseases they drastically changed the overall picture of medical research.

We cannot claim final victory over pathogenic micro-organisms because, obviously, they can mutate (and there exists, for example, penicillin-resistant *Staphylococcus*), thus, we are left with unsolved problems.

On the other hand, it is the chronic diseases, such as heart disease, cancer and others, which to-day have become the major killers. In the United States alone, different forms of cancer kill about 260,000 persons every year, and heart diseases account for nearly 900,000 deaths every year. So it is these diseases which are now attracting major attention, along with neurological disorders, arthritis and the entire spectrum of metabolic diseases, mental health, allergy, and dental disorders, and there we have the titles of the seven Institutes at Bethesda: Allergy and Infectious Diseases, Arthritis and Metabolic Diseases, Cancer, Dental Research, Heart, Mental Health, and Neurological Diseases and Blindness.

In terms of scope, the National Institutes have recognized the necessity of becoming concerned with the entire scheme of man's existence—the whole organism of man in relation to disease and his total environment, including both the psychological and physical.

As one example of the scope of research, the Institutes are providing new and increased opportunities for such para-medical scientists as anthropologists, biometricians and epidemiologists to clarify the relationships between cultural stresses and diseases, for example, as in the relationship between the high intake of fat in certain cultures and the incidence of atherosclerosis.

If, for another example, we consider the field of psychiatry, we find that it, too, is expanding beyond the limits of the office couch into environmental studies, on one hand, and yet also much deeper into the part that biochemistry may play in the origins of, as well as therapy for, mental and/or emotional disorders.

As to the depth of our research penetrations, I would like to quote Dr. James A. Shannon, director of the National Institutes of Health[1]: "Although until recently we were limited in our examination of the economy of the body as a whole—its net energy balance, the net exchange of foodstuffs, and the endocrine factors that were effective in producing changes in these—we are now able to isolate for study important specific systems within cells and to define with precision many of the discrete steps which characterize their activity. We can describe the components of these systems, quantitate the kinetics of the interactions involved, study the effect of superimposed variables upon these reactions, the products of which may be retained or released. By other techniques we can describe the importance of many of these systems to the cell, to the organ, to the organ systems and, in fact, to the body as a whole".

The pattern of much of the biomedical research in the world to-day is the pursuit of a much larger, broader, deeper, more integrated body of knowledge about basic human biology. The National Institutes have accepted this charge, and the acceptance of this new challenge has found wide expression in all the Institutes' programmes, particularly that of the Division of General Medical Sciences.

In effect, the Division of General Medical Sciences is the eighth Institute at the National Institutes of Health, because it functions as an Institute in all respects except that there is no intramural programme. The programmes are devoted entirely to grants to the medical schools, universities, research hospitals and other private research institutions. In the research training programme, which also is oriented toward the basic biomedical sciences, funds are provided to the medical schools and other institutions to aid in the training of new investigators. In the research fellowship programme, awards are made to individuals, at the predoctoral and post-doctoral levels, to aid the general research training development of scientists.

Until recently, nearly all the Division's research grants have been on an individual project basis, and the grants of this type in the Division of General Medical Sciences alone total approximately 2,000. One modification of this procedure came in the establishment of the General Clinical Research Center Program which provides funds to medical schools and research hospitals. The purpose of these grants is to broaden and intensify clinical research, particularly in the sense of achieving careful quantitative measurements of disease. The funds go for the establishment of discrete facilities or centres for the location of beds and laboratories. A variety of different research projects can be carried on in these centres. Another modification of the individual

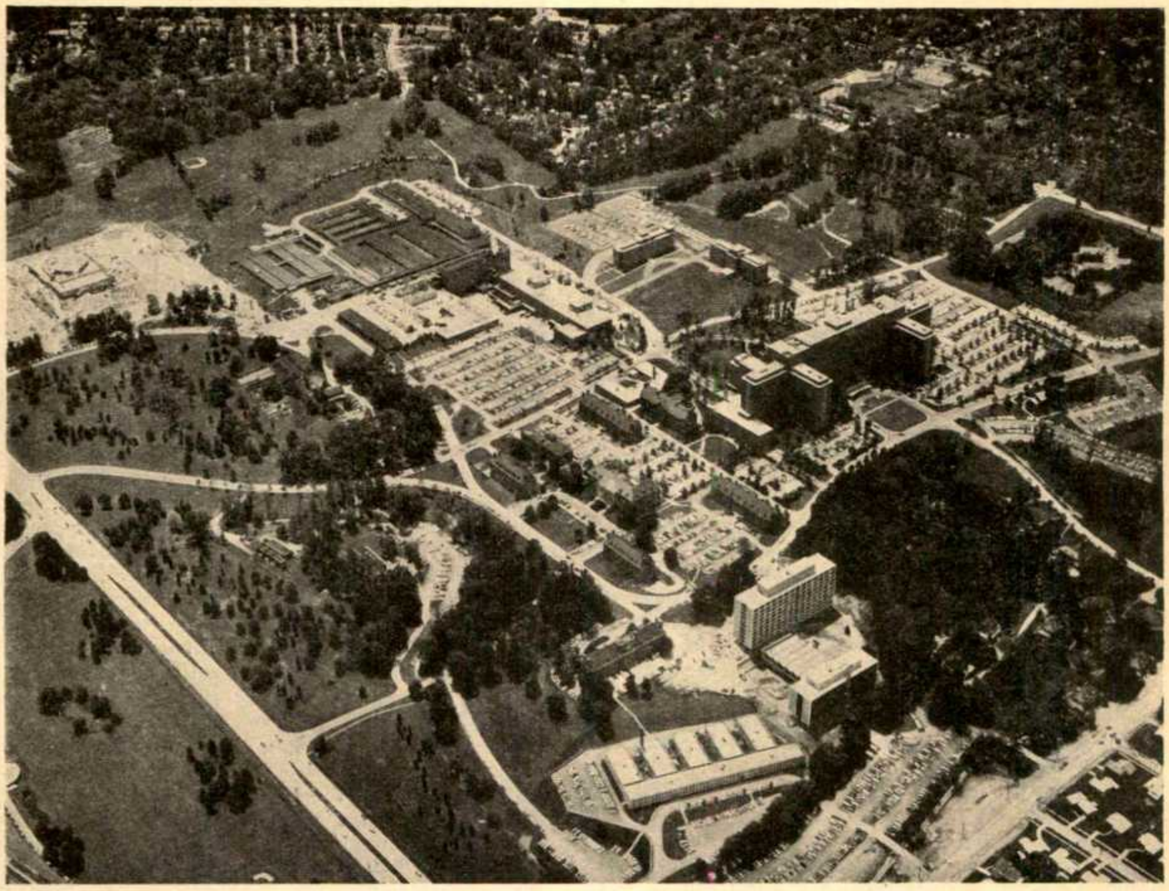

Fig. 1. The National Institutes of Health, Bethesda, Maryland, U.S.A.

project procedure is the new General Research Support Grants Program, which was recently initiated. This will provide the schools of medicine, public health, dentistry and osteopathy and other institutions with funds which these institutions may then use for the support of their research programmes. The feature which principally distinguishes this programme from most other National Institutes of Health and Public Health Service programmes is the even greater freedom given the institution in the use of these funds.

The establishment of the Division of General Medical Sciences just three years ago was an expression of the broader and deeper research approach of the National Institutes of Health. The research programmes of the Institutes have always involved basic research; but for the most part these programmes have been oriented to specific diseases. In the mid-1950's, there was recognized a need for more basic research, and basic research training, which was not disease-oriented. The National Institutes began making a number of grants for such purposes, and by 1958 it became apparent that the demand was such that a unit should be established especially for the administration and management of these grants. The reaction of the biomedical-scientific community to this development was such that expenditures for the Division's programmes have more than tripled since it was organized. During the past year, these programmes totalled nearly 85 million dollars. Further recognition of the importance of these programmes has come in a recent proposal by President Kennedy to the Congress that this Division be established as the National Institute of General Medical Sciences.

While the Division's activities are generally not oriented to specific diseases, their justification is the urgent need for basic biological knowledge essential for progress in the conquest of human diseases.

A simple example—but not a simple problem—pertains to research in the biological process of ageing and, more specifically, to recognition of the distinctions between the physiological ageing process and the pathological disease process.

Molecular biology is a field in which the Institutes are very active. One is sure that work in this area eventually will provide much additional information on such problems as rheumatic syndromes, infertility, tuberculosis, virus infections, cancer, hypertension, schizophrenia and alcoholism, because all these conditions involve disordered chemical processes of cellular metabolism.

In recent years, of course, modern biochemistry has come into its own, with its progress and expansion being such that it has fathered a number of subdisciplines and has become integrated into nearly all phases of medical research.

In the field of developmental biology, the Institutes are seeking to be as broad and deep as in biochemistry. Research is being supported in all phases and aspects of structure and function related to genetics and reproduction. These would include the germ cells and fertilization, the placenta, the fœtal fluids, the embryo and fœtus, teratology, parturition and lactation, and special problems of gestation.

The field of genetics, of course, continues to grow at a tremendous rate, but we do not yet know its full significance in human biology and in disease. Dr. Jack Davies, of Washington University, St. Louis, has said: "How the genetic and environmental factors act and interact during development (of the fertilized egg) is perhaps the fundamental problem of biology"[2]. The implications with regard to congenital malformations and other hereditary diseases are, of course, obvious.

The work on the deoxyribonucleic and ribonucleic acids is beginning to provide a new concept of man and of disease. Among the great amounts of research that the Institutes are supporting in this field is that by some Israeli scientists on the role of the ribonucleoprotein complex of the cell in the synthesis of antibodies.

Modern geneticists are beginning to predict that man some day may be able to control his own biological destiny by influencing the genetic factors. This is one of the reasons for emphasis on medical and human genetics, cytogenetics, viral and bacterial genetics, biochemical genetics and genetic ultra-structure.

There are many other basic fields in which the National Institutes are also active. Examples are: public health and nursing; biomedical instrumentation; research surgery; anæsthesiology; bio-organic and biopolymer chemistry; biomathematics; and biophysics.

As the American interest in biomedical research and improved health is integral to all elements of the U.S. Government, so also is it an interest to be shared with the other countries of the world, for mutual benefit.

Such an effort has two goals: the first is the interchange of newly developed fundamental knowledge among the scientists of the world; and the second is the equally substantive advantage in biomedical research of learning more about the incidence and variable characteristics of specific diseases and also general biological phenomena in different parts of the world, as related to variations in climate, altitude, food, culture and other factors.

Just a year ago, the President of the United States signed the International Health Research Act of 1960. In general, the Act provides for support of research activities in foreign countries, research training and fellowships, the making of grants and loans of equipment, the support of international health research meetings, and the exchange of research scientists.

For the Fiscal Year 1961, just passed, a sum of 5 million dollars was designated for the development of activities under the Surgeon General's authorities provided by this Act. Approximately a half this amount was allocated for the development of a series of international centres for medical research and training, based in United States universities with overseas extensions in the form of research laboratory training facilities.

Each of the centres consists of, first, a professional, university-based organizational entity which provides a stable, continuing framework in which programmes of medical research, research planning and research training may be conducted in the United States, and which is associated with the second part, a research and training establishment abroad where these activities may be pursued under environmental, ethnic, and medical conditions which do not prevail in the United States.

The remaining 2·6 million dollars are to be utilized by the several Institutes and the Division of General Medical Sciences for the support of research projects involving foreign investigators and institutions, and directed toward research and research training objectives of key significance to these projects.

The National Institutes of Health recently established an Office of International Research, within the office of the Director of the Institutes. This new Office has the responsibility of advising the Director and the Office of the Surgeon General on the international aspects of medical research and research training. It also assists the Institutes and the Division of General Medical Sciences in the further development of international projects, and provides a mechanism by which the National Institutes of Health can work with international organizations, foreign institutions and scientists on a planned, predictable, long-range basis.

In years past, there has been talk about the need for an international language. We already have an 'international language' in the field of biomedical research. It is a subject which transcends geographical boundaries and defies language barriers. It is the goal of the National Institutes of Health and the whole American Government to improve and expand this 'language' to bring all the countries of the world closer together in the common cause of good health for all men.

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[1] Shannon, J. A., *Philadelphia Med.*, **56** 699 (1960).

[2] Davies, Jack, *Survey of Research in Gestation and the Developmental Sciences* (Williams and Wilkins, Baltimore, 1960).

RUTHERFORD JUBILEE INTERNATIONAL CONFERENCE ON NUCLEAR PHYSICS

THE fiftieth anniversary of the publication of Rutherford's 1911 paper announcing the discovery of the atomic nucleus was celebrated by an international conference during September 4–8 at Manchester on what is nowadays called 'low-energy' nuclear physics. The conference was attended by more than four hundred people; its wide support will undoubtedly make it the major international meeting in its field in 1961.

As is usual with these almost annual events, progress during the past year was summarized; but on this occasion half a day was set aside from the discussion of present problems for a commemorative session on Rutherford's discovery. There were talks on their reminiscences by Sir Ernest Marsden, Sir Charles Darwin, Prof. E. N. da C. Andrade, and Prof. Niels Bohr, all of whom had worked with Rutherford at Manchester. Sir James Chadwick was present on the platform but did not speak. In these talks there came to life a period which to many of those present had been a legend they had read about only in text-books. Here was the tale of

Marsden whom Rutherford told to look for the reflexion of α-particles from solids, something which neither Rutherford nor Marsden expected to happen, but Marsden knew that if the effect existed and he missed it, that would be the end of him Then there was Darwin's story of collaboration with Moseley, whose methods of non-stop work made him not only an expert on X-rays but also on where to get a meal in Manchester at three o'clock in the morning Niels Bohr closed the session with a few brief remarks in which he laid special stress on the central importance of Rutherford's discovery in providing a testing ground for the theorists during the development of the quantum theory beyond the point to which Planck and Einstein had taken it.

The remainder of the conference was conducted in an atmosphere moulded by the portrait of Rutherford which looked down on the participants over coffee, and the presence of an exhibition of much of his apparatus The complete proceedings will be published soon, so that here there is no need to mention more than a few topics the selection of which from the wealth of material presented is to a large extent a matter of personal taste

There was steady progress to report on several fronts. The question of the fundamental origin of the interaction between nucleons has become clearer in recent years with the accumulation of evidence that the component of longest range is what would be expected from the exchange of single π-mesons. Further evidence of this effect has now come from an extensive analysis of the scattering of nucleons by nucleons in states of high relative orbital angular momentum where the centrifugal barrier makes the effects of longest range predominate, as it should, the strength of the coupling between nucleons and mesons required turns out to be the same in neutron–proton and proton–proton scattering, and agrees with that needed to account for the scattering of π-mesons by nucleons The dependence of the long-range interaction on the spins of the nucleons also agrees with the theory A new development in the field of nucleon–nucleon interactions is a direct measurement of the strength of the interaction of two free neutrons by means of the reaction $D(n,p)2n$ at low energies which is strongly influenced by the interaction of the two outgoing neutrons, the first result is not far off the value to be expected from p–p scattering on the grounds of charge-symmetry Another problem which is at last showing signs of being cleared up is the origin of the spin-orbit coupling between two nucleons which had been introduced phenomenologically to account for scattering at high energies, this is probably connected with the existence of a short-lived quasi-bound state of three π-mesons of angular momentum unity—the '3π-resonance'—the discovery of which was announced at the conference The spin-orbit coupling would arise analogously to that which acts between two electrons due to the electromagnetic field

Some fundamental points in connexion with the shell model are still being elucidated, in particular the origin of the strong spin-orbit coupling seen by each particle in a nucleus, and the existence and properties of the low-lying single particle states which are not normally involved in low-energy reactions The origin of the spin-orbit coupling probably also lies in the newly discovered 3π-resonance, through a mechanism analogous to the production of the spin-orbit coupling in atoms The low-lying single particle states are now beginning to show up in $(p,2p)$ experiments, the incident protons have an energy of about 150 MeV and are able to knock a proton out of the target, the spectrum of the summed energies of the two emerging protons showing clearly defined peaks corresponding to the binding energy of the ejected proton The measurements on isolated nuclei which were previously available have now been extended until the binding energy of the lowest-lying level—the $1s$—has been traced for elements up to atomic number 20 The same experiments can also give the original momentum distribution of the struck nucleon from the angular correlation of the emerging protons.

There was much discussion of two aspects of nuclei which go qualitatively beyond what can be described by the simple shell model built up from the picture of individual nucleons moving in a static potential well These were the structure of the nuclear surface, where there may be important clustering effects, and collective motions in which many particles partake coherently in such a way as to generate an oscillating or rotating potential well On the structure of the nuclear surface, new evidence produced during the previous year supports the view that the surface is probably more 'lumpy' than a simple shell model would lead one to expect This evidence comes partly from new estimates of the probabilities of α-particle emission in some heavy nuclei on the basis of shell model wave-functions which give values smaller by about a factor 10^3 than the experimental ones There is also new information on the absorption of K^- mesons at the periphery of a nucleus from bound Bohr orbits, where the existence of correlations between the nucleons can be detected by the possibility of the production of very energetic hyperons in absorption mechanisms such as $K^- + 2$ nucleons $\rightarrow$ hyperon + nucleon, in addition to the less-energetic hyperons which are emitted in the process K^- + nucleon $\rightarrow$ π-meson + hyperon, which is the only one to occur for free nucleons The situation is not clear because it appears that in the light nuclei, in contrast to the heavy ones, it is possible to account for the emission and absorption of compound particles with a simple shell model without recourse to any additional clustering

The field of collective oscillations is still developing under the new impetus it received from the discovery of the importance of particle-hole pairs and of pairing correlations at the top of the Fermi sea So far most of the emphasis has been on quadrupole and octupole shape oscillations, and on the giant dipole resonance in which the protons oscillate against the neutrons. The theory gives the frequencies of collective oscillations in terms of the energies of the single particle excitations which contribute, and the probabilities of occupation of the single particle-levels involved Measurements of these properties by means of stripping reactions now exist in a limited number of nuclei, and the collective frequencies calculated from them are found to vary from nucleus to nucleus in agreement with the measured values However, in the prediction of electromagnetic transition probabilities the theory is still only in semi-quantitative agreement with experiment

A welcome contribution was a talk under the title of "Rutherford and Nuclear Cosmochronology", by W A. Fowler, who discussed the present estimates of the ages of the Earth, the solar system and the Galaxy, making use of the known abundances of the elements and of calculations of these abundances from the synthesis of the elements in stars and supernovæ It turns out that where Rutherford in 1929 gave the age of the solar system as 3 4 × 10^9 years, the best present estimate is still as close as 4 5 × 10^9 years

The success of the conference amply repaid the members of the organizing committee for their labours If they have one regret, it is that the limited space available made it necessary to refuse a place to many whom they would have liked to have seen If some active and distinguished research workers feel disappointed at our inability to invite them, may we ask them to bear in mind that the places which were due to them probably went to representatives of isolated institutes for whom these conferences are the only real contact with the main stream of research in nuclear physics A HERZENBERG

ENDOCRINOLOGY AND EVOLUTION

DURING the recent meeting at Norwich of the British Association for the Advancement of Science a symposium on "Endocrinology and Evolution" was held under the auspices of Section D (Zoology), with Prof C M. Yonge in the chair

In the opening paper, Prof E J W Barrington pointed out that while far too little is known about the ways in which hormones exert their effects, it seems evident that there must be a closely adaptive relationship between the active hormonal molecules and their target cells This raises the questions when, and in what circumstances, these relationships were first established, questions which are important because the answers to them can scarcely fail to enlarge our understanding of the primary functions of the hormones concerned He developed this theme with particular reference to the thyroid gland, an organ which shows a remarkable uniformity of biochemical organization through the vertebrates, from the cyclostomes to the mammals Its characteristic iodinated tyrosines and thyronines are known to be produced in the endostyle of the ammocœte larva of the lamprey, before this organ has been transformed at metamorphosis into the definitive thyroid gland It would appear, therefore, that any search for clues to the mode of origin of the gland must take us outside the vertebrates altogether

Here we cannot fail to be struck by the fact that iodine has long been known to be present, and often in great abundance, in many marine invertebrates Our knowledge of this phenomenon has been greatly extended in recent years by the use of radioactive iodine, and by employing the techniques of autoradiography and paper chromatography These have shown that the organically bound iodine of marine animals is not strictly of a thyroidal character, for instead of being bound within thyroglobulin molecules it is commonly associated with the scleroproteins of the skeletal tissues These have been closely studied in the Anthozoa and sponges, and it has been found that their iodine content may be surprisingly high, as much as 10 per cent as compared with the 1 per cent of thyroglobulin It seems likely that the amount of iodine taken up by the scleroprotein molecules depends on their tyrosine content, and that it is mainly bound as monoiodotyrosine and diiodotyrosine, although trace amounts of thyroxine and triiodothyronine have been reported present in the horny skeleton of gorgonids

The organic binding of iodine in invertebrates thus appears to be a chance consequence of the molecular constitution of their skeletal tissues, and this raises the question whether there is any evolutionary relationship between it and the highly specialized iodination sequences of the thyroid gland We cannot be sure of the answer to this, but consideration of the results of recent work on the protochordates, which are known to be closely related to the vertebrate line of ancestry, suggests some interesting reflexions Autoradiography has demonstrated the occurrence of iodine binding in certain clearly defined cells of the endostyles of ascidians and amphioxus, and chromatography has shown that iodinated tyrosines and thyronines are present in these animals There is some evidence that these substances may be associated with the specialized outer layer of the tunic of ascidians, as well as with the endostyle, so that these particular animals may eventually be found to show random iodination side by side with the endostylar process which foreshadows the eventual emergence of a true thyroid gland

It is attractive to speculate that iodinated products arising thus by chance in early chordates might have become metabolically available for the animals, by ingestion or by transport in the blood stream, and that increasing dependence on these would have led to the evolution of a more efficient biosynthetic mechanism in the endostyle At the present time this is no more than a thought to guide further investigation, but we certainly cannot dismiss the possibility that the thyroid hormones evolved as a result of animals incorporating into their biochemical organization substances which were initially presented to them as random biological products

Any discussion of the evolution of hormones needs to take account of the fact that they do not act in isolation, but as components of highly integrated systems of mutually interrelated glands Prof J M Dodd was to have discussed this aspect with particular reference to the work of himself and his colleagues on the relationship between the pituitary and the thyroid, but he was unable to attend, so his place was taken by Dr K M Ferguson, who began by remarking that although the controlling influence of the pituitary over the thyroid, mediated by its thyroid-stimulating hormone, is well known in mammals and amphibians, it is not always so easy to demonstrate it in the other vertebrate groups Nevertheless, there is ample evidence of a qualitative nature to demonstrate that this so-called pituitary–thyroid axis is probably common to the entire vertebrate series For example, although neither removal of the pituitary nor administration of mammalian thyroid-stimulating hormone produces significant histological changes in the thyroids of adult elasmobranchs, Dent and Dodd have shown that a distinct thyroid–pituitary interrelationship exists in the immature, newly hatched dogfish, *Scyliorhinus caniculus*, and that its thyroid-stimulating hormone is probably located in the ventral lobe of the pituitary

Quantitative evidence is scanty, but by using the mouse assay of McKenzie it has been possible to demonstrate thyroid-stimulating activity in pituitary extracts from animals ranging over the entire vertebrate series, although at considerably lower concentrations than are found in mammals Certain features of these results, however, present difficulties of interpretation. For example, a large part of the activity is heat-stable, while mammalian thyroid-stimulating hormone is markedly heat-labile Moreover, measurable activity is obtained from the neural lobe, even in the mammal, although there is every reason for regarding thyroid-stimulating hormone as a product of the adenohypophysis The significance of these facts is bound to remain obscure until we are better informed of the chemical nature of thyroid-stimulating hormone, but in the meantime it is necessary to remember that glandular extracts may contain a large number of contaminants, hormonal and otherwise, which are likely to interfere with responses, and which cannot be adequately controlled by injections of indifferent tissues such as brain or muscle It is possible, too, that foreign pituitary material may set up interfering stress responses, while it is known that the relative thyroid-stimulating potency of extracts from different species varies according to the choice of the assay animal

These considerations combine to make it impossible to decide whether the apparently low concentrations of the hormone in extracts of pituitaries of the lower vertebrates reflect quantitative or qualitative differences It may be, however, that the thyroid-stimulating hormone molecule has been subjected to biochemical evolution, but has yet retained sufficient community of chemical structure throughout the vertebrates to allow pituitary extracts from a wide range of donors to stimulate the thyroids of a wide range of recipients

Further aspects of endocrine evolution were brought to light in Dr J G Phillips's discussion of the adrenal gland There is much variation in the gross appearance of this organ, and in the degree of association of its two components, the chromaffin and the adrenocortical tissue, but these variations, characteristic of each of the main vertebrate groups, mask a remarkable constancy in the properties of its secretory cells This is well illustrated by the steroid hormones of the adrenocortical tissue, which appear to be chemically identical throughout the vertebrate series, and which are involved in homeostatic mechanisms contributing in one way or another to ensure a constancy of the internal medium Nevertheless, this fundamental uniformity has not prevented the establishment of a wide degree of functional adaptive radiation In the sphere of water and salt metabolism, for example, these hormones influence tissues such as the nephron, the gills of fish, and the skin and bladder of frogs, all of which share a common capacity for the transcellular conduction of electrolytes and water, and which react to adrenocortical hormones in a manner determined by the nature of the environmental hazards to which the particular species is exposed Thus, marine fish are faced with the problem of retaining water and excreting excess salts, while the converse situation confronts freshwater species, and these hormones, although not the only factors involved, contribute to the necessary physiological adaptations by their action on the kidneys and the gills In sea-water they promote the excretion of excess electrolytes through the gills, and the maximal re-absorption of water in the kidneys, while in fresh water they promote the excretion of large volumes of dilute urine, accompanied by salt retention

It is evident that one important factor in the evolution of endocrine systems is the possibility of change in the character of the target organs, and Dr Phillips illustrated this by reference to recent work on the remarkable ability of marine birds to survive for long periods the ingestion of fluids which have an ionic concentration many times higher than that of their body fluids This ability depends on the nasal glands situated above the eyes Following the ingestion of sea-water, these glands are activated, and, as a result of their remarkable concentrating ability, the bird excretes the excess salt through the nasal gland without commensurate loss of water The action of the adrenocortical hormones is one of the factors involved in this response, and there is evidence that, with the hormones of the neural lobe of the pituitary, they arrest the excretion of the excess salt through the kidney; this is then followed by a shift to the physiologically more economical method of excretion through the nasal glands There is good evidence that the latter are also dependent on the nervous system, but at present it is not possible to judge the relative importance of the two forms of control It is clear, however, that the action of the adrenocortical hormones on the nasal glands is closely analogous with their action on the gills of marine fish, for in both cases they serve to promote the excretion from the body of excess salt without undue loss of water.

The final paper was given by Dr D B. Carlisle, who reviewed some problems of crustacean endocrinology, and this was followed by a lively discussion which ranged over such diverse problems as sex-reversal in molluscs, the function of indole derivatives in crustaceans, the influence of ciliary feeding in the evolution of the thyroid gland, and the possible role of adrenocortical tissue in the metamorphosis of lampreys. The session as a whole must have done much to reassure those who fear that endocrinology, with its para-medical background and its esoteric terminology, is too specialized for comprehension by the general biologist, and it amply demonstrated how widespread are the biological problems which are being illuminated by present developments in the comparative study of endocrine mechanisms

E J W BARRINGTON

OBITUARIES

Prof. H. P. Robertson

PROF. H P ROBERTSON, who contributed to the theories of relativity and cosmology, and was a member of many Government scientific boards in the United States, died on August 26 as a result of injuries sustained in a motor-car accident His death takes from many people throughout the world a sensitive, gay and ever-helpful friend as well as an experienced and wise counsellor

He was born in Hoquiam, Washington, on January 27, 1903, and received his bachelor's and master's degrees in 1922 and 1923, respectively, from the University of Washington, where he was introduced to the Einstein theory of relativity by E T. Bell

He obtained the Ph.D. degree from the California Institute of Technology in 1925 and then spent two years in Germany as a National Research Fellow at Gottingen and Munich. He joined the Mathematics Department of the California Institute of Technology as an assistant professor of mathematics for the period 1927–29.

During 1929–47 he was at Princeton University, occupying successively the posts of assistant professor, associate professor and professor of mathematical physics. In 1947 he returned to the California Institute of Technology as professor of mathematical physics, a post he held until his death.

Although Robertson devoted most of his scientific efforts to the general theory of relativity and to its application to cosmological problems, he was interested in the foundation of physical theories and the application of various branches of abstract mathematics to physical problems. His excellent translation of Herman Weyl's *The Theory of Groups and Quantum Mechanics*, which he completed in 1931, demonstrates his profound knowledge of the theory of groups, quantum mechanics and the relationship existing between these two subjects.

He was a master in applying the theory of groups of motion of Riemannian spaces to problems in relativity. His 1933 paper entitled "Relativistic Cosmology", which appeared in the *Reviews of Modern Physics*, illustrates his use of the technique. In this classic paper he summarizes his own basic work and that of others on the consequences of the symmetry assumptions made in relativistic cosmology and relates these to the astronomical observations. He afterwards studied the implications of the symmetry assumptions alone, divorcing these from the Einstein field equations. The latter work was stimulated by the lively controversy over kinematical relativity, in which he and E. A. Milne engaged, and appeared in three papers and in the *Astrophysical Journal* (1935, 1936) entitled "Kinematics and World Structure".

Robertson's contributions to relativity should not be measured only by his own papers. He engaged in extensive discussions with, and participated in the work of, others. His aid is acknowledged by many authors; in particular, he played an important part in the work of Einstein, Infeld and Hoffmann on the equations of motion of singularities in the gravitational fields and published his solution of the equations for two bodies with comparable masses obtained by these authors.

In 1939 Robertson responded to a call from his friend and mentor, R. C. Tolman, and began his work on scientific problems important to United States military agencies. He stopped his intensive work in relativity, which was just coming to fruition, and answered to the demands made on him with characteristic vigour. He quickly produced a scholarly review of work in terminal ballistics and made contributions to this field. He took an active part in organizing the group that became Division II of the National Defense Research Committee and in directing the early work of this group.

He served with the National Defense Research Committee from 1940 until 1943 and as scientific liaison officer, London Mission, Office of Scientific Research and Development, during 1943–46. From 1944 until 1947 he was an expert consultant to the Office of the Secretary of War. In 1945 he was chief of the Scientific Intelligence Advisory Section of Supreme Headquarters, Allied Expeditionary Force in France. For his war service he received the United States Medal of Merit.

In 1950 he was called back to Washington and served for two years as deputy director and research director of the Weapons Systems Evaluation Group of the Department of Defense until 1952. During 1954–56 he was the scientific adviser to General A. M. Gruenther, the then Supreme Allied Commander in Europe. For four years beginning in 1956 he was member at large and chairman of the Defense Science Board. He was also a member of the President's Scientific Advisory Committee.

In addition to belonging to a number of scientific societies, Robertson was a member of the U.S. National Academy of Sciences, of which he was foreign secretary from 1958 until his death.

Although Robertson never lost his interest in working in the theory of relativity and in teaching this subject, his many Washington duties to which he gave himself unstintingly made it impossible for him to do more than publish a few papers after the Second World War. In some of these he again reviewed the theoretical and observational state of the theory of cosmology. He was much interested in the possibilities of using satellites for experimental tests of theories of gravitation and took part in organizing, and was chairman of, a conference on this subject in July last at Stanford University.

This review of some of Prof. Robertson's achievements and activities must of necessity omit much that should be said about him. Suffice it to say that he made his mark in science, in service to his country, and in the hearts of the many people from all walks of life who were privileged to know him.

A. H. TAUB

Mr. Lewis Eynon

THE news of the death on October 18 at the home of his son at Stoke Bishop, Bristol, of Lewis Eynon will sadden his friends in many countries. The sugar industry has lost a most experienced analyst, who further had done so much to help the analytical profession. Eynon was born in 1878 at Stoke Newington and was educated at the City of London School. On leaving there he became a student at Finsbury Technical College during 1895–99 under Prof. Meldola. He was then appointed a chemist to the Beetroot Sugar Association. Another Finsbury student, J. H. Lane, was also there with him, and the two became partners in 1910 as consulting chemists. Having acquired the laboratories from the Sugar Association of London, as it had then become, and also the business of the analysis of its sugar samples, they had spare time which they used for research on sugar, chiefly on improvement of methods of sugar analysis. Their long search for a sensitive internal indicator for use with Fehling's solution for testing reducing sugars eventually led them to methylene blue. The result was the Land and Eynon method which has become well known throughout the sugar world. It has eventually been accepted as an official method by many countries and also by the International Commission.

Eynon obtained many qualifications and held many important offices among the various professional bodies to which he belonged. In 1900 he gained the associateship of the Royal Institute of Chemistry and was elected to the fellowship in 1903. A year later he graduated B.Sc. at the University of London with honours in chemistry. For many of the years between

1920 and 1949 he served on the Council of the Royal Institute of Chemistry, and during 1930 he was a vice-president. An early member of the Society of Public Analysts (later the Society for Analytical Chemistry) he served on the Council, was honorary secretary during 1936–47 and president during 1947–49 He attended the meeting of the eighth session of the International Commission for Uniform Methods of Sugar Analysis, held in 1932 at Amsterdam, and was elected honorary secretary, in which position he remained until the end of the ninth session in London in 1936 He was also chairman of the Publication Committee, responsible for the production of the *Proceedings* for the eighth, ninth, and tenth sessions In 1932 he became honorary secretary of the newly formed British National Committee of the Commission, which position he relinquished in 1948 to become chairman This post he held until 1954 The same year in Paris at the eleventh session of the Commission he was unanimously elected life honorary vice-president "in grateful recognition of his extensive and valuable services to the work of the Commission and for his distinguished contributions to the literature and practice of sugar analysis"

At the end of more than sixty years of a very busy life, he was still the same gentle-mannered man, a very kindly character, unassuming and loved by all who knew him, his many friends could count on the encouragement and help that was always forthcoming.

H. C S de Whalley

NEWS and VIEWS

Nobel Prize for Physics:

Prof Robert Hofstadter

Prof Robert Hofstadter, who shares the Nobel Prize for Physics with Prof R L Mossbauer, was born on February 5, 1915, he received the bachelor of science degree from the City College of New York in 1935, and his Ph D from Princeton in 1930 He then taught and carried out research at Princeton, Pennsylvania, and the City College of New York until the war years, during which he worked at the National Bureau of Standards and the Norden Laboratory Corporation. For four years after the War he was assistant professor at Princeton, but in 1950 he went to Stanford as associate professor, becoming professor in 1954, and eventually head of the department Prof Hofstadter is best known for his use of high-energy (roughly 1 BeV) electron beams to probe the structure of nucleons A knowledge of the form of the meson clouds surrounding the proton and neutron has been at the foundation of important recent developments in quantum field theory It is not possible to give an adequate idea of this work in a short space, but the success of his work is perhaps best illustrated by the discussion it provoked in the United States of the feasibility of constructing an electron accelerator of still higher energy

Prof. R. L. Mossbauer

The early recognition of the importance of Prof R L Mossbauer's work by a share of the Nobel Prize for Physics will give great satisfaction to all physicists The recognition might have been even earlier if Prof Mossbauer's work, carried out at the Max Planck-Institut at Heidelberg in 1957, had not passed strangely unnoticed for nearly two years The discovery of what is now called the 'Mossbauer effect' is concerned with the spectrum of the γ-rays which are emitted by certain excited nuclei It has been known for many years that the nuclear levels in many cases are extremely sharp, but considering the problems of nuclear recoil and lattice vibrations which introduce Doppler shifts, physicists had resigned themselves to the conclusion that the very narrow γ-ray lines which exist in principle would remain for ever inaccessible Mössbauer's achievement lies in recognizing that in certain specific cases, appreciable fractions of the γ-rays may be emitted undisturbed by recoil or lattice vibrations The imaginative achievement is the more remarkable when one considers that the theory underlying the effect has been known for twenty years, but the relevant inferences had not been drawn The first spate of new results obtained by making use of the Mossbauer effect in experiments to test the predictions of relativity theory, to investigate the properties of the solid state, the nature of magnetism and the properties of some nuclear excited states has now subsided, but there can be no doubt that the application of the discovery will continue to be a subtle tool in all branches of science

Nobel Prize for Chemistry :

Prof M. Calvin, For.Mem R.S

Prof M Calvin, who has been awarded the Nobel Prize for Chemistry for 1961, took his doctorate at the University of Minnesota and afterwards spent two years at the University of Manchester with the aid of a Rockefeller Grant In 1937 Prof. Calvin joined the staff of the University of California. Discovery of the long-lived isotope carbon-14 during the War (prior to which only the short-life isotope carbon-11 had been available) provided an opportunity for a comprehensive study of the intermediates of photosynthesis which was immediately realized by Prof Calvin Success came when the isotope technique was combined with the then recently discovered paper chromatographic method of analysis In work with A A Benson and later others, the first product of the photosynthetic fixation of carbon dioxide was shown to be phosphoglyceric acid and for the first time the importance of the pentose and heptulose phosphates in photosynthesis was realized Calvin postulated a sequence of reactions constituting the 'photosynthetic cycle' in which these compounds formed a synthetic cycle for the incorporation of carbon dioxide. About the same time essentially the same reactions were shown to play an important part in the respiratory process (the pentose phosphate shunt) not only of plants but also of animals The interests of Prof Calvin's group have now moved towards the investigation of the basic mechanism by which light energy is converted into chemical energy in the chloroplast of the green plant Attempts have been made to devise model systems in which light energy can be converted to chemical energy with high efficiency, thus necessitating an extension of the earlier studies of the photochemistry of dyes Prof Calvin was elected a foreign member of the Royal Society in 1959

Nobel Prize for Physiology : Dr. Georg von Békésy

THE award to Dr Georg von Békésy of the 1961 Nobel Prize for Physiology gives recognition to a remarkable achievement in the field of experimental biophysics Dr Békésy has had an unusual career Born in 1899 in Budapest and educated at the Universities of Bonn and Budapest, he has, since 1949, been senior research fellow in psychophysics at Harvard He has displayed outstanding skill as an experimenter, devising at all stages of his work new technical equipment, mechanical, optical and electrical, of great ingenuity His first remarkable contribution was a penetrating analysis of the vibrational responses to pure tones of the basilar membrane of the human cochlea A later outstanding achievement was his use of vibrating electrodes to explore and reconstruct the patterns of amplitude and direction of vibration induced in individual cell elements within the cochlea in response to pure tones of varying frequency and intensity His papers, written in clear, concise and graphic language, have exercised a profound effect on our concepts of the peripheral mechanism of hearing An edited selection of his earlier German papers has recently been translated into English by Prof E G. Wever, one of his American colleagues, and republished together with others in book form, *Experiments in Hearing* This is a substantial volume of 750 pages and incorporates a representative bibliography of the author's papers In 1961 Dr von Békésy was awarded the Gold Medal of the Acoustical Society of America

The Education Bill in Great Britain

THE Education Bill, which received its second reading in the House of Commons on November 13, has two purposes The first is to provide statutory authority for the reforms in the system of grants to students which were announced following the report of the Anderson Committee, the second is to reduce to two the number of school-leaving dates in the year, as recommended by the Central Advisory Council for England and Wales in the Crowther Report In moving the second reading of the Bill, the Minister of Education, Sir David Eccles, said that the local authorities, with which he had fully discussed the provisions of the Bill, readily accepted that the time had come to make the awards automatic when the student had both the qualifications and a place at a university, and Clause 1 laid this duty on the authorities as from September 1962, and gave the Minister power to define the extent of the duty. Sir David said that he had accepted the Anderson Committee's recommendation that the qualifications for an award should be two A-level passes in the General Certificate of Education, but it is necessary to provide for further qualifications which students may gain as they advance in technical colleges Clause 2 ensured that local authorities had full discretion to make awards for further education courses below the standard comparable with first-degree courses

The second fundamental principle of the new system is uniformity of treatment Sir David said that local education authorities now generally accept for the student's own maintenance during term the standard figures reviewed at intervals by their representatives and the Ministry In future there would be nationally prescribed rates and conditions of grant binding on all authorities for all items of a student's grant which could be dealt with in this way It was proposed to retain the means test in a relaxed way which would relieve about 10,000 more families from all contributions and those in the middle range of incomes would pay substantially less Grants for students in teacher-training colleges would be calculated in the same way as for university students, and those for students in technical colleges taking full-time courses comparable with degree courses would be similarly calculated The standard vacation grant had been revised and now applies throughout the income range of parents, while conditions for dependants' grants have been revised and broadened Students may keep up to £100 of private income, including income from scholarships and prizes, without deduction from their grant, and the Minister said he had appointed a Standing Advisory Committee with Sir Francis Hill as chairman to advise on matters relating to the size of the grant and the definition of 'comparable courses' The Committee had been asked to recommend revised rates of grant early in the New Year

Sir David said that under our present policies, the cost of education in Britain is rising at about 5 per cent a year, and awards to students now cost £35 million a year What is proposed under the Bill would add a further £10 million To abolish the means test altogether would add another £10 million, and Sir David said that if he and the Secretary of State for Scotland were allowed to spend a second £10 million, they thought they could spend it to better advantage in other parts of the service Under Clause 3 of the Bill, State Scholarships would disappear, but the Minister intends to retain the scheme for mature State scholars, and his powers to operate the State studentship scheme of awards for postgraduate students in arts subjects are also continued As regards school leaving, under the Bill a child who reaches fifteen in the months September–January must stay at school until the end of the Easter term, if he reaches fifteen after the end of January, he must stay to the end of the summer term The Bill also applies to Scotland the automatic awards, uniformity of treatment, revised income scale and other improvements, but the Scottish Education Department will itself make the awards to students in universities and similar institutions of higher education

Scientists and Engineers in the U.S. Federal Government

A REPORT, *Scientists and Engineers in the Federal Government, October* 1958 (NSF 61–43. Pp. vi+44 (Washington, D C Government Printing Office, 1961) 35 cents), issued by the National Science Foundation, also includes some data on trends in Federal employment of scientists and engineers since the previous report (covering the year 1954) In 1958 the Federal Government employed about 8 per cent of all full-time 'white-collar' workers, an increase of 20 per cent on 1954 Of these 121,000, 55,000 were engineers, 21,000 were biological scientists and 18,000 physical scientists The Department of Defense was the largest employer, and some 75 per cent of the total were employed outside the District of Columbia About 42,000 were engaged in research and development, more than half being in the Department of Defense and 20 per cent in the physical sciences About 100,000 technicians were employed by the Federal Government, 35,000, including 21,000 engaged in nursing, being employed in the health fields, 12,000 were employed in biological and less than 5,000 in the physical sciences, and of the total, less than 15,000 were engaged in research and development, about half of whom were employed by the Department of Defense, mostly (86 per cent) as engineering or mechanics technicians

National Health Service

OVERSEAS interest in the British National Health Service has led the Pharmaceutical Society of Great Britain to prepare an informative booklet which aims to identify the historical roots from which the present Service has grown, to explain a little of the complex financial basis, to describe the general structure, and, in more detail, the pharmaceutical services (*The British National Health Service. a Short Survey with particular reference to the Pharmaceutical Services* Pp. ii+22 London The Pharmaceutical Press, 1961 5*s*) Mention is also made of the main findings of some of the committees that have deliberated from time to time on the various problems affecting the Service Although the booklet aims especially at supplying information to pharmacists and others overseas, it may also provide a suitable introduction to the intricacies of the profession for newly qualified pharmacists or pharmacy students in Britain. It should also serve as a refresher course for those pharmacists who, although familiar with the day-to-day forms and procedures of the Service as it affects them, are sometimes less sure about the general structure of which they form so integral a part

The United Kingdom and the Common Market

UNDER the title *The Common Market and the United Kingdom* the Westminster Bank, Ltd, has issued an admirable concise guide to the European Economic Community and the possible effects on Britain of membership (Pp 30 London Westminster Bank, Ltd., 1961) After a brief introduction which notes the complications arising out of Britain's position in the Commonwealth, her obligations to the European Free Trade Area and the special problem presented by agriculture, the pamphlet describes the European Economic Community, its organization and progress and then seeks to assess the effects on the United Kingdom of entry into the Common Market, including the effects on industry, on agriculture and on the consumer

The Polish Academy of Sciences

IN the *Review of the Polish Academy of Sciences* for January–March 1961 (No 1, Vol VI), W Nowacki assesses the research plans of the Academy's agencies for 1961–65, and S Zolkiewski the present state of the social sciences and the humanities in Poland. Besides B Swietochowski's account of the scientific work of the Wroclaw Agricultural College, which includes a full bibliography, the *Review* includes notes on the several research centres for thermoenergetics, forestry research, pharmacological research and general theory of organization and on the Institute of Biochemistry and Biophysics and the Basic Raw Material Research Centre of the Institute of Physical Chemistry

Nature Conservation in Western Malaysia

A SPECIAL issue of the *Malayan Nature Journal* has been prepared to mark the twenty-first anniversary of the Malayan Nature Society ("Nature Conservation in Western Malaysia", 1961 Edited by J Wyatt-Smith and P B Wycherley. Pp viii+261+44 plates Kuala Lumpur The Malayan Nature Society, 1961 Soft back, 12*s* 6*d*; hard back, 17*s* 6*d*) It contains forty-five articles by forty-one contributors dealing with such topics as the Malayan Nature Society, 1940–61, conservation and education, the Malayan Forest Department and conservation, river control and conservation in Malaya, conservation problems of Malaya's hill stations, conservation in Penang; nature protection in North Borneo, ferns in Singapore, limestone hills near Ipoh, Batu caves, Kuala Lumpur, cave archæology in Malaya, the distribution of elephant, rhinoceros, seledang and tapir in the King George V National Park, Malaya, the Shwe U Daung, a rhinoceros sanctuary in Burma, the Udjung-Kuolon Nature Park, Java, Mount Kinabalu, North Borneo, and the Bako National Park, Sarawak There are also a number of articles dealing with wild-life conservation in other lands E J H Corner, now reader in taxonomy at the University of Cambridge, and formerly assistant director of gardens, Straits Settlement, Singapore, contributes a stimulating introduction.

Insects as Social Animals

THE fourth congress of the International Union for the Study of Social Insects was held in Pavia during September 9–14, at the invitation of the president of the Italian section of the Union, Prof Carlo Jucci Some two hundred people took part and 72 papers were read There were symposia on endocrinology, caste differentiation, symbiosis and gregarism, and sections devoted to bees and wasps, ants and termites Among the contributors were Prof P-P Grassé (evolution of symbiosis in termites), Prof C Jucci (comparative endocrinology of social insects), Prof M Luscher (hormonal regulation of development in termites), Prof K Gosswald (acceptance and rejection of radioactive markers by solitary and social insects), and Prof J B S Haldane (statistical analysis of some data on ant and wasp behaviour) A well-arranged exhibition was another feature of the congress This included historical documents relating to earlier workers on social insects—Grassi, Silvestri, Forel and others, scientific exhibits ranging from photographs of African ant-galls and Australian termite mounds, to anatomical preparations, and a section on control measures, which included exhibits showing the progress of termite control in Italian libraries, and the successful introduction of the wood ant, *Formica rufa*, for the biological control of pests in Italian forests The proceedings of the congress are to be published in the series *Symposia Genetica* of the L Spallanzani Zoological Institute, University of Pavia

Leucocyte Migration in Inflammation

SIR Howard Florey and L H Grant, in an article appearing in a recent issue of the *Journal of Pathology and Bacteriology* (82, No 1, July 1961), report investigations of the passage of leucocytes through the walls of blood vessels inflamed by ultra-violet light in rabbit tissues using electron microscope techniques No clear morphological evidence was obtained that the surface of the endothelium became 'sticky', although occasionally electron-dense material was seen in relation to the endothelium The leucocytes penetrate the endothelium by thrusting out pseudopodia, possibly down cell junctions, and passing through until they reach the basement membrane, collagen fibres and preendothelial cells. It would appear that they then usually move parallel to the endothelium until they gain exit to the surrounding connective tissue. The granules of the polymorphs do not seem to undergo any changes during migration. Monocytes behave in the same way as polymorphs. Vacuoles of unusual appearance are seen in the endothelium after ultra-violet stimulation but not after mechanical 'insult'.

Chalk River Atomic Energy Instrumentation Conference

The complete set of five parts of the proceedings of the Sixth Tripartite Instrumentation Conference, which was held at Chalk River, Ontario, during April 20–24, 1959, is now available (Edited by J. E. Woolston and S. J. Townsend. Part 1: *Reactor Instruments*. Pp. xi+214. 5 dollars. Part 2: *Radiation Dosimetry*. Pp. xi+145. 3 dollars. Part 3: *System and Component Reliability*. Pp. viii+19. 1 dollar. Part 4: *Instruments for Nuclear Physics*. Pp. xi+212. 5 dollars. Part 5: *Radiation Detectors*. Pp. ix+76. 2 dollars. Chalk River, Ontario: Scientific Document Distribution Office, Atomic Energy of Canada, Ltd., 1960. 15 dollars the set). The participants consisted of members of the United Kingdom Atomic Energy Authority, Atomic Energy of Canada, Ltd., and various scientific and industrial laboratories in the United States of America. Some of the articles are abbreviated, but the discussions are given in full. Part 1 (thirty-one contributions) deals with reactor instruments and includes brief reviews of reactor instrumentation practice in the United Kingdom and at Savannah River. In the first three articles in Part 2 (twenty-nine contributions), which is concerned with radiation dosimetry and health instruments, descriptions are given of the health-physics instrumentation at the Brookhaven National Laboratory and at the Hanford Atomic Products Operation, Richland, and of radiation dosimetry at Chalk River. Part 3 (five contributions) on system and component reliability includes a summary of the reliability performance of equipment at Harwell during 1953–56, and an analysis of the component failure at Chalk River during 1956–59. A wide range of electronic, including transistorized, equipment is covered in Part 4 (thirty-three contributions), which deals with instruments for nuclear physics, and in Part 5 (fourteen contributions), on radiation detectors, various forms of ionization chambers, fission chambers, scintillation counters and semi-conductor radiation detectors are described.

Artificial Earth Satellites

The memoir, "Artificial Earth Satellites", recently issued by the Radio-Electronics Section of the British Astronomical Association (39, No. 2, August 1961. Pp. viii+160. Hounslow West, Middx: British Astronomical Association, 1961. 20*s*.; 3 dollars), is divided into two sections. The first section (contributed by J. Heywood) is devoted, first, to an introductory survey of Earth satellites, and secondly, to a historical review of the radio measurements made by members of the British Astronomical Association and Radio Society of Great Britain on *Sputniks* 1 and 2. The second section (contributed by various authors) is concerned with the techniques of visual and photographic satellite observation, the use of charts for the prediction of satellite positions and the graphical derivation of apparent tracks for satellites. The memoir, compiled and edited by J. Heywood, should be of interest to all amateur observers of artificial Earth satellites.

University News: London

The title of professor of theoretical physics has been conferred on Dr. P. T. Matthews, in respect of his post at the Imperial College of Science and Technology. The following have been appointed to readerships: Dr. K. A. Kermack, vertebrate palæontology at University College; C. H. Lack, bacteriology at the Institute of Orthopædics; Dr. J. E. Prentice, geology at King's College; Dr. M. Schacter, physiology at University College; Dr. Gordon Smith, virology at the London School of Hygiene and Tropical Medicine; Dr. Maynard Smith, zoology at University College; Dr. G. L. Watson, mathematics at University College.

The Research Report 1958–61 of the City and Guilds College gives a very readable account of the work carried out in the five engineering departments of the Imperial College of Science and Technology—Aeronautics, Chemical Engineering and Chemical Technology, Civil Engineering, Electrical Engineering and Mechanical Engineering (Pp. v+96. London: Imperial College of Science and Technology, City and Guilds College, 1961). While the account makes clear the importance of the contribution of the College to present-day technological advances, it is left for the reader who is interested in particular researches to approach the relevant Department direct for further information. No names of those responsible for the various researches are given, nor is a list of published papers included.

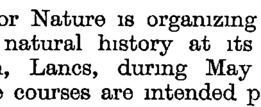
Announcements

The Imperial Forestry Institute at Oxford has been re-named the Commonwealth Forestry Institute.

The next symposium of the Society for the Study of Development and Growth will be held during June 24–27, 1962, at "Asilomar", Pacific Grove, Monterey Peninsula, California. The subject of the symposium will be "Cytodifferentiation and Macromolecular Synthesis".

The Council for Nature is organizing two seven-day courses on natural history at its Brantwood Centre, Coniston, Lancs, during May 12–19 and May 19–26. The courses are intended primarily for amateur naturalists. Further information can be obtained from Stanley Jeeves, Council for Nature, Brantwood, Coniston, Lancs.

Errata. The structural formula of 3-methyl-7-chloro-1,2,4-benzothiadiazine-1,1-dioxide (*SRG* 95213) was misrepresented in the letter entitled "A Non-diuretic Benzothiadiazine with Anti-hypertensive Properties" which appeared on p. 176 of the October 14 issue of *Nature*. The correct formula is:



In the communication entitled "Preparation and Determination of Sulphosalicylo-soluble Proteins using Tannin", by Prof. W. Mejbaum-Katzenellenbogen *et al.*, in *Nature* of October 21, p. 262, par. 2, last line, for "an appropriate conversion factor should be used" read "no conversion factor should be used".

THE WATER RESEARCH ASSOCIATION

NEW RESEARCH STATION

THE Water Research Association (director, Dr. R. G. Allen) invited its members on September 20 to inspect the permanent Research Station on the River Thames at Medmenham, in Buckinghamshire, into which it moved earlier this year. The Association was founded by water undertakings and is now in receipt of grant-aid under the scheme for industrial Research Associations operated by the Department of Scientific and Industrial Research. Previously it had been in temporary accommodation at Redhill, and with the move into specially designed buildings on a site where there is a plentiful supply of raw water, and provided with appropriate facilities for experimentation, it can be seen that the organization has now attained its full stature as an established Research Association.

The removal to new laboratories made it possible both to extend the scope of the research on hand and to begin programmes in new fields. The internal organization was expanded to comprise the following eight divisions: chemistry; physics; biology; hydrology; plant process; information; workshop services; administration. Thus there are now staff and facilities for work on every technical aspect of providing potable water.

Perhaps the most interesting structure in the new buildings is the Pilot Plant Laboratory, which has a total floor area of about seven thousand square feet, with a maximum roof height of thirty-six feet. This laboratory is intended primarily for the testing of equipment and techniques on the industrial scale; raw water is brought in from the River Thames and the construction and fittings are designed specifically for such work.

Specialized laboratories include those for physics, and for physical chemistry, general chemistry, coagulation chemistry, chemical instruments, microbiology and hydrology. There is a well-equipped machine shop. The library is being enlarged with the object of maintaining a unique collection of the literature relating to certain technical aspects of the water industry which are not indexed together elsewhere.

Chemistry Division

In the Chemistry Division the main work has been on the use of coagulation in water treatment. In this commonly used chemical process the fine suspended particles of clay which cause turbidity in water are made to coagulate into a floc of larger particles by the addition of a coagulant such as aluminium sulphate, and are then removed comparatively easily by settlement.

This investigation has thrown a completely new light on the mechanism of coagulation. It has been found that the range of values of *p*H in which the coagulation process is most efficient in removing turbidity corresponds to that in which the hydrolysis product is most rapidly flocculated, that concentrations of various coagulants which are equivalent in terms of aluminium concentration are not equivalent in terms of coagulant efficiency, and that there is at least a rough relationship between the relative coagulant efficiency and the amount of hydrolysis product that is precipitated immediately on reaction of the coagulant with water. This suggests that the mechanism of the clarification of turbid water is not coagulation in its true sense; the only coagulation that takes place is that of the hydrolysis product, and the clay particles which constitute the turbidity are carried down in the mass of flocculating hydroxide particles. The precise nature of the interaction between the clay and the hydroxide is not yet clearly understood, but is being investigated.

A number of coagulants have now been studied in some detail, and other coagulants and materials assisting the coagulation process are also being evaluated.

Coagulation processes are also used for the removal of colour from water. To provide the quantities of the colouring matter (complex organic acids derived from soil humus) required for research, a technique has been developed in which this material can be isolated from raw Thames water at rates of flow of up to 100 gallons per hour. It is proposed in the near future to begin isolating the organic colour from a number of raw water sources in addition to that on the site, and to study the way these materials behave in flocculating systems.

An analytical section has been established within the Chemistry Division, and is making a systematic study of methods used in the analysis of potable waters. This section will investigate new techniques for the measurement of every aspect of water quality, and this experience will be of great value if eventually a water quality network is established in Britain, similar to that which operates in the United States of America.

Physics Division

The Physics Division is concerned with all the physical aspects of water distribution. Its principal research programmes at present relate to the application of plastic materials to the construction of water mains and service pipes, to methods of instrumentation (particularly for the detection of leaks) and to mathematical analysis of flows and pressures in water distribution networks.

In an investigation on plastic pipes it is hoped to establish optimum design criteria for long-term service. Plastics react to variations of stress and temperature in a more complicated way than iron and steel, and as their long-term strengths are not precisely known, deliberately conservative ratings are now being used. Progress has already been made towards determining the long-term effect of variations of pressure in plastic water-distribution mains, employing accelerated tests, and the present programme relates to the measurement and specification of impact strengths. The impact resistance must of course be high enough to withstand reasonable shock loads in transit, when being laid, or when other services are being installed in the ground alongside.

It is estimated that leakage from water distribution systems in Britain exceeds 10 per cent of the

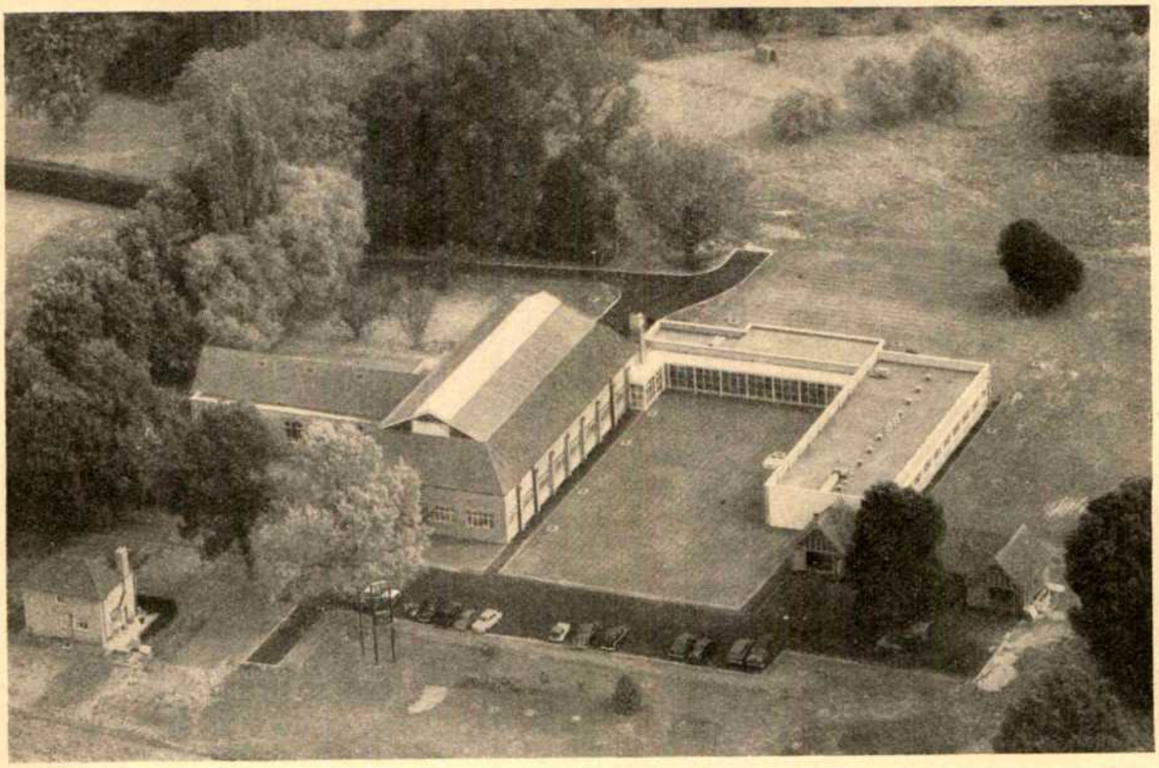


Fig. 1. The new Research Station of the Water Research Association, at Medmenham, near Marlow, Buckinghamshire. The grounds extend nearly to the River Thames, from which water is taken for experimental purposes

input, and a strong economic demand exists therefore for a device which will accurately locate leaks from buried water mains. The object of the programme in this respect is to develop a telemetering system in which an instrument carrier will be drawn along inside the pipeline and will transmit readings to an observer on the ground surface. Plunger devices (termed 'ferrets', 'go-devils' or 'pigs') which are driven through distribution mains by the fluid pressure are already in use for cleaning and other purposes, and such devices would also be suitable for carrying instruments. The first phase of the programme has therefore been the development of suitable signalling equipment, and devices have now been demonstrated which transmit a magnetic signal from a small piece of equipment inside a buried pipeline of any material including iron and steel, through the earth or other top filling, to light-weight portable receiving equipment.

While the work on the development of a complete telemetering system continues, the method of transmitting a signal from inside a buried pipeline is being put to good use in the design of a device to detect the position of a 'ferret' in service. This has now reached the prototype stage. An electro-magnetic beacon is attached to the 'ferret', so that its position can be found by moving the receiving equipment until the signal is loudest. It has been a major objection to the use of 'ferrets' that if they should stop in the pipeline it might be a very difficult matter to find and recover them, and a reliable 'ferret' detector should encourage their wider use.

As areas of water supply are extended, and rising demand brings many of the supply mains nearer their limits of capacity, it becomes increasingly important that it should be possible to calculate pressures and flows rapidly and accurately under existing and postulated conditions of supply and demand. Such calculations are altogether too time-consuming to be done by hand, and investigations have therefore been made of the use of both analogue and digital computers to produce methods of rapid and precise computation.

Plant Process Division

The Plant Process Division was set up when the Association had moved to its new establishment. The present programme is as follows: to investigate existing water-treatment processes; to develop from the findings of the Chemistry Division new processes which may be applied to full-scale waterworks plant; to investigate particular problems which may occur in the plant of members of the Association.

The equipment which is at present being designed will occupy a large part of the Pilot Plant Laboratory, and will include two treatment processes, one as a standard of reference. It will be large enough to ensure that the processes developed in it can be transferred to full-scale plant without further difficulty, while still being small enough to be flexible in operation. This plant will use raw water from the Thames at a maximum rate of about a thousand gallons per hour.

Hydrology Division

The study of hydrological problems is the responsibility of another newly established Division. The work of the Water Research Association in this field is part of an integrated national programme, and its present aims are: to encourage the improvement, both in quality and quantity, of hydrological measurements made by water undertakings; to advise the water undertakings in membership on ways of using their own hydrological data; to co-operate in the scientific study of evaporation and rainfall measurements in upland catchment areas of the British Isles

The Hydrology Division is devoting its main attention at present to surface waters The water industry is immediately interested in calculating effective reservoir capacities, and in the effects of different types of land use on water yield Several continental countries and the United States of America have made considerable progress in this respect and their results, although not directly applicable to the very different conditions in Britain, suggest a number of ways by which substantially increased yields might follow if the necessary data were determined and applied The research aspects of the work of the Hydrology Division work are designed to assist in this.

In addition, since 30 per cent of the raw water taken by water undertakings in the British Isles comes from underground sources, these must be assessed as regards their long-term yield in terms of infiltrating rainfall Natural replenishment by this means is a very slow process, but artificial replenishment by pumping from surface water sources offers storage at low capital cost This method is already in use in Sweden, Germany, The Netherlands and the United States of America, the Association's programme provides for research to assist in collecting reliable data and overcoming technical difficulties

Biology Division

The biological aspects of the water supply process are clearly of the utmost importance, not only because biological purity is required equally with chemical purity but also because biological as well as chemical processes are employed in water purification

The Biology Division has only recently been established, and is still engaged on a preliminary examination of the problems. It is expected that its first programmes will be concerned with aspects of water sterilization, with the development of faster techniques for bacteriological examination and with the control of algae in stored waters

Information Division

The objects of the information services are to see that new information from all sources is readily available to members, to provide assistance with day-to-day problems where the necessary information already exists, and to maintain communication with the water industry, other research establishments and other organizations with appropriate interests throughout the world

In addition to the material supplied to staff and members by the library, numerous technical inquiries from members are answered The staff of the Association is in a position to accumulate extensive experience of such problems, to maintain contact with specialists and to keep abreast of the published information

The problems presented are normally within the experience of the technical staff of an undertaking but outside their special province They cover a very wide range of topics, a typical selection might include complex cases of corrosion, attacks by insects (even on polythene water-supply pipe), the infestation of water supplies by various organisms, the chemical contamination of supplies, and such practical problems as the protection of reservoirs from birds

In addition to its technical papers the Association publishes a periodical, *Water Research News*

The Future

In its recent annual report, the Council of the Water Research Association comments "It is now apparent that considerable changes will be required in the nation's water systems if the rapid increases in agricultural and industrial demands are to be met everywhere The techniques of every stage of the water supply process are open to review, and a large part of the work must properly fall on the Water Research Association"

A visitor to the Water Research Association will realize that this consideration underlies all the planning at the new Research Station

MUSCLE RECEPTORS

THIS year the University of Hong Kong, as part of its golden jubilee celebrations, held six symposia during September 11–16 One of these, under the chairmanship of Prof. Ragnar Granit (Stockholm) was on muscle receptors It was organized by Prof D Barker, professor of zoology There were about thirty participants, who represented both the physiological and histological aspects of work on receptors in mammalian muscle The muscle spindle was considered from many angles and the papers, which ranged from accounts of the exacting work of electrical recording and single nerve fibre stimulation to details of the intricate structure of spindle innervation were given to full audiences and provoked lively discussions

Prof Granit opened the proceedings with a summary of much of the earlier work and a statement of some of the outstanding problems A special lecture by Sir John Eccles (Canberra), who was unable to be present, on the "Central Connections of Muscle Afferent Fibres", was read by Prof A K McIntyre (Otago, New Zealand) In the papers and demonstrations that followed, evidence was given of new advances which we may expect to see developed further in the future H Shimazu and T Hongo (Tokyo) directed attention to brain-stem influences on the motor supply to muscle spindles, while E Eldred (California) spoke of cerebral influences McIntyre considered that any discharges passing up muscle nerves and reaching the cerebrum probably came from Pacinian corpuscles and joint receptors and not from spindle primary endings Y Laporte (Toulouse) described elegant work in which both the primary and secondary afferent endings in a single spindle could be subjected to controlled stimulation of the spindle motor nerves and the responses compared C. Eyzaguirre (Salt Lake City) spoke of spindle motor innervation and described how some of the intrafusal muscle fibres were actually seen to contract slowly I A Boyd (Glasgow) gave a graphic account, with many beautiful colour slides, of the whole structure of muscle spindles in the cat, based on de-afferented and de-efferented material and much work on nerve fibre spectra He directed particular attention to the possibility of a double motor innervation D Barker (Hong Kong) and his co-workers, N K Chin, M N Adal, M C Ip and M Cope, also described much detailed work on the structure, nerve spectra and distribution of spindles in cat muscles It became obvious that there are points of great interest to both physiologists and histologists still needing to be settled about the complexity of the motor innervation of spindles

The mechanisms by which sensory endings initiate electrical discharges were considered by C C Hunt (Salt Lake City), using the simpler Pacinian corpuscle The electron micrographs shown by M C R. Merrillees (Melbourne) gave an insight into the ultrastructure of both spindle and tendon organ endings and their relation to underlying tissues A S Paintal (New Delhi) directed attention to the less well-known muscle receptors, with small nerve fibres, which probably subserve pressure and pain K S Lim (Indiana) also spoke of the responses of these endings Other papers dealt with pharmacological studies of spindles (J E Pascoe, London, and H -D Henatsch, Gottingen), spindle numbers and distribution (Eldred), differentiation of primary and secondary ending discharges (S Cooper, Oxford), the potentiation of motoneurones by spindle discharges and also human experiments on reflexes involving muscle receptors (S Homma, M Kano and K Takano, Chiba, Japan)

The ideal conditions under which the symposium was held both in the Department of Zoology and in the beautiful surroundings of Hong Kong gave many opportunities for discussions in small groups which did much to bring to light problems of structure and function that may be solved by mutual efforts of the workers in the two fields

The Hong Kong University Press plans to publish the Proceedings early in 1962 SYBIL COOPER

CHEMOTHERAPY OF VIRUS DISEASES

AT a meeting of the Fine Chemicals Group of the Society of Chemical Industry held in the Wellcome Foundation on October 6, the subject under discussion was research on the chemotherapy of virus diseases There are two ways in which this subject can be approached What might be called the rational approach requires an investigation of the essential components of virus multiplication with special emphasis on those steps which could be readily inhibited Thus, many substances are known that will inhibit the synthesis of viral nucleic acid or viral protein or that will block the supply of energy required for virus multiplication The problem is not how to inhibit virus growth but how to inhibit it selectively, without at the same time damaging the host cells The second approach, the empirical one, seems to have been the favoured approach, and one which has had more success so far

Dr A Isaacs (National Institute for Medical Research) presented the first paper, on interferon Interferon was first found during an investigation of the phenomenon of viral interference, in which it seems to play an important mediating part Further investigations suggest that it may also play a part in the immune processes that lead to recovery from virus infections

Interferon is a protein of molecular weight 63,000 which is produced by cells of a number of different vertebrates in response to infection with a large number of different viruses Its antiviral activity is usually more apparent in cells of the animal species in which it was made However, it is active against a wide range of animal viruses although there are differences among viruses in their sensitivity to interferon It does not prevent adsorption or penetration of virus but it inhibits the replication of viral ribonucleic acid. This probably results from inhibition of an oxidative process of the cell which supplies the energy for viral synthesis The inhibition seems to affect viral synthesis much more than normal cellular syntheses, however, since human thyroid cells can divide at a rate not grossly different from that of normal cells in the presence of a large amount of interferon

There is growing evidence that antibody may play a less important part in the processes of recovery from virus infections than has been thought hitherto, and that interferon may play a more important part This was shown first by experiments in chick embryos of different ages, in which recovery from virus infections is found to be closely related to development of sensitivity to interferon Secondly, persistence of virus in the lungs of mice of different ages was found to be inversely related to the production of interferon in these animals Thirdly, two ways of inhibiting the interferon mechanism *in vitro*, namely, increased oxygenation and treatment with cortisone, both have a detrimental effect on the course of virus infections *in vivo* These findings point to the conclusion that interferon may play an important part in our ability to recover from virus infections and they suggest that it might be possible to assist natural recovery from virus infections by giving interferon therapeutically This has been carried out successfully in some virus infections of animals and it is hoped that preliminary trials of its action in man will begin shortly

The second paper was given by Drs R Hull and E W Hurst (Imperial Chemical Industries. Ltd.) on structure activity in various heterocyclic compounds Material tested for possible antiviral activity was obtained from products synthesized for other biological assays, from the synthesis of analogues obtained in the course of following up an antiviral lead already found in the department or elsewhere, and from the synthesis of possible antimetabolites to viral nucleic acid Heterocyclic compounds constituted the largest section of organic compounds tested and a review was given of structure–activity relationships that had been found in the acridines 'Mepacrine' was the most active and showed antiviral activity against equine encephalomyelitis, Rift Valley fever and louping ill viruses

In evaluating experimental work it was important to take into consideration the complex of cell and virus that was studied Examples were given of compounds which reduced the growth of viruses in the chick embryo but which were not appreciably active in the mouse. On the other hand, 'Mepacrine', which was active against equine encephalomyelitis virus in the mouse and adolescent rat, was inactive against this virus in the chicken, guinea pig, rabbit and monkey.

Work on simple antimetabolites, in which the furan ring had been substituted for the ribofuranoside moiety in the nucleoside, had led eventually to certain tetrahydropyrimidines which showed activity against viruses of the psittacosis-lymphogranuloma-trachoma group both in the chick embryo and in the mouse. One, 17,025 [5-methoxycarbonyl-6-methyl-4-(5-nitrofuryl)-2-oxo-1,2,3,4-tetrahydropyrimidine] which was sparingly soluble in water, had very low toxicity and was both effective and non-irritant when given by the intramuscular route. It showed, weight for weight, rather less than one-tenth of the activity of chlorotetracycline. Its clinical use might be envisaged (for example, against trachoma) when it was desirable to control an infection by infrequent parenteral therapy rather than by prolonged topical application or by frequent oral dosing.

The third paper, by Drs. D. J. Bauer and P. W. Sadler (Wellcome Laboratories), was concerned with the antiviral action of derivatives of isatin β-thiosemicarbazone on viruses of the pox group. These compounds are known to show antituberculous activity and they were later shown by American workers to have some antiviral action against vaccinia virus. However, the latter work was not followed up until the group at the Wellcome Laboratories commenced detailed investigations of a number of derivatives of isatin β-thiosemicarbazone on a number of different members of the pox virus group. Perhaps the most striking finding has been the selectivity of action of these compounds. Thus, isatin β-thiosemicarbazone and some of its derivatives are very active against vaccinia but have no activity against ectromelia virus; conversely, isatin β-4′ 4′-dialkylthiosemicarbazone and related compounds are highly active against ectromelia but not at all active against vaccinia virus. This specificity should provide an important clue to the mode of action of these compounds. To the authors it suggested that these compounds were not acting on some cellular enzyme required for viral synthesis, but that they were acting directly on the virus itself.

A most interesting aspect of this work is the finding that *N*-ethylisatin β-thiosemicarbazone, in doses of 0·03 and 0·015 mgm. twice daily for five days, protected infant mice (less than six days old) against fatal infection by the viruses of alastrim and variola major. Dr. Bauer discussed at some length the question of the importance of the treatment of smallpox in these days of vaccination and he concluded that there was a strong case for trying out these compounds in man and that it was reasonable to expect that a specific anti-smallpox drug would be in use within the not too distant future.

ALICK ISAACS

PHOSPHOLIPIDS AND SULPHOLIPIDS

DR. M. MACFARLANE, joint chairman with Prof. D. D. Eley of the joint meeting of the Biochemical Society and the British Biophysical Society on "Phospholipids and Sulpholipids", which was held at the School of Pharmacy, University of London, during October 5–6, underlined its significance by a quotation from the Macleans (1927)[1]: "When we consider the obscurity in which the chemistry of the phospholipids has been shrouded, it is easy to understand that many of the properties and functions ascribed to these bodies are based on little more than imagination." Thirty years later imagination remains in plenty, but an immense amount of accumulated factual evidence was presented at the meeting. The chemical, physical and physico-chemical problems surrounding phospholipids challenge all scientists interested in the structure and functions of the living cell.

With the advent of recent analytical procedures involving chemical[2,3] or enzymatic hydrolysis, paper and silicic-acid chromatography[4], the compositional detail of many animal and plant tissues is progressing fast. Consequently, the phospholipid chemist of to-day recognizes phosphatidylglycerols, phosphoinositides, the classical ester phosphatides having either serine, ethanolamine or choline as the nitrogenous base, the plasmalogens, being analogous to the phosphatides, but with an aldehyde in place of a fatty acid, the sphingomyelins, the complex phosphoinositols and finally the lysophosphatides. Among many most recent compositional details reported at the meeting was the observation by Dr. G. R. Webster that the ethanolamine base in brain white matter is predominantly present in plasmalogen instead of the more usual phosphatidyl ethanolamine. Dr. E. Lederer, withdrawing earlier claims, pointed out that mycobacteria do not appear to contain any nitrogenous base phospholipids but do yield mixtures of phosphatidyl-inosito-mono, di- and tri-mannosides. Of great interest, however, was his report on the structure of the phosphatidyl-inosito-dimannoside, and of his proof that the 6-*O*-α-D-mannopyranosyl-α-D-mannopyranose is inositol linked, the dimannosides alone being almost as effective as *BCG* vaccine in the protection of animals against tuberculosis.

In plant chloroplasts the major anionic lipids are phosphatidylglycerol and the sulpholipid, 6-sulpho-α-D-quinovopyranosyl-(1 → 1′)-2′,3′ diacyl glycerol. The precise formulation of the latter compound has been established by Dr. A. A. Benson in an elegant analytical procedure. The presence of lipid lamellæ in plant chloroplasts and the high-rate of incorporation of carbon-14 dioxide, phosphorus-32 labelled phosphate and sulphur-35 labelled sulphate ions into the anionic lipids during photosynthesis points to at least two functions of phospholipids—namely, interfacial orientation with concomitant orientation of adjacent molecules, such as enzymes, and involvement in translocation of metabolic products.

With commercially available gas chromatography apparatus, each phosphatide can now be examined in terms of its fatty acid composition. This further stage in the dissection of phospholipids was reported on by Dr. G. M. Gray, who compared the mean fatty acid composition of normal tissue phospholipids with those from Landschutz ascites tumour cells. Surprisingly, and perhaps of fundamental importance, the tumour cell phospholipids showed far less variation in the kind of fatty acid acylated. For example, stearic is the predominating saturated acid in all tumour phospholipids, thus differing from normal tissues in which palmitic acid is the major saturated acid of phosphatidyl choline and choline plasmalogen

Prof L L M van Deenan and his colleagues at Utrecht rightly argued that a better understanding of the physical properties to be attributed to the fatty acids and to their particular position in the phosphatide molecule would clarify the biophysical properties of the phospholipid Reporting a remarkable programme of syntheses involving re-acylation of L-α glyceryl phosphoryl choline, with fatty acids of varying chain-length, Prof van Deenan was able to contribute experimental results obtained from his semi-synthetic material and relate it to many of the chemical and biophysical aspects covered during the meeting Perhaps the most significant advance so far resulting from the use of these re-acylated phosphatides has been to establish beyond doubt that the phospholipases *A* of snake or bee venoms only release the fatty acid from the β position

Combining biochemical and biophysical techniques, Dr A D Bangham and Dr R M C Dawson presented substantial evidence to suggest that the activation of at least two phospholipases is controlled by the electrokinetic properties of the lipid/water interface The ζ potential of lecithin suspensions was varied either by adding insoluble amphipathic molecules, for example, docosanyl pyridinium bromide or dicetyl phosphoric acid to the lecithin or by the addition of multivalent ions, for example, calcium ions or ferricyanide ions, to the bulk aqueous phase Such experiments showed that *Penicillium notatum* phospholipase requires micelles having a net negative (> 15 mV) ζ potential before hydrolysis of the lecithin occurred Calcium, uranyl and thorium ions, if present, as multivalent gegen ions, inhibited the system The phospholipase *C* (α toxin) of *Clostridium perfringens*, however, required a positively charged lipid interface and required either the addition of amphipathic cations to the lecithin, or divalent calcium or magnesium ions to the bulk aqueous phase That these interactions are confined to the lipid-water interface was demonstrated by following the hydrolysis of unimolecular films using lecithin highly labelled with phosphorus-32 The ability of phospholipase *C* (α toxin) to hydrolyse phosphatidyl ethanolamine, but only in the presence of lecithin, confirms earlier work and fits in well with the hypothesis that lecithin, being a surface-active zwitterion, can dilute the high net negative charge of a pure phosphatidyl ethanolamine micelle with ionically neutral molecules, thereby permitting the negatively charged enzyme to approach the micelle The adjustment of the net charge of lipids by admixture of lecithin may well be an important feature in a variety of biological systems, for example, blood coagulation, fat absorption Endorsing the importance of a definitive lipid/water interface for the activation of phospholipase *C*, van Deenan reported that synthetic phosphatidyl cholines prepared with C_2 or C_4 fatty acids were not hydrolysed and could not be shown to have any lamella structure in electron microscopic preparations

The present-day biophysical interest in phospholipids naturally centres around the structural arrangement of such molecules in various solvents—and in particular in water The accepted interpretation of the results obtained by X-ray diffraction, light scattering and with polarized light, is that most phospholipid mixtures consist of concentric layers of bimolecular leaflets intercalated with water The smallest repeating unit of mixed phospholipids in the presence of protein has recently been examined and the precise significance of the electron opaque lines of methacrylate embedded, osmium-fixed material discussed by Stoekenius, Schulman and Prince[5] Their evidence, together with that of Finean[6], strongly suggests that the smallest unit membrane recognizable in model lipid membranes is a bimolecular leaflet covered on both sides with protein—a structure postulated by Danielli nearly thirty years ago and recently presented by Robertson[7] as a structural entity in some biological cell components

Of particular interest to the biologist is the intermolecular arrangement of the phospholipids and sterols in the plane of any unit membrane or film Dr D G Dervichian and de Bernard[8] have already shown that in monolayer studies, lecithin forms molecular condensations with cholesterol, and Willmer[9] has recently indicated how such mixtures might give rise to a variety of mosaic patterns, conceivably controlling the permeability of membranes or in facilitating the absorption of specific proteins (for example, gonadotrophin)

The precise orientation of the ionic groups of the phospholipid is at present a matter of some controversy, but the concensus of opinion is that with pure phospholipids the charged groups lie in a nearly level plane at the water interface Finean[10], however, has presented evidence that cholesterol might realign the base of a phospholipid away from the water, in so doing, local areas of phospholipid surfaces might present net negative ζ potentials

As it is now possible to prepare large quantities of chemically pure, although inhomogeneous in fatty acid, lecithin, this substance has been studied extensively in terms of its conductivity, viscosity and micelle formation Drs P H Elworthy and D S McIntosh, examining the light scattering of lecithin, found that the micellar weights increased as the polarity of the solvent decreased They also concluded that in their series of solvents—ethanol, *n*-butanol and *n*-hexanol—the polar groups were probably hidden, while the hydrocarbon chains protruded outwards into the solvent This result is of great interest when it is remembered that lecithin exists as very large micelles in water and with the polar groups outwards.

The behaviour of fairly concentrated sols of lecithins in water with salt solutions has formed the basis of a study by Dr L Saunders and was reported to the meeting He finds that a film of some measurable mechanical strength forms at the sol/salt interface and that this film has some of the permeability characteristics of cell membranes Presumably the intermicellar channels restrict the diffusion of divalent and, to a lesser extent, monovalent ions

Finally, it must be remembered that phospholipids and sulpholipids are integral parts of living cells and as such are in dynamic equilibrium with metabolic and structural processes Isotope incorporation studies have repeatedly shown that certain phosphatides can exhibit high rates of turnover in cells undertaking active secretive or ingestive functions[11] L E Hokin and M R Hokin, for example, reported how the salt gland of certain marine birds rapidly incorporated phosphorus-32 when the gland was stimulated with acetyl choline—the normal physiological stimulus Their evidence is suggestive that in this instance sodium transport is coupled to the renewal of phosphate in phosphatidic acid, a process which appears to be catalysed by the cyclic action of diglyceride kinase and phosphatidic acid phosphatase. The questions raised during the course of this meeting suggest that it would be foolish to expect a complete understanding of the function of phospholipids even within the next thirty years A. D BANGHAM

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BUILDING RESEARCH

THE annual report for 1960 of the Building Research Board of the Department of Scientific and Industrial Research (Pp 99 H M S.O, London. 7*s* 0*d*) is written and illustrated with the clarity which has characterized previous reports, and provides a convenient summary of the very diverse activities of the Building Research Station

Before a new building material can be used with confidence, information is needed on many aspects of its behaviour over long periods of time, and the devising of suitable accelerated tests is a major problem in this field of research The present report describes such a test for the weathering of polyester roofing sheets reinforced with glass fibre Work is in progress on the behaviour of rain-water goods made from various plastics; the chief problem here appears to be lack of colour stability

Another group of materials, lightweight aggregates for structural concrete, poses many problems including mix design, bond strength, moisture movement, and corrosion of reinforcement

Work in this field, begun in 1957, is still in progress, and aims to provide the basis for a future code of practice

Like most similar organizations, the Building Research Station is often asked to solve a problem within a few months Their experience has shown that "superficial short-term examination of problems is apt not to be rewarding". Unhappily, the converse is not always true, and one feels sympathy for the workers on stone preservatives who report, after five years investigation, that "no 'stone preservative' treatment has yet served its purpose well enough to merit commendation Some have done more harm than good . . nothing of proven value has emerged . . ".

Another important group of investigations is concerned with the economics of building construction and maintenance A survey of 1,000 post-war houses in Scotland has shown that certain non-traditional forms of construction lead to abnormally high maintenance costs, especially in conditions of severe exposure Prefabricated systems have a tendency to leave small quantities of work to be completed by traditional methods, at disproportionately high cost

The structural designer will be interested in the development of realistic design methods based on more comprehensive data than those now available New surveys of actual floor loads and snow loads on buildings are now in hand, and the problems of wind loading on tall buildings are also receiving attention

An important study of composite action in buildings is in progress Attention to the interaction of beams and slabs, and of walls and structural frames can make more difference to frame design than can the most elaborate frame analysis

The Building Research Station pioneered soil mechanics research in Great Britain, and is continuing the investigation of pile foundations. It would appear from results quoted in this report that the ability of deep cylinder foundations to carry load by both shaft friction and base reaction is still in some doubt

This important conclusion appears to be based on a limited series of tests on a laboratory model, and it would be a great help to readers if statements of this nature were accompanied by references to the list of publications at the end of the report At present it is not at all easy to relate the conclusions reported to the publications giving details of the original work.

The contents of the present annual report cover a very wide field, and this arbitrary selection of topics can do little more than indicate the diversity of matters treated No mention has been made, for example, of the interesting work on the equatorial comfort index for tropical buildings, or the effect of various construction methods on the earth pressures on buried service pipes In both cases an attempt is being made to replace the present trial-and-error approach by a logical theory developed from observed facts

This report should be of interest to all who design buildings or organize their construction.

R PAUL JOHNSON

INDUSTRIAL RESEARCH AND DEVELOPMENT IN THE UNITED STATES

TO obtain information about the economic resources allocated to research and development activities, the National Science Foundation of America has been conducting comprehensive surveys of research and development in the economy on a periodic basis Such surveys yield economic data that assist the National Science Foundation, the Federal Government, and other public and private organizations to assess the present research and development efforts and to formulate science programmes for the future

A report* summarizes the results of the 1958 survey of industrial research and development, the survey representing a continuation of the Foundation's series of annual surveys of research and development in industry that began in 1953.

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* National Science Foundation, Washington Surveys of Science Resources Series, NSF 61-32 Funds for Research and Development in Industry, 1958 Pp xvi+119 (Washington, D.C Government Printing Office, 1961) 65 cents.

Funds for research and development performance in the industrial sector of the economy totalled 8·2 billion dollars in 1958. More than half this total was for research and development performed in the aircraft and parts, electrical equipment and communication industries, which were heavily engaged in Federally financed research and development.

The amount spent on industrial research and development in 1958 was 7 per cent higher than the 7·7 billion dollars total for 1957. Increases occurred between 1957 and 1958 in all industries for which separate data were obtained except aircraft and parts and rubber products. The relative increase in research and development of companies with less than 5,000 employees (15 per cent) exceeded that of companies with 5,000 or more employees (6 per cent) between 1957 and 1958.

More than half (56 per cent) the industrial research and development performance was financed by the Federal Government. Industries with the highest proportions of federally financed research and development performance were aircraft and parts (85 per cent) and electrical equipment and communications (68 per cent).

In contrast to the large Federal research and development financing in certain industries, 90 per cent or more of the total funds for research and development performance were from company funds in the following industries: food and kindred products, paper and allied products, drugs and medicines, other chemicals, stone, clay and glass products, primary ferrous products, and petroleum refining and extraction. The chemical and allied products industry exceeded all others in the dollar volume of company financed research and development.

Companies with 5,000 or more employees accounted for 84 per cent of the funds for performance of industrial research and development, compared with 7 per cent in companies with less than 1,000 employees and 9 per cent in companies with 1,000–4,999 workers.

An estimated number of 11,800 industrial firms carried out research and development in 1958, including 10,300 manufacturing companies and 1,500 non-manufacturing companies. In terms of research and development moneys, however, manufacturing companies accounted for 98 per cent of the total. Funds for research and development in 1958 were distributed by the character of work as follows: basic research, 4 per cent, applied research, 23 per cent and development, 73 per cent. Approximately three-fifths of the 295 million dollars for basic research was expended in the physical and mathematical sciences, about one-fifth in the engineering sciences, and the remainder in the life and other sciences.

The product fields of guided missiles, communication equipment, and aircraft and parts accounted for more than half the 7·9 billion dollars utilized for applied research and development. Although the companies in the aircraft and parts, and electrical equipment and communication industries accounted for most of this, companies in other industries also performed substantial amounts of applied research and development in these product fields.

The dollar volume of research and development performance per research and development scientist and engineer in 1958 was 32,900 dollars for all industries combined. Among major industry groups the average ranged from 16,400 dollars in food and kindred products to 52,500 dollars in motor vehicles and other transportation equipment.

Funds for industrial research and development comprised 3·8 per cent of net sales in manufacturing companies performing research and development. Total performance includes federally financed research and development, which amounted to 56 per cent of the total amount for 1958. Research and development performance funds as percentages of net sales were highest in the following separately reported industries: aircraft and parts (17·7 per cent), electrical equipment and communication (10·5 per cent), and scientific and mechanical measuring instruments (9·9 per cent).

STRUCTURE OF ALPHA-KERATIN

By PROF. S. KRIMM

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THE structure of α-keratin[1] has been assumed to be based on the α-helix[2] following the demonstration[3,4] that the coiling of such helices, possibly arising from periodic axial distortions[5,6], can account for the presence of the 5·15 Å. and 1·5 Å. meridian reflexions in the X-ray diffraction pattern. In particular, a three-stranded rope[6] seemed to account satisfactorily for the main features of the diffraction pattern[3,4]. Recent work[7], however, indicates that this structure may not be entirely satisfactory in explaining the details of the pattern, and other models involving additional layers of helices have been suggested[8]. The present ideas do not satisfactorily account for the changes in the X-ray pattern resulting from treatment with heavy metals[9,10], nor for some of the longitudinal regularities found in the matrix component of α-keratin by electron microscopy[10].

It is the purpose of this article to explore a possibility which has not been considered before and which seems to have merit in accounting for much of the data. Specifically, it is proposed that in the two-phase structure of α-keratin consisting of microfibrils and matrix, the latter, far from being amorphous or disorganized, consists of a relatively highly organized structure. In particular, whereas the microfibrils, as is known, compose the α-helical component, it is suggested that the matrix is built up of a structure similar to that of feather keratin, and that this matrix structure accounts for the 198 Å. fibre period[11,12] and the various orders of it which are observed and which are intensified by heavy-metal treatment.

In considering the evidence which suggests this proposal, it may first be noted that the characteristic α-keratin spacings at 5·15 Å. and 1·5 Å. may not, and indeed need not, be correlated with the 198 Å. repeat. Although accurate diffractometer measurements[13,14] give the former spacing as 5·18 Å., which would be a reasonable 38th order of 197 Å., it is not required that this be true in order to account for this spacing Secondly, the many orders of 198 Å which can be observed[11] bear a striking resemblance to the orders of the 189 Å repeat observed in the diffraction pattern of feather keratin[15], a fibrous protein which contains no α-helix component Thirdly, the uptake of metals occurs primarily in the matrix, which is rich in cystine compared to the microfibrils[16] Since this treatment affects primarily the low orders of the 198 Å meridional repeat[10] (the effect on the low-angle equatorial spots is also accounted for by penetration of the matrix by the reagent[9]), it is not unreasonable to suppose that the component giving rise to this long repeat is distinct from the α-helical component located in the microfibrils

The correlation of the matrix component of α-keratin with a feather keratin type of structure is suggested by the results of recent work on the structure of the latter fibrous protein[15,17] These investigations indicate that a structure which is in reasonably good agreement with the data can be developed from the hypothesis that proline residues are distributed periodically along an essentially extended polypeptide chain Amino-acid analyses, and the presence of a strong 8th order of the 189 Å fibre axis repeat, are consistent with the proposal that in feather every 8th amino-acid along the chain is proline[17]. The 198 Å fibre axis repeat of α-keratin (which although slightly larger than that of feather keratin is nevertheless consistent with similar values found for other proteins giving the feather keratin pattern, cf 197 5 Å for *Varanus* claw[18]) also is characterized by a strong 8th order reflexion[12] Chemical analyses[19–21] have indicated that the matrix component of high cystine content is that which is high in proline The proline content found[21] is of the same order as that found in feather[22], and at the present state of the analysis it can be said that they could indeed be equal The chemical composition therefore is entirely consistent with the presence in the matrix of the proposed type of extended chain structure It should be noted that infra-red spectra of α-keratins indicate the presence of a β-component oriented with respect to the fibre axis[23,24]

If we accept this possibility, then we find further that the proposed feather keratin structure[17] can account for several other of the dimensional features of α-keratin Thus, the predominantly observed fibre axis repeat in feather is expected to be half of 189 Å (ref 17), and this fits in well with the approximate 95 Å longitudinal periodicity seen in electron micrographs of the matrix of α-keratin[10]. Again, the diameter of the predicted fibrillar unit for feather keratin is about 22 Å (ref 17) Electron micrographs of wool[16] show that in the paracortex there are microfibrils of diameter about 60 Å the centre to centre separation of which is about 83 Å. These dimensions are consistent with each microfibril being separated from its neighbour (in an essentially hexagonal array) by one feather keratin unit Twelve feather keratin units will just pack around one microfibril Finally, the observation[25] that hydration of dry α-keratin results in preferential swelling of the matrix in comparison with the microfibrils may be understandable in terms of the open type of structure suggested for feather keratin[17].

The above proposal of two structural components in α-keratin can also help to explain the results of heavy-metal treatments Recent investigations of this kind[26] suggest that cystine is periodically located along the polypeptide chain of feather keratin (with a periodicity of about 47·3 Å) The same may be true for α-keratin Amino-acid analyses[21] show that the matrix of α-keratin has about twice as much cystine as does feather. If these residues were periodically located along the chain, we would expect heavy-metal treatment to enhance in all cases the 8th order of the 198-Å fibre axis repeat This is precisely what is found[9,10] This evidence itself suggests that the matrix component of α-keratin may be a relatively highly organized structure

There is another important characteristic of α-keratin which could be accounted for by the proposed model Recent work on the α → β transformation in keratin[27–29] has indicated the complex nature of this process and the likelihood that a simple transformation of a single chain from α-helix to β-extended chain is not involved Suggestions have been made that the transformation may proceed through an intermediate phase[27,28], or that there may be two crystalline phases[29,30] The foregoing model would fit in with the latter scheme, extension of the microfibrils leading to loss of the α pattern whereas further extension of the (already nearly extended) matrix component would result in the development of the β pattern The detailed mechanics of this process (which may, for example, involve the relative sliding of the two components with respect to each other) perhaps cannot be specified at this point, but the general mechanism does not seem to be eliminated by the available data

In summary, it is suggested that the matrix component of the two-phase structure of α-keratin consists of a relatively ordered polypeptide chain structure basically similar to that of feather keratin, and with which is associated the long axial periodicities in the X-ray diffraction pattern

Support for this investigation is gratefully acknowledged from U S Public Health Service grant *A*–2830

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CRYSTAL STRUCTURES OF SALTS OF METHYLATED PURINES AND PYRIMIDINES

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NUMEROUS patterns of hydrogen bonding have been postulated between purines and pyrimidines in the various models which have been proposed for the structures of nucleic acids and their synthetic analogues. It is a disadvantage of the method of fibre crystallography, which is most directly applicable to the study of such structures, that it does not allow a refinement of the structural parameters based solely on the diffraction data, and hence it is important that structures proposed to explain fibre diffraction diagrams should be both chemically and stereochemically acceptable as well as in reasonable agreement with the diffraction evidence[1]. An examination, by X-ray diffraction techniques, of single crystals of salts of methylated purines and pyrimidines (the simplest analogues of nucleotides) might be expected to yield three valuable pieces of information which would be of use in the formulation of structures for nucleic acids, first, a knowledge of the molecular geometry of the various bases; secondly, an identification of the sites of protonation of the bases at acid *p*H; thirdly, a possible model for the geometry of inter-base hydrogen bonding in polynucleotides. With this in mind, we are conducting a systematic survey of acid salts of such methylated purines and pyrimidines and wish to report some of the results of work on two of these, 9-methyladenine dihydrobromide and 1-methylcytosine hydrobromide.

Crystals of 9-methyladenine 2-hydrogen bromide are orthorhombic with $a = 17.59 \pm 0.05$ Å, $b = 4.88 \pm 0.02$ Å., and $c = 11.75 \pm 0.04$ Å. The space group is $Pna2_1$ with four molecules of the dihydrobromide in the unit cell. The crystal structure was determined, using the heavy atom method, from examination of the (010) and (001) projections and refined, using Fourier and difference Fourier syntheses. In addition, the $(h0l)$ data were further refined by least squares methods. Fig. 1 shows the electron density distribution $\rho(x,z)$. Each molecule of methyladenine is enclosed by a three-dimensional network of Br$^-$ ions, the Br$^-$ . . . Br$^-$ contacts ranging in length from 4.25 to 5.17 Å.

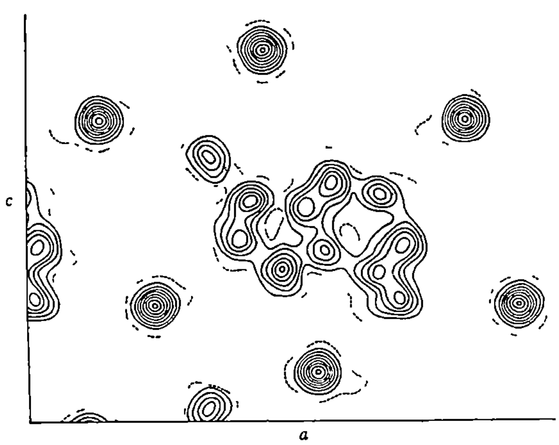

Fig. 1. Electron density $\rho(x,z)$ for 9-methyladenine 2-hydrogen bromide. Contours at equal but arbitrary intervals, intervals for Br$^-$ × 5

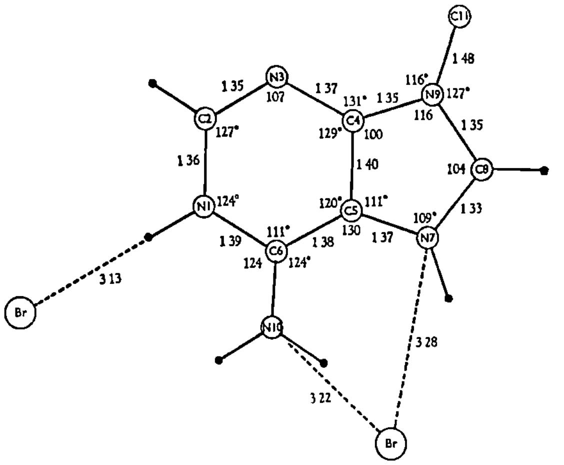

Fig. 2. Interatomic distances and angles for 9-methyladenine 2-hydrogen bromide

The conventional residuals, R, excluding unobserved reflexions, are 0.07 for $(h0l)$ and 0.11 for $(hk0)$. Despite these low values the overall accuracy of the structure determination is only fair, the standard deviation in the position ascribed to carbon or nitrogen atoms being about 0.05 Å., and the good agreement between observed and calculated structure factors may be attributed mainly to the very accurate location of the two bromine ions. The details of the molecular geometry are shown in Fig. 2. Although the dimensions found for the cation of 9-methyladenine are reasonable, no useful comparison can be made between them and the dimensions found by Cochran[2] for the adenine molecule in adenine hydrochloride, nor with those proposed by Pauling[3] or by Spencer[4] for the adenine molecule as it is assumed to exist in nucleic acids. This illustrates the disadvantage of using compounds containing such heavy atoms where particularly accurate molecular dimensions are sought. From our point of view, however, the most interesting feature of the structure is the hydrogen bonding pattern. There are no hydrogen bonds joining the purine molecules directly, each molecule forming instead three hydrogen bonds to neighbouring Br$^-$ ions. This is most probably because access to the only remaining acceptor atom on the adenine molecule, N(3), is hindered by the 9-methyl group. It is likely, indeed, that because of a similar effect exercised by the sugar molecules, no structures involving N(3) as an acceptor atom in hydrogen bond formation can occur in multi-stranded polynucleotide structures. The hydrogen bonds which are formed are N(1)—H ..Br(1), 3 13 Å ; N(10)—H. Br(2), 3 21 Å , and N(7)—H Br(2), 3 28 Å All these bonds are shorter than the more usual length found, 3 4 Å., at least two of them by significant amounts, and we postulate a strong electrostatic contribution to the bond energies All other intermolecular contacts are of normal lengths

The fact that the protonated nitrogen atoms are N(1) and N(7) provides additional evidence for the correctness of the structure proposed by Rich, Davies, Crick and Watson[1] for polyadenylic acid. Fibres of this substance show poor orientation at neutral *p*H, but a high degree of orientation when the *p*H is lowered somewhat At still lower *p*H, however, the orientation again disappears, though the molecular weight remains unchanged. Following Cochran[2], Rich *et al.* assume protonation of the adenine at N(1) with a consequent internal neutralization of the overall charge of the molecule The two strands of the helical molecule are assumed to be held together by pairs of hydrogen bonds between the 6-amino group and N(7) The disappearance of orientation at the very low *p*H can thus readily be explained as due to the disruption of this hydrogen bonding consequent on protonation of N(7)

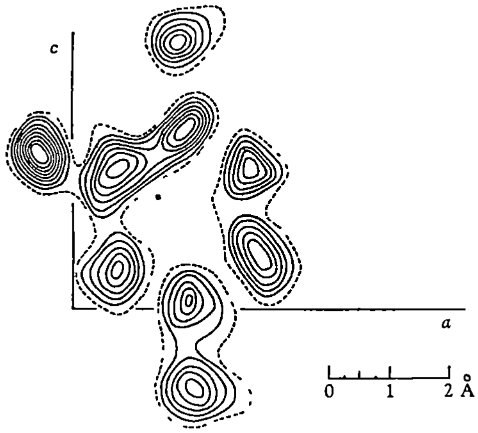

Fig 3 Electron density $\rho(x,z)$ for 1-methylcytosine hydrogen bromide The contribution of the Br$^-$ ion has been subtracted from the structure factors, its position is indicated by the dot

1-Methylcytosine hydrogen bromide also occurs as orthorhombic crystals with a = 12·98 ± 0 03 Å., b = 6 80 ± 0·02 Å , and c = 8 83 ± 0 02 Å. The space group is *Pnma* with four molecules in the unit cell which. in consequence, occupy the mirror planes of symmetry at $y = \frac{1}{4}$ and $\frac{3}{4}$ The structure was solved from the (010) projection by making use of the heavy-atom method The molecular arrangement is somewhat unusual and this caused us some initial difficulty in deciding on a correct trial structure. In projection, a Br$^-$ ion at $y = \frac{1}{4}$ is situated roughly in the centre of a cytosine ring at $y = \frac{3}{4}$. Fig. 3 shows a synthesis of the type $(F_0 - F_{\mathrm{Br}})$ giving the electron density $\rho(x,z)$ with the contribution of the Br$^-$ ion removed. This sandwiching of the bromine ions between the heterocyclic rings leads to a very efficient type of interlayer packing A similar arrangement is found in the case of 9-methylguanine dihydrobromide (work still in progress)

The structure was refined by means of difference Fourier syntheses to a value for the index R of 0 12 for ($h0l$) Structure factors were also calculated, as a check, for ($h1l$) and ($h2l$), giving the same value of

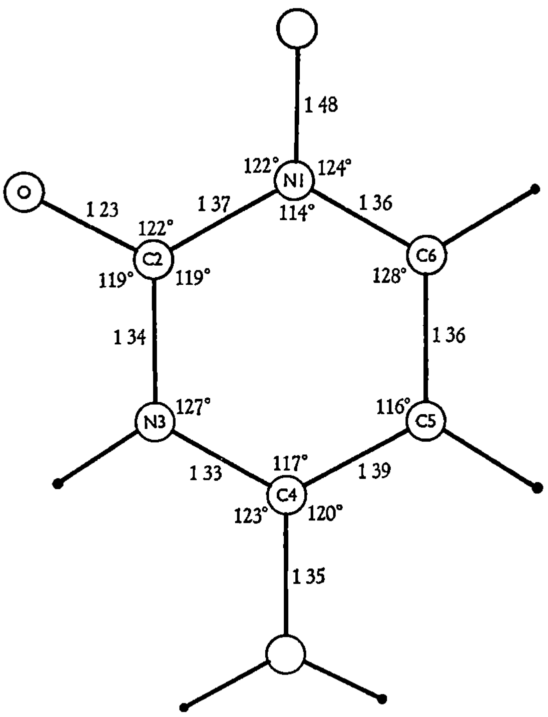

Fig 4 Bond-lengths and angles in the cation of 1-methylcytosine

R as for ($h0l$) in each case. Fig 4 shows the molecular dimensions found for the cation of 1-methylcytosine. The bond-lengths found (σC—C = 0 03 Å) are in good agreement with those suggested by Pauling[3] and by Spencer[4] for the cytosine molecule as it is assumed to occur in nucleic acids The sum of the individual differences in bond-lengths between our dimensions and those of Pauling is 0 10 Å., and for the Spencer model this sum is 0·08 Å. However, some of the bond-angles which we find are in marked disagreement with those suggested by either Pauling or Spencer The average difference is 6°, the maximum difference is 10°. We estimate[5] our standard deviation in bond-angle to be about 2°, and so these differences would seem to be significant in some cases.

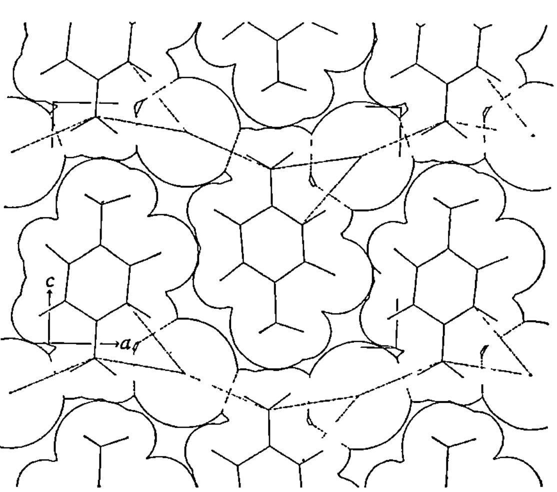

Fig 5 Packing drawing of the arrangement of molecules within a layer of the structure of 1-methylcytosine hydrogen bromide Conventional van der Waals radii are inscribed, hydrogen bonds are indicated by the lines

However, owing to the overlap, in projection, between the Br^- ion and the cytosine ring it is possible that the errors in the positions of some of the atoms in our structure are in excess of our estimate which is based on the assumption of non-overlapping atoms. We are therefore not prepared to offer our values for these angles as being intrinsically better than those in either of the two models with which we compare them. A subsequent three-dimensional refinement may enable us to improve the accuracy of this determination.

The cytosine ring is protonated at N(3) and there are again no hydrogen bonds between individual base molecules. Instead, each ring forms three hydrogen bonds of length 3·36, 3·39 and 3·40 Å to adjacent Br^- ions as illustrated in Fig. 5, which also shows the molecular packing within a given layer. Since the molecules are planar it is possible to inscribe the conventional van der Waals radii of the atoms, and this has been done in Fig. 5. It is seen that the packing is extremely efficient with the molecules forming an interlocking double dovetail arrangement, the backbone of the individual dovetail units being the hydrogen bonds. Particularly noteworthy is the manner in which the Br^- ion lies between N(3) and the 4-amino group and thus takes part in two hydrogen bonds. The deviation from linearity in each of these bonds is in excess of 25° and illustrates the large distortions which can be tolerated in such bonds where there is a compensating electrostatic interaction.

This work was supported by grants from the National Cancer Institute of the U.S. Public Health Service and the American Cancer Society. One of us (R. F. B.) was in receipt of a U.S. Public Health Service postdoctoral fellowship.

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INTERACTION OF LIGHT AND ATMOSPHERIC PHOTOCHEMICAL PRODUCTS ('SMOG') WITHIN PLANTS

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DAMAGE to plants from ozone and peroxyacetyl nitrate, two photochemically formed components of 'smog', has been described[1-3]. However, variations in symptom expression and the degree of damage caused by a given concentration of these components, whether of synthetic or natural origin, have complicated development of an adequate biological assay method for these materials. These observed variations in symptomatology have implicated stomatal action, inorganic nutrition, temperature, genetics, ascorbic acid content, physiological age of tissue and photoperiod[4-8]. Plants grown under artificial illumination differ in their response to the photochemically formed pollutants as compared with plants grown in the greenhouse. Interactions between light and oxidants from the polluted atmosphere within plants, as reported here, might well explain some of the variabilities in symptomatology observed in earlier controlled experiments as well as the unexplained natural variability observed in the Los Angeles area. The results presented also emphasize the importance of standardizing plant growth conditions for future work of this nature.

Lots of pinto bean (*Phaseolus vulgaris*) and petunia (*Petunia hydrida*, var. Rosy Morn) plants were grown under the following light conditions: (1) natural light received in the greenhouse during August–November, (2) a 15-hr. photoperiod of 8·6 Klux from cool white fluorescent lamps in a shaded greenhouse and (3) a 15-hr. photoperiod of 21·5 Klux from Sylvania 'very high output (VHO)' fluorescent lamps also in the shaded greenhouse. Approximately 85° F. day and 70° F. night temperatures were maintained. Plants were exposed to the oxidants in chambers placed under a 21·5 Klux fluorescent lamp panel. Dark chambers in the greenhouse were used for the experimental dark conditions. The greenhouses and fumigation chambers were supplied with carbon filtered air[9] to remove the naturally occurring oxidants.

To ensure that the stomates were open at the time of exposure to the oxidants, all plants pre-conditioned by a dark period were given 15–30 min. light before exposure to the oxidants. Stomatal opening was determined by a resistance porometer[10] with a leaf-cup attached to a snap spring device to ensure a rapid seal to the leaf. In later experiments the porometer measurements were supplemented with transpiration measurements. During the 15–20 min. before exposing the plants to the oxidants the rate of water-loss by transpiration was followed. When this rate of water-loss equalled the rate of water-loss from the pre-light treated plants the fumigation was started.

Plants were exposed to the oxidants for periods of 30 min. or less. After exposure, unless otherwise specified, the plants were returned to the pre-treatment light condition. Symptoms of oxidant damage were usually apparent within 4 hr.; however, assay of damage was made 48 hr. after exposure to the oxidants. The extent of damage was expressed as the percentage of leaf area showing symptoms of the oxidant injury. Eight to twelve plants were used in each treatment and the experiments were repeated several times.

The type and extent of ozone and peroxyacetyl nitrate injury on bean and petunia plants were affected by light intensity under which the plants were grown before treatment. Sensitivity to ozone injury was increased by the low light intensity (8·5 Klux), while sensitivity to peroxyacetyl nitrate was increased by the higher light intensity (21·5 Klux). A total bleaching and collapse of affected leaf tissue characterized the oxidant injury on plants grown under the low light intensity. By contrast, oxidant injury on plants grown in the higher light intensity usually appeared as bronzing of the lower leaf surface, and ozone injury on the bean appeared as discrete dark brown spots on the upper leaf surface.

Sensitivity of pinto bean and petunia plants to peroxyacetyl nitrate was significantly affected by the presence of light immediately before, during or after exposure to the toxicants. A 24-hr. pre-treatment dark period completely protected these plants against peroxyacetyl nitrate damage (Fig. 1 *DL*) regardless of subsequent light régime during and after fumigation. Plants which had received the standard 15-hr. day, with a 5- to 6-hr. light period immediately preceding the fumigation, were severely damaged (Fig. 1, *L*), whereas similar plants transferred to the dark for 24 hr. after fumigation were uninjured (Fig. 1, *LD*). Bean plants returned to the light after the 24-hr. post-dark period appeared normal and did not develop oxidant damage, whereas some oxidant damage did develop on the petunia plants after transfer to the light.

In another series of experiments, bean plants were fumigated with very high concentrations (5 p.p.m.) of peroxyacetyl nitrate for 10 min. All the plants were grown under the 15-hr. light régime and were returned to the lights after the 10-min. fumigation. Darkness during this short fumigation period gave complete protection from damage to petunia and bean plants while identical plants fumigated similarly in the light were severely damaged (Fig. 2). Porometer measurements showed that during the 10-min. dark period the resistance to air-flow through the stomates decreased only 0·028 unit[10], an insignificant change. This indicated that the observed results were related to the interaction of light and some product in the plant and not to the exclusion of peroxyacetyl nitrate by closed stomates.

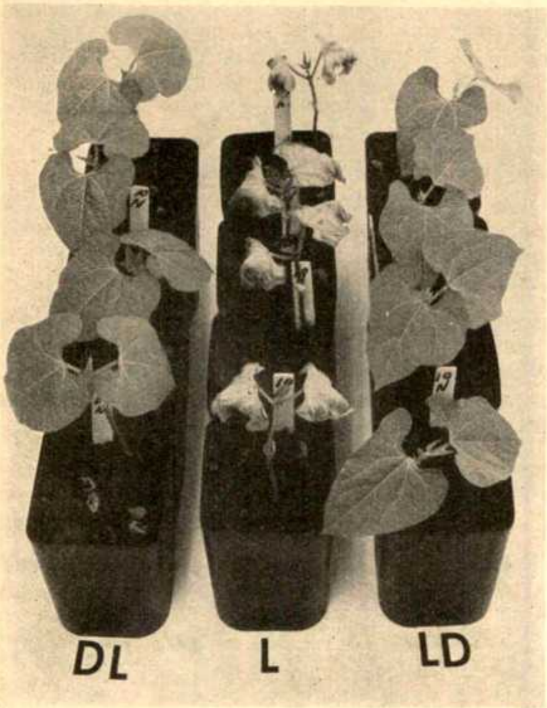

Fig. 1. Pinto bean plants fumigated for 30 min. with 1·0 p.p.m. of peroxyacetyl nitrate
DL, dark before (24 hr.), light during and after fumigation—no damage; *L*, light before (6 hr.), during and after fumigation—severe damage; *LD*, light before and during fumigation and dark (24 hr.) after fumigation—no damage

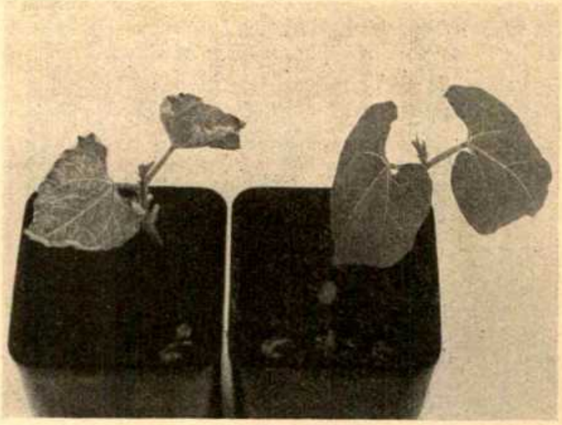

Fig. 2. Ten-day-old pinto bean plants exposed to 5 p.p.m. peroxyacetyl nitrate for 10 min. Left, light before, during and after fumigation—severe damage. Right, light before, dark during (10 min.) and light after fumigation—undamaged

The interaction of light and ozone was unlike that of light and peroxyacetyl nitrate. A considerably longer pre-treatment of dark period (48 hr. or longer) was necessary to provide protection to petunia and bean plants from an ozone fumigation (Table 1). The marked reduction in extent of ozone damage that occurred as the pretreatment dark period was lengthened beyond 24 hr. suggests that susceptibility may be dependent on the level of photosynthate or some carbohydrate intermediate within the tissue. Further evidence of the difference in action of peroxyacetyl nitrate and ozone was shown by the damage response obtained with a short, dark period during fumigation and with 24-hr. post-dark treatment. Both these conditions prevent oxidant injury from peroxyacetyl nitrate but have no effect on ozone damage. There were indications from additional experiments that under the conditions of these experiments the bean required at least 3–4 hr. of light after fumigation for symptoms of damage by peroxyacetyl nitrate to develop.

The distinct interrelationship of light with the oxidizing air pollutants in plants definitely shows that there is a photochemical reaction between the oxidant and some intermediate synthesized by light in the plant. The reactions of this intermediate with the oxidant peroxyacetyl nitrate is further dependent on light to cause a change in the metabolism of the plant sufficient to induce irreversible leaf damage.

Table 1. EFFECT OF VARIED LENGTHS OF DARK TREATMENTS BEFORE, DURING AND AFTER FUMIGATION ON THE PERCENTAGE OF PINTO BEAN LEAF AREA INJURED BY OZONE AND PEROXYACETYL NITRATE (PAN)

Fumigant and concentration	Pre-dark period (hr.)*	Condition during fumigation	Condition after fumigation	Injury (per cent)†
Ozone, 0·6 p.p.m.	0	light	light	76
	24	,,	,,	77
	48	,,	,,	0
	0	,,	dark	80
PAN, 1·0 p.p.m.	0	,,	light	99
	24	,,	,,	0
	48	,,	,,	0
	0	,,	dark	0

* The pre-dark period started at 9 a.m. The plants were grown under fluorescent lamps of 21·5 Klux with a light period of 15 hr. (4.30 a.m.–7.30 p.m.). The plants were moved into the light at 9 a.m. and fumigation started after the stomates had opened (15–30 min.). A resistance porometer was used to determine when the stomates were open.

† Average values from 8–12 plants per treatment.

This work was supported in part by the American Petroleum Institute, the Air Pollution Foundation and the Agricultural Air Research Programme.

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ACID-SOLUBLE NUCLEOTIDES OF *STAPHYLOCOCCUS AUREUS*: MASSIVE ACCUMULATION OF A DERIVATIVE OF CYTIDINE DIPHOSPHATE IN THE PRESENCE OF PENICILLIN

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PENICILLIN is known to cause accumulation of three uridine nucleotides in *S. aureus*[1]. The compounds were identified by Park as uridine diphosphate (UDP)–acetylmuramic acid, its alanine derivative, and a nucleotide composed of a peptide (ala-glu-lys-ala-ala) and UDP–acetylmuramic acid[2]. Several aspects of nucleotide accumulation in the presence of penicillin or other anti-bacterial substances have been investigated extensively[3]. On the basis of their findings, Park and Strominger have suggested that penicillin might be a specific inhibitor of transfer of the peptide from the most complex of the above nucleotides to the cell wall structure[4]. Relatively little attention has been paid to other nucleotides present in penicillin-treated cells. Strominger has investigated the acid-soluble extract of penicillin-inhibited *S. aureus* by means of anion exchange chromatography[5]. In addition to the UDP-derivatives, cytidine-5′-monophosphate (CMP), adenosine-5′-monophosphate (AMP) and small amounts of unidentified ultra-violet-absorbing substances were detected. Penicillin was reported to cause a doubling in the amount of CMP, when compared with untreated cells. Park and Strominger have noted, according to a personal communication to Armstrong *et al.*[6], the accumulation of cytidine diphosphate (CDP)–ribitol in *S. aureus* during treatment with penicillin and crystal violet. A report of the accumulation of this nucleotide induced by crystal violet has been published[7]. In the course of another investigation, indications were found of the presence of a large number of nucleotides in *S. aureus*, prompting the present work.

S. aureus 209 Pfeiffer (obtained from Dr. I. Rantasalo of this Institute) was grown in a medium adapted from that of Park and Johnson[1], with shaking at 37° C. At approximately half maximal growth, penicillin (benzylpenicillin, the Distillers Co., Ltd., London) was added to a final concentration of 10 µgm./ml. culture medium. After incubation for 1 hr., the cells were gathered rapidly in a refrigerated centrifuge and frozen. The pellet was extracted with cold 0·6 *N* perchloric acid and the acid-soluble fraction was neutralized and chromatographed on a 'Dowex-2' column at 4° C., using the gradient elution system of Hurlbert *et al.*[8]. Details of the procedure have been described in ref. 9.

Typical elution diagrams of normally grown and penicillin-treated cells are presented in Fig. 1. In both cases, a number of ultra-violet-absorbing compounds can be detected. Fraction *A*, which represents a large portion of the total ultra-violet-absorbing material, contains several substances. Chromatography of this fraction in a system with better resolution revealed in addition to diphosphopyridine nucleotide and CMP a third major compound in the pencillin-treated bacteria. This compound appears to be a nucleotide giving a positive ninhydrin reaction. Fraction *B* was identified as AMP. Analysis of peak *C*, characterized by its high 275/260-ratio, will be given here. Derivatives of adenine, guanine, uracil and cytosine have been found in the subsequent fractions. Peaks *D* and *E* were shown to be uridine nucleotides containing amino-acids, and presumably correspond to the known derivatives of UDP (ref. 3).

When the two patterns of nucleotides are compared, a striking increase in the amount of fraction *C* is seen in the penicillin-treated cells. Material corresponding to this fraction was collected from several preparations and purified by means of paper chromatography in neutral ethanol – ammonium acetate – water[10]. The small amount of the compound recovered from repeated runs in this system was subjected to further analysis. The substance was found to be uniform in several other solvent systems. The mobilities were close to the values reported earlier[7,11] for CDP–ribitol (in propanol–ammonia–water: R_F 0·36, in neutral ethanol–ammonium acetate–water: R_{UMP} 1·23. The base, which was isolated by means of paper chromatography after vigorous acid hydrolysis[12], gave a typical spectrum of cytosine in 0·01 *N* hydrochloric acid and in 0·01 *N* sodium hydroxide. A colorimetric assay[13] yielded 1·88 moles of phosphorus/mole cytosine. Paper chromatography in propanol – ammonia – water[14] after acid hydrolysis yielded in addition to CMP and inorganic phosphorus other breakdown products, two of which gave a positive reaction for phosphoric esters[14] and with ammoniacal silver nitrate[15]. While it has not yet been possible to identify the phosphoric ester moiety with certainty, the evidence available suggests the compound to be identical with CDP–ribitol[11,16].

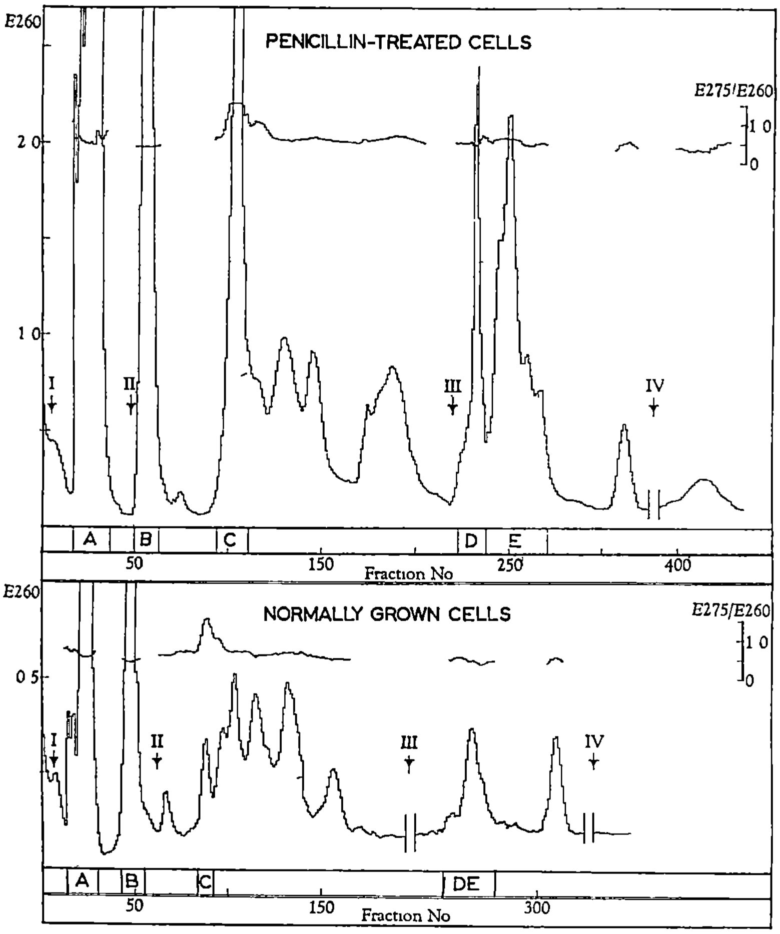

Fig. 1. Anion exchange chromatograms of extracts from penicillin-treated *S. aureus* and from normally grown cells. Bacteria from 4 l. of culture were extracted in both cases. The column was 'Dowex-2', $HCOO^-$, 200–400 mesh, 8 per cent cross-linked. A 1.0 × 18 cm column was used in the first case, a 1.2 × 15 cm column in the latter instance. The volumes of the mixing flasks were 450 and 375 ml., respectively. After washing with distilled water, the column was eluted with the following solutions in the reservoir: I, 0.5 *N* formic acid, II, 4 *N* formic acid, III, 4 *N* formic acid and 0.4 *N* ammonium formate, and IV, 4 *N* formic acid and 0.8 *N* ammonium formate, as indicated on the diagrams.

The present work shows that a large number of nucleotides is found both in penicillin-treated and in normally grown *S. aureus*, apparently forming a pattern similar to the distribution discovered in some other micro-organisms and in many animal tissues (cf. refs. 9 and 17). The various uridine nucleotides from the extracts of penicillin-treated bacteria have not yet been identified. It is assumed that they belong to the group of UDP-derivatives involved in cell wall synthesis. Ito *et al.* have reported that the UDP–acetylmuramic acid peptides of this strain contain aspartic acid and glycine[18]. The accumulation of uridine nucleotides is paralleled by the massive accumulation of the CDP-derivative, which in many cases surpasses the increase in the amount of individual uridine nucleotides.

The difference between the present results and earlier findings could be due to a number of factors. As pointed out by Strominger in the case of bacteria treated with crystal violet, the accumulation of CDP–ribitol cannot be demonstrated with all strains of *S. aureus*[7]. Possible differences in the composition of the culture medium and in other experimental conditions could also have influenced the results. Baddiley and Mathias, who originally discovered CDP–ribitol and CDP–glycerol in *L. arabinosus*[19], found the compounds very labile during extraction. This was attributed to the presence of potent phosphatases. For this reason, heat-killed bacteria have been used for later investigations. Strominger, who inactivated the enzymes by heating a washed bacterial suspension at 100° C for 10 min., found great variations in the amount of CDP–ribitol from crystal-violet-treated cells, accompanied by a corresponding change in the amount of CMP (ref. 7). In the work described here, the bacteria were grown in small batches, chilled rapidly and extracted at low temperature. Even a small delay in chilling was found to reduce the amount of the nucleotide considerably. With proper care, this method seems to give high yields of the nucleotide.

It has been suggested that CDP–ribitol might be a precursor of teichoic acid, a component of bacterial cell wall containing ribitol phosphate[6]. While the biology of the accumulation of CDP–ribitol is not well known, it is interesting to note that both penicillin and crystal violet cause accumulation of this nucleotide. These two agents appear to interfere at different points with the uridine nucleotide pathway leading to cell wall synthesis[7]. Whether the accumulation of CDP–ribitol is a result of an independent action of penicillin on the cytidine nucleotide metabolism or whether the accumulation of the two types of nucleotides is caused by an interference with a single reaction in the metabolic sequence, cannot yet be decided.

This work was aided by grants from the S. Jusélius and J. K. Paasikivi Foundations.

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SENSORY DEPRIVATION AND FŒTAL CIRCULATORY HOMEOSTASIS

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EMBRYOLOGISTS seem to overlook the effect of a flotation-type of existence *in utero* on physiological activities within a fœtus as compared with physiological capabilities once development has proceeded through the period of morphogenesis Morphological differentiation must precede functional capability This is true of single-organ systems or of two or more interdependent systems such as homeostatic regulatory mechanisms However, full function of one or more completely developed secondary organ systems may not occur because a primary organ system essential to the secondary one is not yet called into action by the exigencies of the environment It is this sort of system–system interaction that appears to be involved in the circulatory adjustments of the fœtus (lamb) *in utero*

The problems of fœtal heart-rate, blood pressure and reflex mechanisms have been reviewed by Windle[1] and Barcroft[2] In 1954, when fœtal blood pressure and heart-rate were recorded *in utero* with the membranes intact[3], fœtal circulatory characteristics could be studied in nearly normal circumstances In such a preparation, responses to a variety of stressful situations were determined

Parasympathetic and sympathetic nervous system capabilities were explored, largely with the fœtus *in utero* Included were the responses of heart-rate and blood pressure to (*a*) increased intrauterine pressure[4], (*b*) graded degrees of hypoxia[5], (*c*) disturbances of the maternal circulation[6], (*d*) occlusion of the umbilical cord[7], (*e*) vagotomy[8], (*f*) stellate and thoracic ganglionectomy[8], (*g*) excision of the fœtal adrenal glands[8]

The resting fœtal heart-rate decreases when intrauterine pressure increases by a weight placed on the uterus and decreases when the weight is removed These are transient episodes evoked by a brief and dynamic stimulus The bradycardia is vagal in origin since it is abolished by atropine There is therefore, a competent vagal mechanism which is brought into temporary action by this change in the environment

A lamb *in utero* and its mother respond differently to the same asphyxial stimulus When nitrogen is given to a ewe, her heart-rate and blood pressure rise The fœtus shows bradycardia and no change in blood pressure When atropine is given to the fœtus during bradycardia the heart-rate becomes rapid The bradycardia is vagally induced Again we see that the autonomic system of the lamb responds to this stress by parasympathetic output and the fœtal response is unlike that of the adult

Less stressful stimuli have an effect also When a pregnant ewe is subjected to progressively stronger hypoxia, the fœtal heart-rate decreases or it shows a 2 1 heart block which is vagal in origin Similarly, if there are acute disturbances of the maternal circulation involving transient congestion of the maternal portion of the placenta, evanescent decreases in fœtal heart-rate occur This is the result of induced vagotonia

From these and other experiments we conclude that the parasympathetic system of the fœtal lamb is functionally competent, but it lacks a stimulus to maintain parasympathetic tone

Most of the present observations were made with ewes lightly anæsthetized with diallylethylabarbituric acid It might be argued that this anæsthetic abolishes existing vagal tone This is unlikely, however, since vagotonia is the easiest response in the lamb to exhibit under this anæsthesia and experiments made with the ewe operated under local anæsthesia ('Procaine') alone make no difference in the fœtal responses to stress or in the resting characteristics of the fœtal circulation

Barcroft found progressive vagotonia as a fœtal lamb becomes larger However, the *in utero* rates observed by him were recorded by auscultation and by palpation which involved some pressure on and handling of the uterus which, as we have shown, induces vagotonia From Barcroft's experiments on lambs at Cæsarean section, it appears that the acute response to stress of handling induces vagotonia, since he operated and observed very rapidly He says, "Let me emphasize again, that if the phenomena which have just been described [fœtal vagotonia] are to be observed they must be observed at once on the delivery of the fœtus—a delivery which must be carried through with no strain on the uterine and umbilical blood vessels and no exposure of the cord or fœtus—it may seem wasteful to do an experiment on a sheep which only takes 10 min and produces but a single lesion, but it is less wasteful than for him to misinform himself on 3 or 4 matters" (ref 2, p 137)

Concerning sympathetic activity in the fœtus, evidence is meagre but equally clear When normal resting fœtal heart-rates in lambs are compared with resting heart-rates after bilateral excision of the stellate and other thoracic sympathetic ganglia, no change occurs in resting fœtal heart-rate This is true whether the vagi are intact or sectioned or both carotid sinus areas are excluded from the circulation One must conclude that there is no appreciable resting sympathetic tone in the fœtus so far as heart-rate is concerned

One type of evidence has been obtained which resulted in sympathetic system activity in the fœtus With the heart completely denervated severe anoxia was produced by temporary occlusion of the umbilical cord The heart-rate showed a slow but profound decrease, due to myocardial depression On release of the cord, an extremely fast and prolonged tachycardia was seen The fœtus struggled, exhibited strong expiratory effort and passed meconium The experiment was repeated after incision of both fœtal adrenal glands After anoxia, the heart-rate returned to normal without tachycardia *In extremis*, therefore, there was mass stimulation of the sympathetic system

One must conclude that the sympathetic system of the lamb fœtus, like the parasympathetic system, is functionally competent but inactive in a resting fœtus One asks, therefore, why a developed and competent autonomic system is silent except in the face of transient and dynamic stresses

Examination of the development of cardiovascular responses in lambs soon after birth is crucial to the hypothesis suggested above A few observations have been made in lambs 4–12 days of age, averaging about 8 days Responses to the following stresses have been studied (*a*) carotid artery occlusion (6 lambs), (*b*) bilateral vagotomy (8 lambs), (*c*) severe hypoxia (9 lambs) The results are summarized in Tables 1, 2 and 3

Table 1 shows that carotid artery occlusion in lambs yields a rise in blood pressure like that of the adult, the heart-rate response is more or less intermediate between the adult and the fœtus

Table 1 COMPARISON OF THE EFFECT OF CAROTID ARTERY OCCLUSION IN FŒTUSES, LAMBS AND EWES

	Blood pressure (per cent)*			Heart-rate (per cent)*		
	+	○	–	+	○	–
Non-pregnant ewes (ref 10)	100	0	0	50	20	30
Lambs (ref 6)	100	0	0	33	0	67
Fœtuses (ref 5) (last third preg)	20	60	20	0	20	80

* Percentage of animals showing increase (+), no change (○) or decrease (–)

Table 2 shows that vagotomy in adult ewes and lambs causes a fall in blood pressure and a decrease in heart-rate while blood pressure in fœtuses is unchanged, although the decrease in heart-rate occurs The effect of vagotomy in the lamb parallels more closely the change in the adult than in the fœtus

Table 2 COMPARISON OF THE EFFECTS OF VAGOTOMY IN FŒTUSES, LAMBS AND EWES

	Blood pressure (mm Hg)		Heart-rate (b p m)	
	Before	After	Before	After
Non-pregnant ewes (ref 12)	101 5 (±8 9)/ 81 8 (±8 2)	87 3 (±8 5)/ 64 (±6 6)	110 4 (±8 5)	99 5 (±6 5)
Lambs (ref 6) (mean blood pressure)	85	70 5	234	194
Fœtuses (ref 5) (mean blood pressure)	37 9 (±4 4)	37 8 (±2 4)	166 7 (±13 7)	158 8 (±14 0)

Although there is a great variability of response in the effects of severe hypoxia in all three groups, Table 3 shows that the blood pressure of the fœtus was, with one exception, unaffected by acute hypoxia The responses in lambs and adults are rather similar The heart-rates were variable but once again the lambs and ewes resemble each other to the extent that some of each showed cardiac acceleration which was not seen in the fœtus

These results suggest that new-born lambs resemble adults in their responses to the three stresses employed More refined experiments are needed to establish this point, but the evidence seems clear that circulatory homeostasis begins soon after birth in the sheep

Table 3 COMPARISON OF EFFECTS OF HYPOXIA IN FŒTUSES, LAMBS AND EWES

	Blood pressure (per cent)			Heart-rate (per cent)		
	+	○	–	+	○	–
Ewes (ref 15)	33 4	46 6	13 4	46	46 6	13 4
Lambs (ref 8)	55 5	33 4	10 1	33 4	20 2	44 5
Fœtuses (ref 7) (last trimester)	0	86 7	13 4	0	42 8	57 2

Recent experiments on men given bed rest[9] or immersed in water for a day to a week[10] show that cardiovascular homeostasis depends on proprioceptive input to the central nervous system Following immersion there is a progressive decline in blood pressure, an increase in heart-rate, and a loss of the diurnal variation in heart-rate The response of heart-rate to the mild stress of a tilt table was much exaggerated An elevation of diastolic and a decrease of systolic blood pressure occurred, signifying diminishing cardiac output With more severe stress of exercise on a treadmill, the deficiency of homeostasis was more exaggerated In short, prolonged reduction of proprioceptive input by weightlessness leads to loss of effective homeostatic regulation in man and exaggerated sympathetic system output

The concept that homeostatic regulatory mechanisms depend on sensory input to the nervous system is nicely set forth in a recent book by Stanley-Jones[12] in which the roles of monitoring positive and negative feed-backs in a wide range of biological systems are reviewed Feed-backs acting on physiological mechanisms result in stabilization, consequently they prevent by negative feed-back a runaway to a maximum (for example, excessive sympathetic activity as in the deconditioned man[10])

Stanley-Jones distinguishes between two main types of sensory inputs (1) those in which brief reflex responses occur and are dynamic in the sense that they elicit reflexly a response of limited duration and a purposeful nature, and (2) those that serve to produce a sustained sensory input, activating the nervous system on a long, continuing basis The former are responses to quickly adapting sense organs, the latter, to slowly adapting sense organs. In the main, the second type have their origin in postural input nervous mechanisms from otoliths and from muscle spindles Speaking of antigravity feed-back in higher vertebrates he says "The new feature is the augumentor system It is Nature's response to the challenge of gravity It employs a positive refection (feed-back) to maintain the posture of the body when at rest, instead of the negative refection used in fishes The muscle spindle is the little machine inside the big machine". Speaking of weightless adults deprived of all sensory input, Stanley-Jones says "The physical status of adults in these circumstances resembles nothing so much as a return to the fœtal condition of isothermal floating in the amniotic fluid of the womb, it is not surprising therefore that the body regression to prenatal conditions should cause a similar regression on the mental plane" With respect to the latter reference, the reader should consult an editorial on the subject[13]

The fœtus *in utero*, like the immersed man in Graveline's experiment, is a free-floating organism As in that experiment, the fœtus is in a sort of zero-gravity situation so far as weight is concerned It is not called on to use its proprioceptive reflexes to offset the effect of gravity in order to maintain posture. The fœtus is subject to no unequal forces It lacks, therefore, the action of a monitoring feed-back from the environment Consequently there is a deficiency of autonomic nervous system controls when the fœtus is in a resting, unstressed situation It is as if the fœtal heart were like that in a heart-lung preparation, subject to its intrinsic controls on a chemical basis unless the autonomic system is called into play by sensory input from its environment The fœtus does possess from a time early in development the capacity for well-characterized patterns of behavioural (dynamic) responses, as Coghill[14] and Hooker[15] have shown, although Windle[1] is of a different opinion regarding their nature

After delivery in those species that are mature at birth antigravity input to the central nervous system is established rapidly It appears, therefore, that such sensory input to the central nervous system is an essential factor in contributing to sustained autonomic system output, at least so far as cardiovascular homeostasis is concerned. The effect is one of negative refection mediated by the parasympathetic system This problem is part of the larger one of sensory deprivation in general on the body.

How effective homeostasis will be after birth must vary with the degree of morphological maturity at birth Opossums, hamsters, mice, rats, rabbits, kittens, puppies, piglets, human babies and many other species will lack the capacity for homeostasis at birth because they do not respond to gravity. Others, such as guinea pigs, foals, lambs, calves, kids, and many other species will possess to a marked degree the capacity for effective homeostasis since they can stand, walk (or run) and regulate fairly well their body-temperature when they are born Most notably, they possess a capacity for proprioceptive input to the central nervous system when they are delivered from a condition of weightlessness during development

It is significant that the uterus is the original space capsule of mankind, and that all men have, so to speak, been in weightless orbit before they were born

This work was supported by the U S. Public Health Service grant *RG*4728

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TRANSAMINATION AND THE CONTROL OF MITOCHONDRIAL PATHWAYS

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RECENT work in several laboratories has demonstrated that the most important pathway for glutamate oxidation is via transamination and oxidation of α-oxoglutarate[1,2]

$$\begin{array}{c} \text{oxaloacetate} + \text{glutamate} \rightarrow \text{aspartate} + \alpha\text{-oxoglutarate} \\ \underline{\alpha\text{-oxoglutarate} + 1\tfrac{1}{2}\, O_2 \rightarrow \text{oxaloacetate} + CO_2} \\ \text{glutamate} + 1\tfrac{1}{2}\, O_2 \rightarrow \text{aspartate} + CO_2 \end{array}$$

Jones and Gutfreund[2] have shown in their detailed work on guinea pig liver and mammary gland mitochondria that under *in vitro* conditions liver mitochondria oxidize some glutamate via glutamic dehydrogenase, but that the above transaminase pathway is more important and the latter is probably the only pathway under physiological conditions. Mammary gland mitochondria have no detectable glutamic dehydrogenase activity[2,3] and yet they oxidize glutamate at approximately the same rate as liver mitochondria. These findings indicate that the concentrations, activities and specificities of mitochondrial transaminase are most important data for the analysis of the control of mitochondrial metabolism

We shall attempt to present a discussion of· (1) the concentrations of transaminase and other enzymes in various mitochondria, (2) the relation between transaminase concentration and the concentrations of pyridine nucleotide - linked enzymes in mitochondria, (3) some special aspects of the enzyme kinetics of transaminase; (4) the so-called latency of mitochondrial transaminase and its relation to the control of pathways in intact respiratory particles

The purpose behind a detailed examination of various enzyme systems in the pure isolated form and in intact respiring mitochondria is to differentiate between various possible factors which control the direction and intensity of multi-enzyme pathways from junction points A thorough knowledge of an isolated enzyme under various conditions and data on the behaviour of this enzyme in mitochondria should give information about the conditions and surroundings of this enzyme in respiratory particles To get a complete picture of chemical organization in mitochondria in this way is necessarily a long-term programme; but some of the investigations reported here are intended to illustrate this approach to the transaminases of mitochondria and their relation to the enzymes of the citric acid cycle More details of similar work on enzymes of the citric acid cycle are reported elsewhere[2,3].

Enzyme Concentrations in Mitochondria

Investigations on the concentrations of various active components of mitochondria from different tissues are too numerous to be referred to individually Bucher and Klingenberg[4] review data on enzyme and coenzyme concentrations, Birt and Bartley[5] discuss pyridine nucleotide movements in mitochondria, and Estabrook and Holowinsky[6] discuss the cytochrome content of various mitochondria The present investigation was carried out to provide data on the concentrations of a number of enzymes from assays on the same mitochondrial preparation, which has also been used for work on intact mitochondria as discussed below and elsewhere[3].

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Table 1 contains results for the assay of activities of a number of mitochondrial enzymes at 25° under specified conditions All the assays were performed on samples of preparations of mitochondria which were first used intact for respiration experiments[3] Three samples of rat liver mitochondria were kindly provided by Dr. W N Aldridge. These samples were frozen at $-20°$ and just before use they were diluted ten times and aliquots of such disrupted mitochondria were added to appropriate reaction mixtures and the rates of the oxidation or reduction of NAD (nicotinamide–adenine–dinucleotide) or NADP (nicotinamide–adenine–dinucleotide–phosphate) were followed spectrophotometrically at 340 mμ The transaminase assays were coupled to dehydrogenases in the following manner

(1) aspartate + α-oxoglutarate $\rightarrow$ oxaloacetate + glutamate

the rate of oxaloacetate formation was determined by following the rate of oxidation of NADH (reduced nicotinamide–adenine–dinucleotide) in the presence of excess malic dehydrogenase In broken mitochondria the endogenous concentration of malic dehydrogenase was sufficient for this assay

(2) oxaloacetate + glutamate $\rightarrow$ aspartate + α-oxoglutarate

The rate of α-oxoglutarate formation was determined by following the rate of oxidation of NADPH (reduced nicotinamide–adenine–dinucleotide phosphate) in the presence of ammonia and excess glutamic dehydrogenase Glutamic dehydrogenase will oxidize NADPH at approximately half the rate of NADH, while the oxaloacetate reduction in broken mitochondria was completely specific for NADH

At this stage the data obtained for the activity of transaminase, glutamic dehydrogenase, isocitric dehydrogenase and malic dehydrogenase are mainly of comparative interest. To establish the absolute concentration of these enzymes one has to find out whether their possible latency—which is discussed later—has been completely overcome and whether there are any significant losses during preparation of the samples of broken mitochondria We have found that transaminase as well as glutamic and malic dehydrogenase are still partially bound to particulate material while the isocitric dehydrogenase is in a freely soluble form. The latter observation is of some significance in connexion with the variability of isocitrate oxidation by mitochondria, especially in the case of 'leaky' particles such as those prepared from mammary gland.

For the calculation of the percentage concentration of individual enzyme protein in mitochondria the activity per mgm. of pure enzyme has to be known. None of the enzymes discussed has been purified from guinea pig or rat liver, but a preliminary estimate is of considerable interest. Assuming for the rat liver enzyme the turnover and molecular weight of pig heart isocitric dehydrogenase[7] one can calculate that 2 5 per cent of the protein of rat liver mitochondria and twice this amount of guinea pig liver mitochondria consist of this enzyme Similar calculations for the other enzymes estimated give values of the same order of magnitude

We were particularly interested in the relative rates of the dehydrogenases, respiration and transamination The relation between enzyme concentrations and respiration is discussed elsewhere[3] Here we shall concern ourselves with the question as to how the concentration and kinetic characteristics of transaminases fit in with the observations on mitochondrial metabolism of glutamate, aspartate, α-oxoglutarate and oxaloacetate.

Table 1 SOME ENZYME ACTIVITIES OF DISRUPTED MITOCHONDRIA AND THEIR RELATION TO THE RATE OF RESPIRATION OF INTACT MITOCHONDRIA

Reaction	Rat liver	Guinea pig liver	Guinea pig mammary gland*
Glutamate + oxaloacetate to aspartate + α-oxoglutarate	1 2, 1 0, 0 92	1 0, 0 5	0 8
Isocitrate + NADP$^+$ to α-oxoglutarate + CO$_2$ + NADPH + H$^+$	0 14, 0 13, 0 13	0 21, 0 41, 0 31, 0 54	0 1
α-Oxoglutarate + NH$_4^+$ + NADH to glutamate + H$_2$O + NAD$^+$	0 16, 0 17, 0 19	0 18, 0 30	none
α-Oxoglutarate + NH$_4^+$ + NADPH to glutamate + H$_2$O + NADP$^+$	This reaction proceeded at about 55 per cent of the rate of the reaction in the presence of NADH		none
Glutamate + H$_2$O + NAD$^+$ to α-oxoglutarate + NH$_4^+$ + NADH	0 081, 0 035, 0 074	0 03, 0 08, 0 06, 0 04	none
Glutamate + H$_2$O + NADP$^+$ to α-oxoglutarate + NH$_4^+$ + NADPH	This reaction proceeded at about 55 per cent of the rate of the reaction in the presence of NAD$^+$		none
Malate + NAD$^+$ to oxaloacetate + NADH + H$^+$	0 36, 0 28, 0 30	0 06, 0 0925	
Oxaloacetate + NADH + H$^+$ to malate + NAD$^+$	3 25, 2 75, 2 98, 3 96	2 50	0 6, 1 4, 0 8
Respiration-rate of intact mitochondria in the presence of ADP inorganic PO$_4^{=}$ and citric acid cycle substrates	0 12–0 14	0 04–0 05	0 04–0 05

The results given in this table are in μmoles of substrate utilized per min per mgm mitochondrial protein at 25° C under conditions of optimum *p*H, substrate, coenzyme and metal ion concentrations, with the exception of NH$_4^+$ during glutamate formation which was 3 33 m*M*

* Examination of the supernatant obtained after centrifugation of mammary gland mitochondria indicates considerable leakage of enzymes from these respiratory particles

Kinetic Characteristics of Transaminase

Two types of enzyme preparations were used for kinetic measurements on various transaminase reactions The transaminase of mitochondria from guinea pig liver and mammary gland and from rat liver were examined in suspensions of broken mitochondria as used for the enzyme assays described above Secondly, we used a highly purified preparation of transaminase from pig heart, which corresponded to that described by Jenkins, Yphantis and Sizer[8] This gives a basis of comparison with an enzyme preparation most extensively described in the literature The kinetics of transaminase catalysis can be described by the scheme shown in Fig 1, in which each step is reversible

This scheme is formally the same as that described by Gutfreund and Sturtevant[9] for hydrogen transfer via enzyme-bound flavin adenine dinucleotide, but the rates of the individual steps of the transaminase reaction are so much faster that even rapid reaction techniques have so far only helped to confirm an order of magnitude for the rates involved

It has been shown[10] that the reaction of the pyridoxal enzyme from pig heart with alanine to form pyridoxamine enzyme and pyruvate is fairly slow (half-time approximately 6 min with alanine 2×10^{-2} *M*), the reverse reaction with pyruvate is at least 100 times faster The reactions of the pig heart enzyme with aspartate or glutamate was known to be much more rapid but no attempt had previously been made to determine its rate We have made some preliminary observations on the rate of forma-

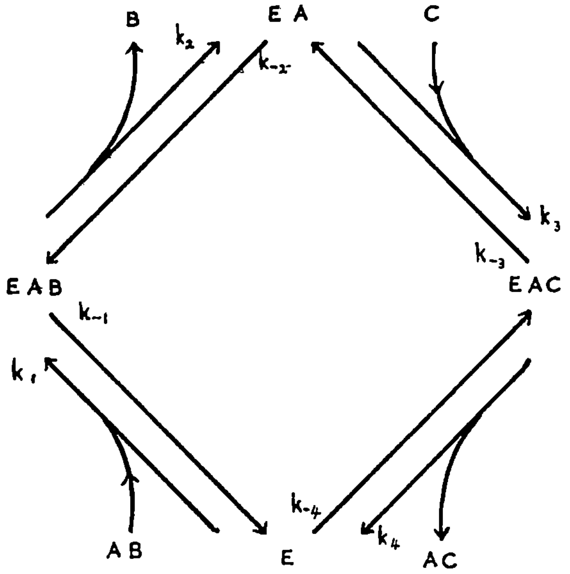

Fig. 1. *E*, pyridoxalphosphate enzyme; *AB*, amino-acid substrate; *EAB*, Michaelis complex; *B*, ketoacid product; *EA*, pyridoxaminephosphate enzyme; *C*, ketoacid substrate; *EAC*, Michaelis complex; *AC*, amino-acid product

tion of pyridoxamine enzyme in the presence of 0·05 *M* aspartate. The reaction was followed in a rapid-reaction spectrophotometer at 365 mμ. The method used was only capable of giving an approximate value for the half-time of the reaction (5 m.sec at 25°). The difficulty of obtaining accurate kinetic constants is due to the fact that the earliest observation is made at a time at which the reaction is about half-way completed and the complication of the reverse reaction discussed later puts special demands on accurate observations over the whole time course of the reaction. It is, however, of great interest to get this approximate value for the velocity of the very rapid pyridoxamine enzyme formation, especially in view of the fact that this reaction is characterized by k_2 (see Fig. 1), which will be shown to be the smallest of four forward reaction constants. The following calculation is of interest, though it is based on a number of approximations. Jenkins and Sizer[11] give the results of an experiment designed to study the equilibrium

$$\frac{(\text{enzyme-pyridoxamine})\ (\alpha\text{-oxoglutarate})}{(\text{enzyme-pyridoxal})\ (\text{glutamate})} = K$$

The data given over a wide range of the two forms of the enzyme do not fit any simple model of a reversible reaction, but at low glutamate concentration one can conclude that the rate of the reaction of the pyridoxamine enzyme with α-oxoglutarate is approximately 100 times as fast as the reaction of the pyridoxal enzyme with glutamate. In any discussion of metabolic control at the point of one enzyme, one is concerned with the relation between the Michaelis constant and the steady-state concentration of the substrates. If two substrates—donor and acceptor—can influence the rate, then one is also concerned with the conditions under which the concentration of one or the other of these determines the rate of the overall reaction. The tissue concentrations of α-oxoglutarate are usually much lower than those of glutamate and the concentration of oxaloacetate is only of the order of 10 μM [1,12]. The value for *Km* for oxaloacetate with mitochondrial transaminase is therefore of particular interest. We have determined this in the following manner with our rat liver mitochondrial preparation. The assay for glutamate to oxaloacetate transamination was carried out over a range of substrate concentrations under initial conditions of equal amino donor and amino acceptor concentrations.

After the optimum substrate concentration (5×10^{-3} *M*) had been determined a series of experiments at constant glutamate concentration (7×10^{-3} *M*) and a range of oxaloacetate concentrations ($0·83 \times 10^{-5}$ to $1·67 \times 10^{-2}$ *M*) was carried out. The *Km* for oxaloacetate was found to be approximately 9 μ*M*. At high oxaloacetate concentrations (above 5×10^{-3} *M*) inhibition of the transfer reaction occurs. This inhibition is of no biological significance since oxaloacetate is a very potent inhibitor of succinic and malic dehydrogenase at much lower concentration and could never build up to this level. It is, however, important to take this inhibition into account when kinetic investigations are attempted with less sensitive assay procedures.

A series of determinations of *Km* for the aspartate to α-oxoglutarate transamination were carried out with mitochondrial preparations from guinea pig liver and mammary gland and the purified pig heart enzyme. When the acceptor and donor concentrations are equal the following values for *Km* are obtained: guinea pig mammary gland, 1·15 m*M*, guinea pig liver, 0·77 m*M*, pig heart transaminase, 4·0 m*M*.

When the α-oxoglutarate concentration is reduced below that of aspartate, no significant reduction in the rate of the reaction is observed until the acceptor concentration is one-tenth of the donor concentration. This indicates that during the conditions of the *Km* determinations the reaction of aspartate with the enzyme is rate determining and the *Km* for the α-oxoacid is much lower than that for the amino-acid. This is analogous to the relationship between glutamate and oxaloacetate discussed above.

Latency and Metabolic Control

The latency of dehydrogenases in intact mitochondria has recently been discussed by Bendall and deDuve[13], and Boyd[14] describes similar phenomena for mitochondrial transaminase. In the report[14] on transaminase it is concluded that the activity for transamination from aspartate to α-oxoglutarate is increased up to three-fold on disruption of mitochondria. The following phenomena can be responsible for the so-called latency of transaminase and perhaps also of other mitochondrial enzymes.

(1) *Compartmentation and permeability* may make available only limited amounts of substrate to the enzyme and may select preferentially either internally generated or externally added substrates. Local accumulation of substrates and/or products can either accelerate or inhibit.

(2) *Structural alterations* of enzymes bound to mitochondrial enzymes or concentrated within compartments can also affect their activities. Several non-mitochondrial enzymes are known to be less active when they aggregate in solution. Fasella and Turano[15] have shown that transaminase forms a dimer with a dissociation constant of 10^{-8}–10^{-7} *M*. The dimeric form has only about 5 per cent of the activity of the monomeric form. Glutamic dehydrogenase, on the other hand, dissociates under a variety of conditions to form inactive sub-units[16]. It appears, however,

that many of the enzymes discussed are, at least partially, bound to the structural material of mitochondria and one has to be cautious in accepting that the conditions causing structural changes in enzyme molecules in free solution would cause similar changes in the bound form of the enzyme

Under physiological conditions a number of factors must co-operate in primitive control of mitochondrial pathways This is the type of control not involving the formation of new enzyme protein The transaminase activity of mitochondria is more than sufficient to convert all the oxaloacetate which can be produced by the citric acid cycle into aspartate, provided a reasonable concentration of glutamate (0 1–1 mM) reaches the transaminase The results of the present investigation show that the enzyme concentrations, kinetic parameters as well as the relation between oxaloacetate concentration and Km are consistent with the pathway for glutamate oxidation outlined in the introduction If it were indeed true that all the oxaloacetate is converted into aspartate, which in turn is used for the biosynthesis of proteins and nucleic acids, then the citric acid cycle would be disrupted There is insufficient information about the potential activity of condensing enzyme and about its affinity for oxaloacetate to predict whether the condensation reaction can compete successfully for oxaloacetate with the transamination system even in the presence of glutamate

The elucidation of the control of the citric acid cycle and of its supply of substrates and disposal of products is both extremely important and, because of its many complex control points, a great intellectual challenge This problem will be readily soluble when the type of information discussed for some enzymes in this article has been obtained for all the enzymes concerned and when tracer kinetic investigations with labelled intermediates have given full information about the relative availability for intramitochondrial enzyme reactions of externally added and internally generated substrates and products

This work was supported in part by a grant from the U S Public Health Service *RG*-4725 (*C*4)

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DIAPAUSE IN AN AQUATIC MYMARID

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CARAPHRACTUS CINCTUS Walker parasitizes the eggs of various Dytiscidae[1] and is easy to rear in captivity on the eggs of the water beetle *Agabus bipustulatus* L The entire development of this mymarid occurs under water within the host egg, the shell of which is transparent The larvæ, usually 2 or 3 in each egg, are clearly visible during their later stages, so the time of pupation is easily determined

Four to five generations of *Caraphractus* have been bred in a year in an unheated north room with a large uncurtained window at my house in St Andrews The last generation of the year undergoes diapause in the full-grown larval (prepupal) state. Such diapausing larvæ may be found in some eggs in September and usually in all eggs by the end of October Pupation occurs in the spring and the majority of the adults emerge from March to May The eggs of *A bipustulatus* do not diapause but develop at all times of the year when the temperature is suitable

In some parasitic Hymenoptera which diapause in the last larval instar[2,3], it has been found that maternal physiology may influence the incidence of diapause in the offspring, either by diet[2,3], by ageing of the females[3], or by rearing the females at low temperature[2,3] In *Caraphractus* the adult female has never been seen to feed, and observations on the laying of old females previously deprived of host eggs showed that retention of the eggs in the ovaries of this insect (which has a very short adult life) was not a factor promoting diapause in the offspring By exposing host eggs at various stages of parasitism to low temperature (5° C) in August, development was retarded, but there was no diapause in the chilled generation and no increase in diapause in the offspring

A succession of generations was obtained during the diapause season', September–March, by rearing *Caraphractus* in a constant-temperature room at 17 5° C in continuous fluorescent light of 9 ft -candles, each generation developing in a little more than three weeks, normally without diapause (Table 1, exp 2) When host eggs with young larvæ were transferred from this constant-temperature room to the north room where diapause normally occurs, the majority of the larvæ went into diapause, while the reverse experiment (moving parasitized eggs from north room to constant-temperature room) resulted in direct development of all the larvæ When females, reared in this constant-temperature room, were transferred to the north room to breed, they produced, from the middle of September to the end of December, 98 1 per cent diapausing offspring in 264 parasitized eggs Since the temperature of the north room, though variable, was mostly 14°–16° C and so not very much lower than the constant-temperature room, it seemed probable that the long hours of darkness at this season were responsible for inducing diapause in the developing larvæ

Table 1. PHOTOPERIOD EXPERIMENTS WITH *Caraphractus cinctus* WALKER

Ref No	No of separate experiments	Temperature (°C) av min. / av max	Dates experiments were started	Hr of light (tungsten)	Intensity of light (ft -candles)	Hr of darkness	No of host eggs with direct developing parasites	No of host eggs with diapausing parasites	Percentage of eggs with diapausing parasites
1	2	17 5 C T R	Dec 29, 1960	24	0 9	0	35	0	0 0
2	52	17 5 C T R	Aug 1960–Feb 1961	24	9 0	0	467	2	0 4
3	3	13 5/14 5	Nov 23–25, 1960	24	8 0	0	63	0	0 0
4	2	25/26 C.T R.	Nov 4, 1960	24	12 0	0	17	0	0 0
5	5	13 0/16 5	Feb 21–March 8, 1961	20½–21	8 0	3–3½	105	1	0 9
6	6	14 0/15 0	May 16–28, 1961	19	7 75	5	209	0	0 0
7	10	13 0/17 5	Jan 26—Feb 5, 1961	15	42 0	9	20	187	90 3
8	12	12 0/14 5	Dec. 16, 1960–Jan. 18, 1961	14	8 0	10	5	239	97 9
9	5	17 0 C T R.	Jan 11–14, 1961	8	7 5	16	0	27	100 0
10	11	13 5/15 5	Dec 15, 1960–Feb. 12, 1961	7½–8	8 5	16–16½	0	206	100 0
11	5	15 2/17 2	June 28—July 9, 1961	7½	7 75	16½	0	173	100 0

C T R, constant temperature room

Experiments with different photoperiods were then tried, first by exposing newly parasitized eggs during December to daylight from a north window and supplementing this with artificial tungsten light of 30 ft -candles in the evening, a method similar to that used for mosquito larvæ by Baker[4] The temperature averaged min 10 5°, max. 13 5° C With only 3 hr. of artificial light, all the larvæ went into diapause, with 6–6½ hr of artificial light the diapause was 88 3 per cent, though the total light (including morning twilight) was about 15–16 hr Controls, covered during artificial light, gave 97 per cent diapause Further experiments, some in ventilated tea-chests and others in constant-temperature rooms, showed that direct development occurred during continuous light, or with 3–5 hr of darkness (Table 1, exps 5 and 6)

The effect of a short photoperiod (7½ hr.) in promoting diapause from ovipositions in April and May has recently been demonstrated, for 98 6 per cent of 306 parasitized eggs so treated went into diapause, while 221 controls, exposed to daylight in the same (north) room at the same time, have all given direct developing *Caraphractus*

Experiments have been undertaken to find out the lowest intensity of light required during the night to prevent diapause By using daylight from a north window in February and March and two 5-W neon bulbs all night from 4 30 p m to 8 30 a m, and placing 264 parasitized eggs at varying distances from the light, direct development of all the parasites occurred, though the light they received at night was only 0 03–0 3 ft -candle Controls kept in the same room, in darkness during artificial light, gave 82 5 per cent diapause The temperature averaged min 12 5°, max 15 5° C Three further experiments were conducted in the spring in tea-chests, using for 15 hr a tungsten 25-W bulb giving 12 ft -candles and for 9 hr cresyl blue filters adapted to give light intensities of 0 8, 0 25 and 0 05 ft -candle As a result, all the successfully parasitized eggs in each experiment (a total of 513) gave direct developing *Caraphractus* The average minimum and average maximum temperatures in these three experiments were 13 5° C and 16 5° C

These results show that *Caraphractus* is sensitive in the larval state to light of extremely low intensity It seems likely that in ponds direct development will only occur with long daylight hours combined with faint light at night (twilight), or with a few hours of darkness. In other insects[5–8], long day-lengths at medium temperature promote direct development, with *Caraphractus*, 9 hr of complete darkness suffice to induce diapause in the great majority of specimens (exp. 7) Experiments have shown that low temperature in this insect is not the deciding factor, and in autumn, when reared in the north room, with an open window, diapause started before the cold weather set in. At the commencement of the 'diapause season' there is individual variation in the susceptibility of the larvæ, and eggs, parasitized by the same female within 5 min of each other, may yield either diapausing or non-diapausing *Caraphractus*. Moreover, it sometimes happens that the development of two individuals within the same host egg may be of both types, though the eggs producing them were laid at one thrust of the ovipositor This variation disappears as the hours of darkness lengthen

From May until August direct development normally occurs and this coincides with the long twilight of the summer nights and the absence of astronomical darkness The onset of diapause in the autumn sets in at a time when there are about 6 hr of astronomical darkness, which increases in early October to more than 8 hr ; this may well be the 'clock'[9] to which the larvæ respond. Once in diapause. *Caraphractus* can withstand some desiccation, even if the host eggs collapse during drought Diapausing larvæ can also survive for days in freezing water (0–1 to 0–3° C) and when removed to a suitable temperature, pupation may occur promptly When the larvæ are not subjected to frost, the diapause runs its course and usually extends over several months, but is sometimes greatly prolonged

The experiments in the constant-temperature rooms were carried out in the Natural History Department of the University of St Andrews and I am grateful to Prof H G Callan for affording me these facilities and to Dr C Muir for the valuable help he has so generously given me with technique and light measurement Prof and Mrs Stibbs have kindly provided me with useful information regarding the duration of astronomical darkness in the latitude of St Andrews

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Supplement to NATURE

No 4805 DECEMBER 2, 1961 Vol. 192

HUMANITIES OF SCIENCE

Science since Babylon

By Prof Derek J de Solla Price Pp x+149 (New Haven, Conn , and London Yale University Press, 1961) 4 50 dollars 37*s* 6*d*

THIS book, Prof Price tells us, had its origin in a set of five lectures given under the auspices of the Department of History at Yale University in 1959 It makes no claim to be a history—even a historical sketch—of 'science since Babylon', but rather by reflecting on certain special aspects of that history to gain some insight into why, or in any event how, science has come to be what it is, and thence to deduce the likely course of its future development The whole thus constitutes an essay in what Prof Price wishes to call "the humanities of science"

In five chapters the author approaches the question along three fairly distinct roads the thesis that the character of a scientific epoch is largely conditioned by the instruments available, hence by the special skills of the contemporary 'practitioners', a somewhat detailed examination of the great 'mutation' in physics consequent on Roentgen's discovery of X-rays, and a roughly statistical analysis of the growth of science deduced from such 'pointers' as primary periodical publication, abstracts, individual papers, numbers of active scientists and universities

Despite Prof Price's well-known virtuosity in the first field, here amplified by some fascinating 'revelations' of his methods, belief in the thesis is not likely to be strengthened by some of the arguments which are adduced What are we to make of the statement (pp 51, 13) " that it was the Germanic region of Luther rather than Catholic Italy that saw the revival of astronomy by Regiomontanus [d 1476], Kepler and Copernicus" ? Or by the description (p 61) of Hooke as an ingenious artisan " . who though reasonably educated [Westminster School and Christ Church, Oxford] had no special training in science other than by apprenticeship ['research assistant' to Willis and later Boyle] ?" Who among the early Fellows of the Royal Society, we may ask, had a better preparation in 'science' ?

On the other hand, Chapter 5, entitled "Diseases of Science", " its superabundance of literature, its manpower shortages, its increasing specialization, its tendency to deteriorate in quality"—together with the epilogue, entitled "The Humanities of Science", constitute for the reviewer alike the most convincing analysis of the terrifying *djinn* that optimistic Western man has released from its bottle, and a carefully considered justification for the establishment of 'humanities of science'—call it what you will—as a separate discipline For this reason *Science since Babylon* is an important, possibly a very important, contribution It is all the more regrettable that it is exorbitantly priced

W P D WIGHTMAN

A SHORT HISTORY OF SCIENCE

The Mechanization of the World Picture

By Prof E J Dijksterhuis Translated by C Dikshoorn Pp viii+539 (Oxford Clarendon Press , London · Oxford University Press, 1961) 105*s* net

TRIPLY laden by its burden of price, bulk and title, this book cannot be expected to join the ranks of best-selling general histories of science However, its contents should and probably will be made known to the ever-increasing audience for the history of science Since the publication of Burtt's pioneering work some thirty-five years ago, the 'metaphysical' side of the rise of modern science has received increasing attention, and there is now a fair selection of popular books presenting this story Up to now each author has had to dig for himself in the secondary literature, scattered among many journals and written in all the European languages Now those who teach and write on the history of science have a coherent and scholarly account, written in English and with a comprehensive bibliography There is no longer any excuse for ignorance Also, those who want to learn something of the history of science and are not satisfied by vulgarization, however high, can study this book with profit and satisfaction.

Prof Dijksterhuis gives us an example of how to write the history of science In each of the many topics he covers, he describes briefly what he is going to discuss, and (more important) mentions relevant topics which he is not going to discuss The history of science has been plagued too long by smash-and-grab methods where one 'scientific' contribution of a man is described and analysed, while the philosophical, religious or social thought in which the contribution was originally embedded is left unmentioned Also, it is clear that he has thoroughly mastered the technicalities of each problem, and not contented himself with understanding only that part which could easily be popularized This produces greater accuracy on small and large points His is the only general account of seventeenth-century mechanics which mentions the significant fact that Newton's laws do not use time-derivatives , "$F = ma$", as an explicit statement, dates from Euler Another example is his simplified diagram of the Ptolemaic world-system no other such diagram shows both the deferents of the inner planets, and the epicycle-arms of the other planets, as roughly parallel to the line joining Earth and Sun

The first half of the book is devoted to the background in ancient and medieval thought The two most attractive problems, cosmological speculation and the causes of motion in inanimate bodies, receive their proper place in the complex of philosophical problems on the nature of reality and the nature of change Prof Dijksterhuis covers the whole range of the exact sciences and philosophical thought in classical antiquity. He never describes a result without criticizing it, and from his criticisms emerges a coherent picture of the intellectual limitations of the 'science' of the time. He also has a good sense of what can be compressed, and what cannot; where the scientific technicalities are not important for the general story, they are pruned drastically (with a reminder of what is being left out). Aristotle's philosophy is given detailed attention, and the beautiful coherence of the whole system is exhibited clearly.

The section on scholastic 'science' is most illuminating. Through the work of Dr Crombie and Prof Clagett, we have been approaching a balanced appreciation of the achievements of this tradition, and developing general ideas on the causes of its fatal limitations. In each of his discussions Prof Dijksterhuis illustrates the interpenetration of scientific thought with problems of logic, metaphysics, and theology. He also sketches in the medieval world-picture, and shows clearly how this conditioned the manner of approaching, and solving, scientific problems. The characteristic 'double-think' solution of the practical problem of the conflict of science with theology is described as well as can be done by a scholar living in a free society.

The second part of the book, on the Renaissance and the seventeenth century (concluding with Newton), offers somewhat less. We are not within sight of a synthesis of what happened between Oresme and Galileo; and on the development of mechanics in the seventeenth century there is already a fair literature. This is not to say that Prof Dijksterhuis provides only well-worn material; his discussion of the continuous tradition in the problem of change and composition (What happens to the qualities of the original substances?) lights up untrodden pathways. But nowhere does he discuss in general terms the astonishing intellectual mutation which took place at the beginning of the seventeenth century, when a world-picture much like our own was suddenly conceived and propagated, rendering nearly irrelevant centuries of philosophical thought, and providing a framework in which modern science could be created.

It is unfortunate that the author was not able to revise the text (originally published in Dutch in 1950), for the present book is essentially based on work carried out in the 'thirties, with additions from the War and early post-war years. Much has been achieved in the history of science in the past decade; it is a tribute to Prof Dijksterhuis's maturity of judgment that his history could be enriched, but nowhere seriously challenged, by more recent work.

J. R. Ravetz

COSMICAL LANGUAGE

Lincos

Design of a Language for Cosmic Intercourse, Part 1. (Studies in Logic and the Foundations of Mathematics.) By Dr Hans Freudenthal. Pp. v + 224. (Amsterdam: North-Holland Publishing Co., 1960.) 45s.

ONE needs must take seriously a monograph in the series Studies on Logic and the Foundations of Mathematics edited (*inter alia*) by so distinguished a mathematical logician as L. E. J. Brouwer. If its ostensible aim were self-evidently *en rapport* with the title of the series, the editors of *Nature* could have chosen few people more unfitted than myself to respond to a request to review it. Their only conceivable motive for doing so is that I once (ten years ago) gave, at its request, an address (*Astroglossa*) to the British Interplanetary Society on the theme which the subtitle of this monograph discloses. The recapture of echoes from radar signals transmitted to the Moon had then lately made conceivable, if none the less unlikely, intercommunication between ourselves and beings, if such there be, on Mars or Venus.

This was confessedly a *jeu d'esprit* for an evening's entertainment, but it had an undertone of serious intention, as there disclosed. Should contact between ourselves and extra-territorial occupants become possible, the success of the enterprise would be a challenge compelling us to retrace our steps from traditional methods of teaching existing terrestrial languages parrot-wise by paradigm or by repetition. We should indeed have to undertake an exacting revaluation of the semantics of so-called natural languages and of syntactical obscurities which do not impede communication between individuals who share explicitly, or at least implicitly, a common social context of discourse. However fruitless *vis à vis* the ostensible outcome, the undertaking might therefore prove to be worth while if it put the spotlight on semantic refinements we should advisedly incorporate it in an ideally constructed global auxiliary, or if it exposed defects of imparting instruction in a so-called natural language if adopted as a medium of international communication.

Accordingly, I would be the last to dismiss as crackpot a project so grandiose as *Lincos*. Dr Freudenthal and I at least have common ground with respect to the initial stages of the programme. If we postulate the existence of hypothetical extra-terrestrial beings at least as intelligent as ourselves, our only common denominator of communication in the first phase of a protracted tuitional process would be the integers. Whence we may conceivably proceed by easy stages from conveying simple algorithms to non-visual geometry, for example, by exemplary definition of a radio signal *and so on and so on* to specify trigonometrical ratios as infinite series. How far the introduction of the symbolical logic of Dr. Freudenthal would help engineers on the job to carry out this assignment is not fully clear to me, but I concede that I dismissed lightly as too obvious the theme of a second stage here set forth by himself in a symbolism tailored as elsewhere to the Russell–Quine tradition of logistic. Following stage 1 (that is, the domain of mathematical discourse), Dr Freudenthal devotes a chapter to the concept of time. Here he proposes that we can make use of varying signal frequencies to establish what we each adopt as our units of time, whence *pari passu* our concept of frequency.

Thereafter, Dr Freudenthal (whose next chapter is "Behaviour", that is, relations involving different individuals at the transmitting or at the receiving end) parts company abruptly with me. I have stated, and still hold, that the next imaginable step would be to establish a common factual framework of semantic reference. At this stage, the only common field of factual experience which the parties could explore would be celestial occurrences (for example, eclipses, conjunctions and occultations), and this our vocabulary of time signals would have now made practicable. Thereafter, we might conceivably learn to convey the structure of our several planetary crusts and atmospheres in terms of spectrum frequencies It seems to me that the last topic about which we could hope to achieve understanding would be the actions of persons in general, and the concept of the ego in particular

How Dr Freudenthal sidesteps this hurdle, I do not follow It may be that he will make himself more intelligible later in Part 2 However, in justice to him, one must here say that his main pre-occupation is not with the factual sequence of the tuitional process In accordance with the aim of the series in which the monograph appears, his paramount concern is to equip the syntax of interplanetary (or intergalactic) communication with a symbolism consonant with the requirements of a two-valued logistic rejected by some mathematicians in the forefront of bull-dozing the foundations of mathematics as such and (in my view) totally inadequate for the requirements of the biological sciences. The outcome may usefully confer on symbolic logic an agreeably topical flavour for the relief of students compelled to take an examination in the subject From any other point of view, Dr Freudenthal has failed to convince me that his programme is very profitable, and my reluctance to concede that it is so gains force from two considerations

One is that examples cited to show how symbolic logic makes manifest semantic and syntactical pitfalls of common speech are not unfamiliar to students of comparative linguistics, who draw on a much richer (and more relevant) fund of material The use of two particles of relation should make this sufficiently clear In Teutonic and Romance languages we have one word for our *always* (German *immer*, Swedish *altijd*, French *toujours*), but Welsh distinguishes *bob amser* (= *each time*) from *yn wastad* (= *ceaselessly*) Again English *with* and French *avec* cover both Welsh *ag* (= *by means of*) and *gyda* (= *in the company of*)

Another doubt will disturb the reader who is unrepentantly sceptical about the extent to which logistic can make the semantic and syntactical deficiencies of common speech more explicit than can the study of comparative linguistics On p 104 ("Behaviour") we meet

$$\text{Ha Inq Ha}\cdot\text{—}\,t_1t_3\ \text{ Hb Enu ? = .Car Etc}$$

$$\text{Sed }t_1t_3\text{ Hb Cpu ? = Car Etc}$$

The intention in this context is to introduce a distinction between two uses of *but*, symbolically denoted TAN (Lat. *tamen*) and SED (Lat) Do we have to invoke logistic analysis to do so ? Assuredly no Dr Freudenthal has to explain this distinction in the text through the medium of common speech, but it will not necessarily be front-page news to a philologist or classical scholar already familiar with *tamen* and *sed* Since the engineer will have to recode in appropriate radio signals any symbols in which we formulate our curriculum of interplanetary (or intergalactic) tuition, all that is relevant to the credentials of what we use is whether the terms and constructions conform to the requirements of rigorous semantic rectitude If so, it is therefore difficult to see why we should add to his or her difficulties by using an unfamiliar code for what a more familiar one could do equally well with a little premedication

LANCELOT HOGBEN

A ROMAN CATHOLIC VIEW OF EVOLUTION

Evolution and Christians

By Philip G. Fothergill Pp xix + 15 plates (London Longmans, Green and Co , Ltd , 1961) 42*s* net

DR. FOTHERGILL, in addressing his fellows of the Roman Church on the matter of evolution, has undertaken an important and difficult task, and has carried it out with a meticulous thoroughness and a scrupulous integrity His book is an encyclopædia of information about the history, progress and present position of the case for evolution so far as this can be stated within the strict limit of Catholic dogma He has presented with a wealth of detail the familiar story of Darwin's evidence in the *Origin of Species*, of the subsequent developments in taxonomy and palæontology, of the impact of genetics and of the varied fields of study since involved He has then dealt at even greater length with heredity, the matter of chromosomes, genes, and mutations, with the relevance of cytology and serology, and with innumerable records of experiments with *Drosophila*. Finally he discusses problems of isolation, of the effects and limitations of selection and of hybridization, and faces the question how far does all this elaborate and highly technical research enable us to point to the appearance of new species arising under the conditions of neo-Darwinian theory and precisely scientific control

In these long and difficult records he does his best to state and expound the innumerable polysyllabic names that each research department applies to its subjects—a heroic task which makes his book almost incomprehensible to the layman—to furnish it with good and relevant diagrams and illustrations, and to insert quotations, short and long, from a large number of writers more or less well known He also quotes a few critics of his views, especially Dr Douglas Dewar and Prof G Gaylord Simpson He is himself very ready to admit that much of what he describes is inconclusive—some of it is also irrelevant His own final conclusion appears to be that though the mass of data give a strong case for accepting evolution as a hypothesis, the only clear examples of new species recent or demonstrable are those of allopolyploidal plants and fertile hybrids like the common hemp-nettle or Townsend's cord-grass—our notorious enemy in the battle of the Western Approaches

The fact is that his task as limited by Catholic orthodoxy is scarcely possible, and that for three chief reasons First, he realizes and complains that without a philosophy of science it is impossible to relate evolution to any coherent interpretation of the world as we know it, the details which he reports so carefully become trivial and meaningless Yet he is debarred by ecclesiastical authority from making any reference to philosophic evolutionists unless, like Sir Julian Huxley, they are avowedly hostile to the faith or like the one or two unknown Catholics whom he quotes accredited by it It is astonishing for an Englishman who has spent all his life with the problems of evolution to read a book on it in which there is not a single mention of Bergson or Lloyd Morgan, Samuel Alexander or A N Whitehead, of emergence or of holism or any other of the systems which have interpreted the process and made the details meaningful.

Secondly, and presumably because of the dogma that "animals have no souls", he never touches on the large and exciting field of comparative psychology, or on the evolution of the behaviour-patterns and instinctive responses which mankind has so obviously inherited and to which all post-Freudian analysis has directed attention. The idea that each and every "intellectual soul" of man is a special divine creation if tenable must surely be adjusted now so as to allow room for an age-old substratum of animalisms conditioning our mind and morals as they do our bodies.

Thirdly, he writes always within the strait-jacket of Papal orthodoxy, much of it formulated in pre-Copernican and all of it except one encyclical in pre-Darwinian days. Though much of this may be held to deal with matters outside the range of present-day science, it has, even so, corollaries of dogmatic status, for example, the historicity of Adam and Eve, which bear directly on his theme. Moreover, does he really think that in matters demanding not only religious but also scientific, historical and ethical experience, the Vatican is the proper and infallible authority to which every other expert must yield unquestioning obedience? CHARLES E. RAVEN

CLIMATES OF THE PAST

Descriptive Palaeoclimatology

Edited by A. E. M. Nairn. Pp. xi+380. (New York: Interscience Publishers, Inc., London: Interscience Publishers, Ltd., 1961.) 11 dollars; 80*s*.

GEOLOGISTS who have struggled to interpret past climates will welcome this book for its wealth of factual information and for the critical way in which most of the authors have approached their special interests.

The first chapter, apart from a brief introduction by the editor, is a summary by Lamb of the nature and causes of the world's climate to-day. It is not easy to read, but it is extremely informative. Then follows a series of chapters each written by a specialist and dealing with the separate criteria which can be used as a means of assessing at least some facet of climate. These may be pure physical measurements (palæomagnetism, the ratio of oxygen-16 to oxygen-18), geological observations (the dips of æolian sandstones, the evidence of the presence and extent of ice), physical chemistry (evaporites and red beds) or biological deductions (from vertebrate, invertebrate or plant fossils). Finally, there are four chapters in which are traced the climatic changes over a large part of geological time in Europe and North America, the Far East and Gondwanaland.

Flint, dealing with glaciation, perhaps stands on the firmest ground, for the conversion of water to ice has a well-known climatic relationship and the presence of glaciers or permanently frozen ground in many cases gives rise to conclusions about climate which are without ambiguity. Much more difficult to interpret are evaporites and red beds, and the authors of these sections are understandably cautious in their conclusions. This welcome attitude is indeed apparent throughout. In dealing with the oxygen isotopes, for example, Thorley does a great service in pointing out the assumptions which have to be made before this method can be used for temperature determinations—assumptions which can too easily be ignored by stratigraphers eager to use a physicist's measurements to support a theory.

Perhaps the biologists are the most cautious of the authors, for all stress the difficulty of using the present as a key to the past when ancient floras and faunas are specifically and generically so different from those of to-day. On the whole, the greatest reliance is placed on anatomy, for it seems that certain modifications of structure, similar in widely diverse creatures, are adaptations to certain extremes of climate.

Throughout the book, emphasis is placed on polar wandering and continental drift. The palæomagnetists accept it without question, for their results would be largely meaningless without it, but they still have a long way to go before they present a conclusive and agreed story. Thus Nairn's equator-positions for the Trias of Europe (Chapter 7, Fig. 9), Africa and North America (Fig. 7) seem to provide an insoluble problem of drift if his three lines are to join into a continuous girdle at one moment of time. The palæontologists are more non-committal about polar shift, while conceding the necessity for great climatic change in the present polar regions. Van Houten's examination of the significance of red beds tends to support wandering rather than static poles, but on the other hand, Green feels able to explain the distribution of evaporites without recourse to drift or polar change.

As a whole, the book is a mine of information and both a stimulus to enthusiasm and a corrective to over-enthusiasm in approaching a difficult problem. Its copious references are international in character though scanty from beyond the Iron Curtain. It would be churlish to point out a few misprints and errors of translation and more to the point to congratulate the editor on welding together the contributions of fifteen authors from seven countries.

F. W. SHOTTON

BIRDS OF TROPICAL PARADISE

Birds of Trinidad and Tobago

By Dr. G. A. C. Herklots. Pp. 287+20 plates. (London: William Collins, Sons and Co., Ltd., 1961.) 42*s*. net.

THE islands of Trinidad and Tobago, the southernmost of the West Indian chain, have a combined land area of about 2,000 square miles, Tobago being very much the smaller, and at 10° N. and about 10 miles from Venezuela on the South American mainland, they are truly tropical. Being small islands they possess a high proportion of coastal land; in Trinidad there is a range of mountains (in the north) topping 3,000 ft. in two places, abundant deep forests, lowland plains, savannahs and extensive swamps; a wide range of habitats that encourage a wide variety of bird species both exotic and interesting.

Without undertaking an expensive safari (of the type so popular with programmers of television) one may seek out and enjoy the sight of a flock of flamingoes (*Eudocimus ruber*) either feeding in swampy land or, at sunset, a blaze of scarlet glory winging its way to roost—a sight not easily forgotten. I have, indeed, photographed a group of these birds feeding quietly on a plot of low-lying coastal land not two miles from the capital city, Port of Spain.

From the islands off the north-west peninsula one may film with ease the superb aerobatic displays of flocks of frigate-birds (*Fregata magnificens*—wingspan up to 7 ft.) as they swoop gracefully to snatch small fish from the surface of the sea with a downward flick of the head, then wheel aloft again, frequently challenged in mid-air by their comrades eager to snatch their prize

In the cool of the thick forests of Trinidad one may see the gaily dressed toucan (*Ramphastos vitellinus*) perched high in the tree-tops or hear its hard crackling call, or one of several parrots and parrakeets common in the forests Even more spectacular are the brilliantly coloured macaws which may be found in and near the Nariva swamp But one may have to look longer to discover the giant nightjar (*Nyctibius griseus*), which is rare and evasive, assuming an erect posture to simulate the elongation of its perch, when danger is nigh It is said that a museum once offered £100 for one of its eggs There is the fascinating guacharo or oilbird (*Steatornis caripensis*) to be found in the caves at Aripo and elsewhere The oilbird nests from year to year in the same place on ledges high on the walls of the cave, so that a truncated cone of guano is built up, in the cupped top of which the new eggs are laid The effect is of rows of artificial stalagmites They feed on the oil-rich berries of certain palms and the young become veritable 'balls of fat' from this rich diet When disturbed an alarm call, like "the cries of tortured men", rings through the cave Being completely nocturnal they are provided with a form of 'radar' which enables them to move freely in the dark caves

On the islet of little Tobago is another interesting ornithological attraction, for here may be seen the greater bird of paradise (*Paradisea apoda*), which was brought from the Aru Islands off Dutch New Guinea to this sanctuary by Sir William Ingram in 1909 His sons donated the island to the British Government on the condition that it be maintained, *in perpetuum*, as a bird sanctuary

Then there is a group of what must be among the most beautiful of all birds—the hummingbirds (Trochilidae) Trinidad has for long been known as the land of the hummingbird' and it is re-assuring to hear from Dr Herklots that there is little reason to suppose that they are not as abundant as ever In all, 17 species are listed, of which Herklots has seen 15 in his seven years on the island Some species are certainly very common and may be observed in gardens in the heart of town, flashing like priceless jewels in the strong sunlight as they sip nectar from flowers such as the ixora or Barbados pride (*Caesalpinia pulcherima*), hovering, humming, perkily prying at each bloom in an inflorescence, then away, with incredible speed

These are but a few of the 415, or so, birds described in this most welcome book One may say that it has been awaited for half a century, since the last comprehensive, descriptive guide to the birds of Trinidad was written (in French) by A Leotaud in 1866 While in its own way Leotaud's book is a classic, it could scarcely be said to be *au courant*, and is in any event rare and almost impossible to borrow Unfortunately, Dr Herklots' book does not bring us quite up to date as he has not managed to find a proportion of the birds listed in the book and has drawn heavily on Leotaud and on the publications of Sir Charles Belcher and G D Smooker, *On the Birds of the Colony of Trinidad and Tobago*, to be found in *Ibis* between 1934 and 1937 This in no way detracts from the practical utility of the present volume and Dr Herklots must be congratulated on the efficiency and clarity of his presentation The glossary, blessedly, precedes, instead of follows, the descriptions and there is a short introduction with two maps For each species there is a precise description followed by notes on nidification, habitat, voice (not in every case), distribution and field identification The plates illustrate some 260 species, about 180 in colour, and are, on the whole, very good indeed, but the majority are head-and-shoulders only Since most of the species are also to be found on the neighbouring mainland, this book is a useful guide for English-speaking naturalists in Venezuela, the Guianas and Brazil, but above all to those interested in the birds of Trinidad and Tobago, this is now the guide of prime importance and, as such, an indispensable one, 'completing' Dr James Bond's *Birds of the West Indies* which omits these islands My only regret is that no photographs were included which would have given those who have not visited the islands some small impression of their charm

L B. QUESNEL

AN ARACHNOLOGICAL ANTHOLOGY

Spiders, Men, and Scorpions

Being the History of Arachnology By Theodore H Savory Pp. 191+15 plates (London University of London Press, 1961) 30*s* net

IN spite of their relatively small numbers, arachnologists have a liking for writing about each other as well as about their subject, and without doubt this has enriched the literature We have already had biographical accounts by W S Bristowe and Pierre Bonnet, and Mr Savory has now taken this a good deal further in his latest book

He has again demonstrated his versatility as a writer in this history of man's relationship with spiders, scorpions and other arachnids, which begins with legend in Ovid's story of Arachne to the outstanding names in arachnology in modern times The first book devoted entirely to spiders was apparently published in 1544 in Basle, but until the seventeenth century the literature appears to have added more in the way of superstitions to the knowledge of arachnology than original observations The story of the development of tarantism, a curious disease which was supposed to be caused by the bite of the tarantula (*Lycosa tarantula,* a wolf spider), is full of interest, however, even though the mystery of how it came about remains The distressing effects supposed to have been produced by the spider's bite could only be relieved by music and dancing, and to-day the only vestige which remains is a dance, the tarantella, probably one of the few contributions made by an arachnid to human culture

Inevitably in a work of this kind a good deal of reference must be made to numbers of minor and insignificant writers for the sake of keeping the record complete Occasionally this makes the reading rather tedious in spite of one's admiration for the industry of the writer in tracing historical data Most of the great names which are familiar to modern students of arachnology are referred to in the later chapters of the book, and Mr Savory's enthusiasm and style give a fresh and personal picture of people who to most of us have never been more than a name attached to monographs or scientific papers The outstanding workers, such as Simon, Pickard-Cambridge, Koch and Thorell, worked mainly on the Araneida, and a good deal of the book is concerned with work on this order Mr Savory considers that the foundation of spider taxonomy was laid by French and German students in the early nineteenth century, of which the publication of Hahn and Koch's *Die Arachniden* in 1831 was the most striking example John Blackwall for Britain made a major contribution in 1861 with his Ray Society volume on *British Spiders*, and the three countries Britain France and Germany perhaps remained pre-eminent in this field until recent times Mr Savory's review does not include post-1958 works and consequently misses H Wiehle's monograph on the Micryphantidae, one of the most important taxonomic works since the War He is perhaps a little unfair to the younger generation of German zoologists, which, he says, "has only just begun to appreciate the Arachnida as subjects for research" In fact, since 1942 more work has been carried out in Germany on the biology and ecology of spiders than probably any other country

This and recent work in other countries emphasize an important historical point not made by the author that the taxonomy of European spiders is now at least sufficiently well known for the main interest of the younger generation of arachnologists to concern themselves largely with population and biological problems This would appear to be the field of investigation for the future, although outside Europe, faunal surveys and taxonomic studies will continue to be necessary for many years to come.

E Duffey

BRITISH MOTHS

The Moths of the British Isles

By Richard South. New edition, edited and revised by H M Edelsten, D S Fletcher and R J. Collins First Series Sphingidae, Endromidae, Saturniidae, Notodontidae, Thyatiridae, Drepanidae, Lymantriidae, and Noctuidae Pp 427+148 plates Second Series Lasiocampidae, Arctiidae, Geometridae, Cossidae, Limacodidae, Zygaenidae, Sesiidae, and Hepialidae Pp 379+141 plates (The Wayside and Woodland Series) (London and New York Frederick Warne and Co , Ltd , 1961.) 35*s* net each volume

INTENDED primarily as a guide to the identification of the larger British moths, this new edition of a standard work fulfils its function more successfully than the editions of 1923 or 1939 mainly through the introduction of improved and completely new colour-plates The original paintings, from which the colour-plates were prepared, are with very few exceptions excellent In the few copies of the new edition examined by me, however, the colour-plates do not always attain the same high standard as the artist's paintings there is a lack of register in many plates and in several of them the red element is weak, resulting in unnatural greenish tones In spite of these drawbacks the plates are far superior to those in any except the first edition, and several plates (especially those of the genus *Eupithecia* Curtis) are as good as any illustrations previously published in Britain Many of the plate legends (for example, Series 1, plate 97) are unfortunately most confusing and will clearly have to be rearranged by the publishers in subsequent editions. References to the corresponding pages in the text have been omitted, as in previous editions, and numerous species exhibiting little sexual dimorphism continue to have both sexes illustrated Of some significance is the introduction of references in the text to figures of the genitalia of some of the more difficult genera (for example, *Procus* Oken and *Hydraecia* Guenée), an indication that externally the species of these genera are extremely difficult to separate and that the colour-plates are of little use as a means of identification The reproduction of many of the half-tone figures is very poor.

Since the publication of the first edition in 1907 the bulk of the text has remained unaltered and, although a thorough revision was clearly overdue, to remedy the haphazard and inconsistent treatment of many species, the opportunity to do this has not been taken New text is therefore restricted mainly to passages of supplementary information, leaving most of the remainder almost exactly as it was more than half a century ago

An important addition is the detailed treatment of several little-known moths, such as *Calamia tridens occidentalis* Cockayne A further improvement is the adoption for the first time in this book of the correct form of scientific nomenclature in accordance with the International Code

The unsystematic arrangement of the various families of moths is regrettable but, as the editors state in the introduction, this is the only way in which both plates and text could be arranged to avoid dividing the section on Noctuidae between the two volumes (a disadvantage of previous editions)

It is remarkable that in spite of the attention given to Lepidoptera by British entomologists, the editors have seldom been able to cite the sub-specific identity of the British representatives of a particular polytypic species—a result of the paucity of taxonomic work on our fauna at the specific and subspecific levels

Although several references to other literature are given in the text, there is still a need for a short bibliography, which could have included some of the more useful general works on British and European Lepidoptera No more than the usual number of minor errors seem to have arisen, but some of these lead to unfortunate confusion concerning identification, for example, on page 347 of Series 1 ab *carthami* Herr -Schaf is wrongly stated to be illustrated by Fig 11 on pl 126

It is disappointing to find that while this new edition is certainly an improvement on those it follows, it does not fulfil the expectations of many entomologists who had hoped for a more radical revision of the text

A Watson

THE GROWING TIP OF PLANTS

Apical Meristems

By Dr. F A L Clowes (Botanical Monographs. Vol 2) Pp viii+217+32 plates (Oxford Blackwell Scientific Publications , Springfield, Ill Charles C Thomas, 1961) 37*s* 6*d* net

DURING the past twenty years or so, there have been many investigations of the apical meristems of plants, an interest which is understandable since these meristems are in a sense regions of permanent embryogeny responsible in most plants for all longitudinal growth and the continued production of new organs The early investigations of shoot and root apices were mainly anatomical, but in a considerable number of the recent investigations biochemical and experimental approaches have shed new light on the behaviour of the apical meristems Although special aspects of this recent work have been reviewed previously, there has hitherto been no reasonably comprehensive account of both root and shoot apical meristems This need has now been met by the new book written by Dr Clowes

Although Dr Clowes' own research work has been mainly on root apices, shoot meristems receive adequate consideration; in fact eleven of the seventeen chapters deal mainly with shoot apices Recent work involving new techniques is discussed in some detail, but the anatomical side is not neglected Chapters on planes of division in shoot and root apices include discussions of the tunica-corpus hypothesis of the shoot apex and of the similar, but less well-known, Korper-Kappe hypothesis of the root meristem A complete chapter is devoted to the bearing of the evidence from chimeras on controversies relating to the shoot apex The sections of the book dealing with the rates of cell division in both root and shoot apices are particularly informative, and it would seem that the newer techniques, including autoradiography, have demonstrated the existence of a 'quiescent' centre in the root apices investigated It has also been shown that, in opposition to the views of the French school, there is no 'méristème d'attente' in the shoot apex

The book is not restricted to a consideration of apical meristems in the narrow sense, but covers in addition such topics as the development of leaves, the determination of sites of leaves, the differentiation and origin of procambium, latitudinal growth in apices and the formation of floral apices There is also a detailed consideration of differentiation in the root apex

The text figures of apices are not very numerous, but are supplemented by 32 photographs including some striking autoradiographs of shoot and root apices The clear readable style is an admirable feature of the book, which is a useful addition to the new series of botanical monographs. A ALLSOPP

ELECTROLYTES AND PLANT CELLS

Electrolytes and Plant Cells

By Prof G E Briggs, Dr A B. Hope, and Prof R N. Robertson (Botanical Monographs, Vol 1) Pp x+217 (Oxford Blackwell Scientific Publications; Springfield, Ill · Charles C Thomas, 1961.) 40*s* net

THE stated purpose of this new series of Botanical Monographs is to inform the advanced student, assist the lecturer and be a point of departure for the specialist The first volume admirably fulfils these aims

Forty pages at the beginning are devoted to an account of the physical properties of electrolytes in solution The principles of ionic exchange receive little attention, but diffusion and Donnan equilibria are dealt with handsomely Plant cells merit no more than passing mention until Chapter 4, which contains an elementary account of the gross structure of three types Chapter 5 deals with fine structure as revealed, albeit still imperfectly, by electron-microscopic investigations One is left wondering how soon it will be possible to apply the physico-chemical principles outlined earlier to the immensely complex and ever-changing cytoplasm At present only the inert cellulose wall seems amenable to such an approach

The concept of 'free space' receives detailed consideration in Part 2 For the benefit of the uninitiated, free space may be defined as that part of a cell or tissue into and through which solutes move readily by physical processes The authors have at various times separately suggested that cytoplasm forms part of the free space They now conclude that it can be adequately accommodated in water-filled spaces in and around the cell wall This is a return to the classical view—never relinquished by more conservative plant physiologists, who are unable to accept the idea of cytoplasm being freely accessible to the vicissitudes of the environment The possibility that a small but more interesting fraction of the free space may be associated with the cytoplasm is still not excluded, but adequate evidence is lacking

Part 3 is concerned with the active absorption of salts and its connexion with metabolism The principles of an ion-carrier mechanism are set out, and a general scheme linking anion transport to electron flow is outlined. Cation movement is said to occur passively along an electro-chemical potential gradient established by anion movements No explanation is offered for the active extrusion of sodium ions which occurs in some algae and in yeast It is surprising that Conway's 'redox pump' is not mentioned in this context since it seems to differ fundamentally from the mechanism proposed only in that it is designed to transport cations rather than anions Presumably in the authors' minds yeast is not looked on as a respectable plant cell, but surely it is unwise to confine one's attention narrowly in discussing such a fundamental biological process as ion-transport

In a short final section, some aspects of salt absorption and transport in organs and intact vascular plants are reviewed It is evident that adequate understanding of these processes which involve yet another level of complexity must await further knowledge of ion movements at the cellular and sub-cellular levels JAMES F SUTCLIFFE

ORCHIDS OF TRINIDAD AND TOBAGO

Native Orchids of Trinidad and Tobago

By Dr. Richard Evans Schultes (International Series of Monographs on Pure and Applied Biology Division Botany, Vol 3) Pp x+275+21 plates. (London and New York Pergamon Press, 1960) 100*s* net

THE third volume in the Pergamon Press's Botanical Division of its new series of Biological Monographs endeavours to maintain the standards set by the two previous volumes of Dr Turrill and Dr Bor on general botany and Indian grasses respectively

Constituting the second largest plant family in Trinidad and Tobago the 180 species of Orchidaceae occurring there merit an account The continued use of Trinidad as an exporting centre for American plants, combined with the fact that so many of its orchids are at the same time beautiful, horticulturally amenable and scientifically interesting, doubly merits an account of the colony's orchids This was realized long ago, and Grisebach (1864), Cogniaux (1910), Broadway (1918–32) and Wilson (1925) all published accounts of the species of the area, but it has been left to Dr Schultes to produce this comprehensive account dealing with Trinidad and Tobago only The classical Flora style that he adopts is admirably suited to the needs of present-day botanists, growers and collectors

The first pages are concerned with a general treatise on the orchids followed by a historical account of collecting in Trinidad and Tobago and the subsequent research There are dichotomous keys to all genera and species, and constant usage during the past few weeks has shown these to be as useful and accurate for determining pressed specimens as for living plants

The main text is composed of a description of each genus followed by a specific key and the descriptions and synonymy of each species The arrangement of genera follows that of Schlechter, which most herbaria nowadays adopt To have the genera grouped according to their 'affinities' is obviously of great use, but, unfortunately, the arrangement of species follows the same rule as in other recent American Floras, that is, alphabetical Affinities between species are therefore not indicated and this can be disastrous when attempting to identify species of a critical nature especially when the keys are also avowedly artificial The species are numbered, but this is quite pointless as the numbers follow the alphabetical sequence

Taxonomic science is a matter of opinion and no criticism should be levelled at Schultes concepts, but nomenclature and phytogeography are more absolute sciences and it is in the interests of all that a few errors be mentioned

The Jack Spaniard, a commonly cultivated species, is named (p 180) as *Gongora maculata*, but this should be *G. quinquenervis*, a name published for this entity thirty-five years before *G maculata*. as indeed the author states in his synonymy *Stanhopea grandiflora* (p 177) is an incorrect name as the epithet *grandiflora* was not published in Loddiges' *Botanical Cabinet*, which was just a book of coloured plates with none of the descriptions or dissections necessary for validation of the names The correct name is *Stanhopea eburnea*, this epithet being published earlier than the validation of *grandiflora* A much more complicated case is that of *Polystachya luteola* (p 154), correctly *P extinctoria* (for a full discussion of this see *Taxon*, 9, No 5, 150, 1960)

A more serious misleading mistake is that the term *ex* is erroneously used Its use is reserved as a conjunction linking the authors' names when the later author published an earlier author's manuscript name In his citation of *Maxillaria liparophylla* (p 210) Schultes authorizes it with Summerhayes *ex* Sandwith, whereas it should be Summerhayes *in* Sandwith Sandwith did not take up an unpublished name of Summerhayes but Summerhayes actually published the name in a general account by Sandwith

A common misconception in the United States is perpetuated in page 162 concerning *Eulophia alta* being introduced into Africa from America Its widespread distribution in Africa suggests that this is impossible and that this species should join the list of those common to the Old and New Worlds

The book is typographically well produced and well printed on a white paper that does credit to the most excellent line-drawings selected from the archives of the Oakes Ames Orchid Herbarium at Harvard Species representative of most genera are illustrated by the pens of such artists as Elmer Smith, Gordon Dillon, G C K Dunsterville, Blanche Ames and many others, and although they have all appeared in previous publications this in no way distracts from their utilization here

It is unfortunate that photographs were used so obviously as an afterthought, probably under the erroneous impression prevalent to-day that photographs boost sales Good photographs of well-selected species probably would help the publication, but poor photographs, ill chosen (of species already amply illustrated by the line drawings) as are those in this book, increase the price but detract from the value The photographs are of no conceivable æsthetic or scientific value whatsoever

In all fairness the book does enable one to achieve the prime object of a Flora—to identify plants , but just a little more care and thought would have rendered it so much more useful

P Francis Hunt

ECOLOGICAL BRANCH OF RUSSIAN PARASITOLOGY

Parasitology of Fishes

By V. A Dogiel, G K Petrushevski and Yu I Polyanski Translated by Dr Z Kabata Pp x + 384 (Edinburgh and London Oliver and Boyd, Ltd , 1961) 84*s* net

THIS book was inspired by the wish of Valentin Alexandrovich Dogiel to institute collective discussion of theoretical and practical problems involved in the parasitology of fishes Wearied by lists and descriptions of new species, he yearned for knowledge about the whole life of the parasitic fauna which he foresaw as a weapon in the fight against parasitic diseases In 1927 he founded this ecological branch of Soviet parasitology and in 1930 the parasites of fishes became a priority study The vast territory of the U S S R comprises most of the habitats of fishes which exist on the Earth, and ultimately all the main rivers, most of the large lakes and both internal and bordering seas were being examined in great parasitological detail He wrote the first chapter of this book himself, and most of the others were written with his guidance Regrettably, he died on June 1, 1955, but in 1958 his wish was to some extent fulfilled when the Russian original of this book was published

Dogiel recognized the prime peculiarity of the parasite, that its environment is furnished by another living organism, the host I do not believe that his choice of fishes as hosts was accidental, because this is the most ancient class of the vertebrates and it harbours more parasites than does any other class Moreover, fishes are important in relation to food supply, and in this book we can recognize a great urge to implement research for economic reasons Dogiel's study of the dynamics of assemblages of parasites inhabiting one host ('parasitocœnoses') convinced him that the environment of the parasite comprises the host (the micro-environment) and the habitat of the host (the macro-environment) The composition of the parasitic fauna of fishes depends on changes in the habitat of the host and changes in the physiological states of the host Geographical location, the season of the year, characteristics of water and bottom in rivers, lakes and the sea, related organisms in and around the habitat, and many other biotic and abiotic features of the environment provide the means of life for host and parasites alike The composition of the parasitic fauna is determined also by the nature of the food and digestive equipment of the host, and other influential factors are age, general condition as indicated by states of nutrition or inanition times of spawning, migration, hibernation and even speeds of locomotion The nature of the integument of the host and its secretions influence parasites which penetrate the skin, some of which are parasites of man Many patterns of correlated environmental factors fluctuate periodically and modify the composition of the parasitic fauna

Dogiel's influence is seen in various chapters of the book dealing with such topics as the ecology of parasites of marine and freshwater fishes, questions concerning host-parasite relationships, specificity, life-cycles and zoogeography Some consideration is given to the parasites of fishes living in reservoirs, changes in the parasitic fauna of acclimatized fishes, parasitic diseases of fishes living in natural waters and also cultured fishes, and fishes as transmitters of human helminthic disease The parasites considered are mainly Protozoa, Platyhelminthes, Nematoda, Acanthocephala and Copepoda Many isolated observations provide interesting revelations but extensive regional surveys may give the specialist more satisfaction The format is pleasing, the translation is readable, and the text is illustrated with diagrams and maps and supplemented by comprehensive tables More than 700 references to the original literature are cited, nearly two-thirds of them from the Russian Errors are few and mainly typographical but sometimes surprising the statement on p 105, "Monogenoideans with a single host", refers to several digenetic trematodes but no monogenetic forms

Students and teachers will find in the book a fund of readily assimilable information, and if the appetite of the specialist is sometimes unsatisfied the gaps more often than not indicate the opportunities for research workers in the future All concerned with the production of the book deserve credit for giving biologists this opportunity to evaluate Russian progress in a branch of parasitology which has been neglected in Britain BEN DAWES

SALT MARSHES AND SALT DESERTS

Salt Marshes and Salt Deserts of the World

By Prof V J Chapman (Plant Science Monographs) Pp xvi + 392 + 45 plates (London Leonard Hill (Books), Ltd , New York Interscience Publishers, Inc , 1960) 95*s* net

LET the good things be placed on record first This book brings together a wealth of information from widely scattered and often inaccessible sources and illuminates it with the author's own long experience of salt marshes Any student undertaking work in this field in the future will certainly start by "looking it up in Chapman" For this service all botanists will be grateful, but gratitude need not blind us to the book's many faults, nor will it arm the struggling reader against exasperation For the book is, frankly, not so much written as thrown together

It opens inauspiciously with a chapter which hovers uneasily between a statement of intentions and a summary of findings This is followed by three chapters on general features of the habitat, the chief fault of which is that they are insufficiently general, all too often a promising principle collapses into a detailed study of a specific example In the next five chapters we are taken on a conducted tour of the salt marshes of the world, and here the author is at his best, we realize that information about the different areas will be uneven, and presentation of these scattered and largely unrelated data is congenial to the author's discursive style Elaborate and detailed successional schemes are, however, given with an off-hand confidence which is likely to arouse misgivings Finally, there are three miscellaneous chapters The first of these summarizes the author's own views on the successions and phytosociological entities represented, and these are most welcome, but the chapter continues with a quantitative study of the affinities between communities which suggests that the author is unaware of the difficulties in devising and interpreting parameters suitable for this type of comparison The chapter ends, unaccountably, with a brief account of salt-marsh fucoids Then follows a disastrous chapter on "Physiology of Halophytes", in which the whole of the thinking concerning transpiration is out of date, and the statements concerning ion uptake simply fail to make sense The book ends with a brief but interesting account of economic uses

It is all clearly the work of a man passionately devoted to his subject, avidly and uncritically welcoming any scrap of information which comes his way, but still too involved in minutiæ to discern underlying patterns If Prof Chapman, secure in his remarkable knowledge, had brooded over his information for a year or two, and then written, and re-written, and revised, he might have given us a new classic, as it is, botanists can only weep for what might have been and go and buy the book W T WILLIAMS

MICROSCOPY IN A MAZE

The Encyclopedia of Microscopy

Edited by Prof George L Clark Pp xiii + 693 (New York Reinhold Publishing Corporation, London Chapman and Hall, Ltd , 1961) 200*s* net

THIS is not an encyclopædia in the ordinary sense, but a collection of 140 articles There is no index, and the subject titles are not always helpful It is thus very difficult to look up any specific point The articles are strung together with little correlation or cross-reference, leading to useless repetition or omission of important topics The two articles on interference microscopy cover much common ground and contain several similar diagrams, but no adequate discussion of phase contrast is to be found There is a good article on the design of the polarizing microscope for detecting very weak birefringence, but no proper description of the basic instrument or its use in crystallography Fluorescence microscopy is represented by two pages from a commercial brochure Fluorescence labelling of proteins, which has led to one of the most important advances in modern biological microscopy, is not mentioned

The section on ultra-violet microscopy contains three pages on methods, with almost no discussion of results or applications, one page on colour translating television systems (with no references) and more than nine pages on "Image Formation by a Fresnel Zone Plate" The latter article might find a place as an original paper in a specialist journal, but why it was given so much space when so little was devoted to ultra-violet microscopy itself is a complete mystery

This lack of balance and bad choice of material is seen throughout the book There is virtually no discussion of resolving power, no description of light sources, no section on photomicrography, yet 30 pages are devoted to non-microscopic refractometry This section is remarkable, for although it includes descriptions of many refractometers and interferometers, it contains not a single diagram of any sort Electron microscopy is equally unbalanced Biological microscopy has been revolutionized by new methods of fixation, embedding and ultra-thin sectioning, but the article on specimen preparation deals only with special non-biological methods used at Los Alamos Such an omission can only be described as fantastic.

The choice of biological subjects is quite extraordinary Thirteen pages are devoted to "kidney ultrastructure", but this is the only organ dealt with Why kidney ? Why not liver, glands, digestive or nervous systems, for example ? One turns to the heading "Pathology" in the hope of reading a much-needed general article on the use of electron microscopy in this field, only to find that another thirteen pages are devoted entirely to the kidney Unfortunately, one could go on writing in this vein for quite a long time Any book of 700 pages is likely to contain some useful information, but it is a pity to see so much effort wasted by bad editing and poor selection of material R Barer

STUDIES IN ENDOCRINOLOGY

Études d'Endocrinologie

Séminaires 1960 de la Chaire de Morphologie Expérimentale et Endocrinologie du Collège de France dirigés par le Professeur Robert Courrier. (Actualités Scientifiques et Industrielles, No 1286) Pp xiv + 350 (Paris Hermann, 1961) n p.

SINCE 1959 some of the advanced courses of lectures which it has long been the tradition for professors of the Collège de France to deliver have been replaced by public seminars at which eminent research workers are invited to discuss the results of their studies The records of two such occasions, both arranged by the Department of Experimental Morphology and Endocrinology, have now appeared

The first deals with the gonadotrophins, and was organized jointly by Prof Robert Courrier and Dr Marian Jutisz The published proceedings provide an up-to-date account of the various gonad-stimulating hormones which can be extracted from pituitary tissue, from the urine of pregnant women, from human urine after the menopause, and from the blood serum of pregnant mares An account is also given of the relationship of sheep pituitary prolactin to other gonadotrophins The papers which describe these various hormonal substances are published in full, with a record of the discussion which followed each day's presentation Four general papers concluded the seminar, and each raises some issue of wide importance The first, by Prof Max Aron, takes up the much-debated question of the degree to which, both qualitatively and quantitatively, luteinizing and follicle-stimulating hormones are distinct hormones The experiments on the guinea pig which he reports tend to indicate that as entities they are not as separate as is usually taught Dr Robert Henry's contribution on the biological dosage of hypophysial gonadotrophins leads to a contrary view, however, with respect to follicle-stimulating hormone and interstitial cell-stimulating hormone, and the paper by Dr Pierre Aschheim—the son of the Aschheim whose name is associated with that of Zondek in the discovery of human chorionic gonadotrophin—also supports the view of the separate chemical identity of various gonadotrophins In the general discussion following the presentation of these papers, follicle-stimulating hormone, interstitial cell-stimulating hormone, the post-menopausal urinary gonadotrophin of women as well as pregnant mare serum were, none the less, set aside as substances which had not yet been isolated in a state of sufficient purity to be regarded as chemically separate Human chorionic gonadotrophin and prolactin, on the other hand, seem to satisfy the criteria of chemical purity, although it was made plain that the criteria of purity are themselves arbitrary

The broad conclusion which emerges from the whole discussion is that much more work will need to be done before we can expect to understand the underlying chemistry of the reactions of the ovaries and testes to the numerous gonadotrophic preparations which the biochemist has already isolated Of facts about gonadotrophins we have plenty, what we lack is an underlying hypothesis which strings them together

The second part of the volume records a seminar arranged by Dr Roger Guillemin on the functional relations of the hypothalamus to the adenohypophysis, with particular reference to the release of adrenocorticotrophic hormone In this field of research the facts, while also numerous, seem somewhat more untidy than those which were dealt with in the first seminar—while of hypotheses there is not a single one which is not characterized more by empty speculation than by predictive value

The opening paper in this symposium was by Prof André Delmas, and dealt with the anatomy of the hypothalamus and its connexions Prof Delmas was followed by Dr Henri Duvernoy, who reported a series of observations on the vascular connexions between the adenohypophysis, the neurohypophysis and the median eminence Contrary to the fashionable belief that has been so assiduously disseminated in recent years, it is now clear that the vascular system of the anterior pituitary connects freely not only with the pituitary portal vessels, but also with the vessels of the neurohypophysis and, through the portal vessels, with those of the hypothalamus This observation is a further nail in the coffin of the theory, based on 'negative' observations, that the pituitary portal vessels form a unique connexion between the hypothalamus and the anterior pituitary, and that through them passes some chemical mediator of hypothalamic origin which stimulates the anterior pituitary to release its various hormones as demanded by the physiological state of the body Dr Duvernoy's new data make it plain that any chemical mediators which the hypothalamus might produce and which enter the pituitary portal vessels could reach the posterior pituitary as well as the anterior pituitary and if any local hormonal action were involved in the control of the anterior pituitary, there is no reason for supposing that there is any physical barrier to the passage of substances in whatever direction one cares to suppose But all this is pure speculation

The second symposium also dealt with the technique of measuring changes in the level of secretion and action of adrenocorticotrophic hormone, as well as with the variety of nervous stimulations which leads to the discharge of adrenocorticotrophic hormone The evidence in favour of the alternative views that the release of adrenocorticotrophic hormone is mediated by some neurohumoral substance of unknown chemical nature (the so-called CRF) or by vasopressin, or by melanophore-stimulating hormone, was reviewed by Dr Guillemin, but there seems little that is precise about these matters, and as the general discussion at the end of the seminar indicated, considerable doubt exists about the assumed localization of hypothalamic function with respect to the control of pituitary function.

The verbatim record of what passes at endocrinological symposia has on occasion detracted from the value of what might otherwise have been regarded as scientific meetings of high quality On this occasion this is not so Not only do the papers which were delivered at the seminars of the Collège de France provide a first-rate account of the matters with which they deal, but it is also plain from the record of the discussions that Prof Courrier kept the several sessions of the two seminars very much to the point He is to be congratulated for having inaugurated a series of seminars which will, one is certain, continue to enjoy the high standard of the two now published S ZUCKERMAN

HÆMOGLOBIN: NORMAL AND ABNORMAL

Hemaglobin and Its Abnormalities

By Prof Vernon M Ingram (A Monograph in American Lectures in Living Chemistry) Pp. xiv + 153 (Springfield, Ill Charles C Thomas, Oxford Blackwell Scientific Publications, 1961) 60*s*

HÆMOGLOBIN is the most intensively studied of proteins Many of the techniques available to the physiologist, biochemist, biophysicist and geneticist have at one time or another been applied to hæmoglobin All this work has led to spectacular progress in our understanding of the structure and function of hæmoglobin and its near relative, myoglobin

Last year saw the publication of papers that represent the culmination of long and detailed analysis by the investigators concerned Kendrew and his colleagues have extended the resolution of data from their X-ray diffraction patterns of myoglobin to the 2 Å -level, so that for the first time the helical arrangement in the polypeptide chain of a protein can be unequivocally demonstrated At the same time, Perutz *et al* published the structure of hæmoglobin at a resolution of 5 5 Å Because of its very great similarity to the myoglobin structure, they have been able to infer considerably more than the actual resolution allows From these X-ray diffraction patterns detailed information about the secondary and tertiary structure of proteins in crystalline form can be deduced There is already correlation of structures deduced from X-ray patterns with the amino-acid sequences which are now known for the human hæmoglobin chains and nearly all known for horse hæmoglobin chains and myoglobin

In 1949, Pauling *et al* demonstrated that the basic defect in sickle-cell disease is an abnormal hæmoglobin The term 'molecular disease' introduced by Pauling for conditions of this kind has caught the imagination of many research workers and provided a great stimulus to research in the field Eight years later Ingram applied Sanger's technique of peptide analysis to normal adult and sickle-cell hæmoglobins. He found that the two molecules differ only in a single peptide, and later this difference was shown to be due to the substitution of the amino-acid valine for glutamic acid which occupies the sixth position from the amino-terminus of the β-polypeptide chain of the normal molecule Ingram and his co-workers, and later others, have shown that several other abnormal hæmoglobins differ from the normal by single amino-acid substitutions in the β- or α-chain There was formal evidence that genes controlling the synthesis of normal and abnormal β-chains were allelic with one another but not with genes controlling synthesis of α-chains

Ingram's important discovery could not have come at a more opportune time The importance of proteins in mediating genetic effects transmitted by nucleic acid had emerged from the publications of Crick and others, and there was a great need to know the sort of effects on protein structure produced by mutation of the genes controlling their synthesis The studies of Ingram and his colleagues provided precisely the required information The technique of peptide analysis, re-named by Ingram 'fingerprinting', came to be used in many laboratories for establishing exact structural differences between related proteins

In the book under review Ingram gives a general account of these developments, beginning with the structure and function of the normal hæmoglobin molecule and describing the structural differences in some of the known abnormal molecules The arguments are well presented and easy to read. There are few mistakes (in caption 2 to Fig 46 *S* should be replaced by *F*) Diagrams and references have been carefully selected and provide an excellent introduction to the field So much has been done that it is, of course, impossible to expect a book of this length to serve as a comprehensive reference work Although the main emphasis of the book is biochemical, the diseases associated with the abnormal hæmoglobins are briefly discussed and a few of the interesting problems in population genetics raised by the high frequency of certain abnormal hæmoglobin genes in some human populations are mentioned In connexion with the latter, Ingram quotes the extraordinary and unfounded suggestion that "hæmoglobin E might have a selective advantage because in the presence of this gene the likelihood of an allelic thalassæmia disease occurring in homozygous form is removed"

This book is likely to be read by many people with an interest in biochemistry and genetics, and it seems a pity that, although only 150 pages long, it should cost £3 A C ALLISON

ADVANCED ORGANIC CHEMISTRY

Advanced Organic Chemistry

By Prof G W Wheland Third edition Pp xi + 871 (New York and London John Wiley and Sons, Inc , 1960) 17 50 dollars, 140*s*

THIS book constitutes an extended, revised edition of Prof G W Wheland's well-known book, *Theoretical Organic Chemistry*, which, for more than a decade, has been of such considerable value to both university students and lecturers The new edition has been well produced with most legible printing on good quality paper and the spatial and conventional organic chemistry formulations are reproduced with considerable clarity

The historical approach employed throughout the text is thought-stimulating and interesting, the earlier chapters dealing with the general theories of valency bonding, inter-atomic force theories, etc Electrostatic, covalent, semipolar double bond systems, etc, are clearly described, together with valuable excursions into the fields of dipole–dipole interactions, solvated chains, polarization and orientation phenomena. The first chapter, on valency and configuration, forms a natural precursor to discussions on the determination of structure by various physical methods, and general comment is made on the application of nuclear magnetic resonance, rotatory dispersion and parachor applications

Other chapters deal with the Lowry–Brønsted theory of acids and bases, and the allied Lewis concept, a survey is made of clathrate inclusion compounds, of nucleophilic displacements, S_N and their relation to S_N1 kinetics and to S_N2 reactions of the Walden inversion type

A most extensive chapter deals with the stereochemistry of carbon, which is well illustrated with numerous examples illustrating resolution, solvent effects, configuration studies, optical phenomena, etc This is supplemented by a further chapter on stereochemistry of elements other than carbon, notably those of nitrogen and sulphur Free radical chemistry also receives the most detailed treatment

The Baeyer strain theory, steric hindrance and conformational analysis receive much attention A memorandum by Prof F W Westheimer on electrostatic affects in organic chemistry forms the basis of Chapter 11, which concludes with a rather abbreviated discussion of the Hammett rho-sigma relations References are, however, given to H H Jaffe's excellent review of this quantitative empirical relation between the rate or equilibrium constants of reactions involving organic compounds The value of kinetic data is frequently stressed throughout the book, and in all chapters the mathematical treatment is of reasonably simple character and will not prove over-burdensome to those who find the more abstruse mathematical concepts somewhat difficult to follow

This book is an able successor to its authoritative predecessors, and will do much to stimulate original thought and constructive discussion in the ever-extending and complex field of theoretical organic chemistry D T Lewis

TEXT-BOOK OF INORGANIC CHEMICAL ANALYSIS

Quantitative Inorganic Analysis

By Prof R Belcher and Dr A J Nutten Second edition Pp x+390 (London Butterworths Scientific Publications, 1960) 35*s*

It is highly commendable that the authors have so thoroughly revised a text-book that is only six years old Among the novel features introduced are choice of appropriate redox indicators for particular systems, and criticism of uncertainties involved in the dichromate–ferrous reaction It is a pleasure to see classical gravimetric analysis treated with due respect and yet a pity to miss a reference, presumably due to shortage of space, to the solvent extraction technique

The reviewer disagrees with the condemnation of the swinging-point balance In its simplicity and versatility, especially as regards control of sensitivity, it ought to remain for a long time a basic instrument in the hands of a chemist Some minor criticisms may be mentioned briefly Cleaning of pipettes by immersing overnight in a dichromate–sulphuric acid mixture is in the long run detrimental to the pipette inscription On p 150, it is pointed out categorically that laboratory glassware should on no account be warmed which, without defining temperature limits or stating reasons, sounds very dogmatic On the other hand, the temperature control in a drying oven to within ± 1° C (p 10) seems exceptionally good for an average student drying oven In the volumetric section, the conventional ferric alum is recommended as indicator in Volhard's method, while in the colorimetric section the authors state that sulphate ions tend to form complexes with iron(III) Why not propose ferric nitrate as indicator in the first instance ?

The book has been edited with great care and the occurrence of misprints such as that in the legend to Fig 40 is exceptional

This clear, concise, and up-to-date text-book can be whole-heartedly recommended for use by students and practising chemists G Kakabadse

THE POLYNUCLEOTIDE FIELD

Polynucleotides

Natural and Synthetic Nucleic Acids By Robert F Steiner and Roland F Beers, Jr Pp viii+404 (Amsterdam Elsevier Publishing Company, London D Van Nostrand Company, Ltd, 1961) 85*s*

In the introductory chapter of this monograph, the authors point out that the nucleic acid and associated fields have expanded at such a remarkable rate in the post-war years that it is no longer possible to attempt a complete coverage of them in a single text They go on to list a few of the main subdivisions such as chemical genetics, virus biochemistry, protein synthesis, polynucleotide synthesis and the physical chemistry of polynucleotides The extensive developments in the last two subdivisions in the five-year period ending 1960 have prompted Dr Steiner and Dr Beers to write this book In the final chapter they touch on some of the other main subdivisions by discussing the biological roles of nucleic acids

The introduction, which indicates the scope of the book, is followed by a chapter on the organic chemistry of nucleotides Then the chemical and enzymatic evidence for the nature of the inter-nucleotide linkages in ribonucleic acid, deoxyribonucleic acid and synthetic polynucleotides is discussed This is followed by two chapters on nucleotide-polymerizing enzymes In the first, a description of the procedures for isolating and assaying these enzymes is given, and in the second, the properties of the different enzymes are discussed The next three chapters deal with some physical methods such as ultra-violet spectroscopy, optical rotation and X-ray diffraction, and their application to the determination of the macro-molecular configuration of natural and synthetic polynucleotides The final chapter, mentioned above, is preceded by a discussion of the helix-coil transition and the binding properties of polynucleotides Besides the main text are included some seventeen appendixes which outline the experimental procedures for the isolation of nucleic acids and the analysis of their degradation products, and for the preparation, assay and use of some nucleotide-polymerizing enzymes

The authors are certainly justified in writing a book on a subdivision of the nucleic acid field as no one or two people could possibly review every aspect of the subject critically Such is the scope of this book that, if anything, they have tried to cope with too much for two people, although it is certainly very useful for workers in the polynucleotide field to have all this material collected in one volume For the latter reason, Dr Steiner and Dr Beers are to be thanked for sorting out such a vast amount of the present-day literature

However, it might have been wiser for the authors to have co-opted specialists to write the chemical chapter in the beginning of the book and help with the accounts of some of the other areas to which they themselves do not appear to have contributed I would single out the chapter on the chemistry of the nucleotides for the greatest criticism This contains formula, chemical, and factual errors and some important omissions, but it is nevertheless an adequate account of this aspect Perhaps the only other adverse criticism of this very useful book is that it is not always written in a clear and concise style The appendixes are a good idea and should be useful The printing and layout are good with the exception of some of the chemical formulæ On the whole, this book can be recommended as being of considerable value to workers in the polynucleotide field, for whom it is designed, and to other people interested in this important subject C B Reese

TECHNOLOGY OF ETHYLENE POLYMERS

Polythene

The Technology and Uses of Ethylene Polymers Edited by A Renfrew and Philip Morgan. Second edition Pp xxi + 781 + 93 plates (London Iliffe and Sons, Ltd , New York Interscience Publishers, Inc , 1960) 165*s* net

THE growth of polythene production has been one of the major features of the plastics industry in recent years The interest aroused by the development of newer types of polythene and the many recent technological developments in the field of ethylene polymers have necessitated a considerable revision of the text of this well-known book The present edition—the second—contains approximately 300 more pages than the previous one , chapters have been so revised that the original text has been largely re-written in order to bring the book in line with rapid developments, particularly in relation to materials of higher density In fact, it has been so changed and enlarged that it may be regarded as virtually a new book

The most important additions are as follows The chapter "Manufacturing Processes" now appears as four contributions describing high-pressure, Zeigler, Phillips, and Standard Oil Co polymerization processes , the chapter on the theory and mechanism of ethylene polymerization now becomes two chapters to cover, respectively, polymerization by free radicals and by ionic growth Additional chapters are "Ethylene Copolymers and Polymer Blends", "Vacuum Forming", "Reprocessing" and two chapters on packaging which describe those varied and useful applications not classified as films Valuable appendixes are a table of world manufacturers of polythene and their production capacities, and a list of world proprietary names for ethylene polymers

The thirty-six chapters of this book deal with every aspect of polythene, its manufacture and properties (structure, mechanical, electrical, chemical, testing, radiation treatment, and modification) , processing techniques (compounding, pigmentation, extrusion as applied to extruder design, cable covering, tube, rod and profile, film and sheet, injection and blow moulding, centrifugal cast, coating and laminating, dip and spray coating, vacuum forming and reprocessing), and applications (cables, film, coated packaging products, pipes, chemical plant, household goods, textile yarns and fibres) Each of the chapters, written by an expert either from Europe or the United States, provides a most complete and authoritative account of the technology and uses of this important polymer The book remains the standard work on the subject C E H Bawn

GAS DYNAMICS IN THE U.S.S.R.

Physical Gas Dynamics

Edited by A S Predvoditelev Translated from the Russian by Dr R C Murray and D R H Phillips Pp v + 183. (London and New York Pergamon Press, 1961) 50*s* net

THIS book is a translation from the Russian of a symposium of twelve papers covering a wide range of topics under the general heading of the title

Five of the papers cover the field of gas kinetics, the longest being concerned with calculating the properties of air in the temperature-range 1,000° K – 12,000° K , and the pressure-range 0 001–1,000 atmospheres Two of the other papers calculate the changes across normal shock-waves in air considered as a real gas

The next two papers consider experimentally the supersonic flow at an oblique-cut nozzle and an oblique-cut base. These are related to the flow in turbine nozzles and blades

The next three papers again present a sharp contrast, the subject under discussion being the pressure field resulting from electrical discharges under water, while the last two papers in the book discuss shock waves associated with flames Four of these five papers discuss experimental work

One of the difficulties encountered by publishers of a book of this nature is that of making the information available sufficiently quickly after completion of the work on which it is based The references to the various papers indicate that most of the work presented at the symposium was done prior to about 1956, so in this sense the book is not up to date, but, despite this, there is no doubt that it gives some of the most up-to-date information in English on the work being carried out in the U S.S R in the field of gas dynamics

It is fair to say that the translation into technical English is in general excellent, though it is noted that in some references which were originally in English, but have been translated into Russian, the authors' names are not correctly translated back into English. The printing is in non-letterpress type setting, but this in no way detracts from the book, perhaps the only thing which does being the quality of schlieren photographs on which several of the papers depend The photographs are in general poor, but there is no indication whether this is due to the printing process used or to the poor quality of the originals

I S Donaldson

SURVEY OF ASTRONOMY

Vistas in Astronomy

Edited by Arthur Beer Vol 3, Part 1 Dynamics—Geophysics—Instruments—Solar System Vol 3, Part 2 Stellar Astronomy—Stellar Evolution—Photometry—Galaxies—Spectroscopy—Cosmology (Special Supplement No 7 to the *Journal of Atmospheric and Terrestrial Physics*) Pp vii + 345 (London and New York Pergamon Press, 1960) 120*s* net

The third volume of *Vistas in Astronomy*, published, as in the case of the two preceding volumes, by Arthur Beer, opens with an article by R. van der R Woolley, Astronomer Royal, on "The Dynamics of Stars in the Neighbourhood of the Sun" The author deals in particular with the movements of stars near at hand, the permanence of very open clusters and the equipartition of energy R A. Lyttleton poses the following question "What is the simplest theoretical approach and minimum amount of calculation that could have led to the discovery of the planet Neptune ?" Tisserand had already posed this question in his *Mécanique Céleste*, when he suggested that it would suffice to assign successive values to the radius of the planet. which is supposed to have a circular orbit Lyttleton shows that one can simplify the problem considerably without altering the numerical solution much, by attributing circular orbits to both Uranus and Neptune The position which he finds for the hypothetical planet does not differ more than one degree from the observed position, as for its mass, he approximates the real value more closely than Adams and Le Verrier But he has the advantage over his two predecessors of knowing that Neptune exists, whereas in 1845 or 1846 it was doubtful, as the question posed by Airy to Adams at first and then to Le Verrier proves What is more, we know that the orbit of Neptune has a slight eccentricity The paths of discovery are often tortuous, but one does not perceive this until the goal has been attained

The contribution of H von Kluber is also of great interest and of more immediate application It is a discussion which takes into account all the determinations, made since 1919, of the displacement of stars around the Sun when in eclipse The existence of this effect is not in question, but the extent of displacement remains very uncertain It appears, however, to be greater than that provided by theory The observations are very scattered and, in consequence, the hyperbolical variation of the displacement as a function of the distance from the Sun cannot be considered as established The recommendations directed by H von Kluber to future observers must be acclaimed without reserve

Of interest are articles, one by W Cochran, the other by V Fock, on "The Clock Paradox", some studies by G M Sisson on the "Design of Large Telescopes", by Marschal A Wrubel on "The Electronic Computer as an Astronomical Instrument", by A H Jarrett on "Techniques in Auroral Investigations" and by D W Beggs on "The Russian Satellites" Finally, four notes on the brilliant comets of 1957 complete the first part of this volume

John B Irwin opens the second part with an article entitled "The First 70,000". It is the exposition of a vast programme of observations destined to yield the following for all stars down to magnitude 8 5 position, proper motion, magnitude, multicolours, spectral type, luminosity, parallax, radial velocity and polarization. The author emphasizes, by an apt title, the paucity of information we have on the southern hemisphere He discusses the use of the transit circle for the determination of positions and proper motions, but he does not mention the use of the impersonal astrolabe for establishing the fundamental catalogue Altogether, the programme outlined by John B Irwin is excellent, but only international co-operation will allow it to be executed It would be an interesting task for the International Astronomical Union

Modern aspects of stellar photometry are dealt with in two articles, one by Gerald E Kron on "Multiple Colour Photometry", the other by A N Argue entitled "Photometry with the Cambridge Schmidt Telescope" A long study by B E J. Pagel provides interesting ideas on "The Excitation of Emission Lines in the Spectra of Early-type Stars".

Olin J Eggen has prefaced his article, "Colour, Luminosity and Evolution of the Stars", with some opinions of this evolution by celebrated men of science, from Helmholtz (1854) to Eddington (1926). This comparison of ideas put forward by very high authorities produces an effect of shock on the reader of to-day and inspires him to salutary reflexions on what H Poincaré called "La Valeur de la Science" Eggen shows, with diverse examples, all that photometry of stellar clusters in several colours teaches us about stellar evolution The volume ends with a representation of "The Visual Milky Way" by Sergei Gaposchkin and with two theoretical studies, one by L Mestel entitled "Star Formation and the Galactic Magnetic Field" and another by D. W. Sciama called "Observational Aspects of Cosmology".

Presented with the same care as the two preceding ones, Volume 3 of *Vistas in Astronomy* contains an abundant bibliography and a very detailed "Name and Subject Index", it is an excellent piece of work. The editor announces the publication of two other volumes, the authors of which will be drawn from fifteen countries or so *Vistas in Astronomy* is indeed of immense utility for all those who wish to know the state of advancement of the diverse branches of astronomy.

A Danjon

THE NATURAL HISTORY OF GALAXIES

The Hubble Atlas of Galaxies

By Allan Sandage Pp viii + 32 + 50 plates. (Washington, D C Carnegie Institution of Washington, 1961) 10 dollars

This is a remarkable and fascinating book. Conceived by Edwin Hubble, who did not live to see it finished, and now completed by Hubble's pupil and successor, Dr Allan Sandage, it can be recommended to a very wide range of readers, from mature specialists in cosmology to young people with a good sense of curiosity and wonder It consists of reproductions of photographs of 176 galaxies, taken almost entirely with the large reflectors of the Mount Wilson and Palomar Observatories, and many made by Hubble himself The photographs are of the highest technical quality and are unlikely to be surpassed until we learn to build much better telescopes, or invent new techniques, perhaps involving artificial satellites Although reproduction is by a half-tone process, such care has been taken that one can often see detail down to about 1 second of arc in diameter In a number of cases two, or even three, prints are given, since the range of surface brightness is too great for one print to show all structure of interest

A preface describes the origin of the *Atlas* and this is followed by a chapter entitled "Galaxies", giving a summary of immediately relevant information, including some very instructive, and sometimes amusing, history that ought to be compulsory reading for all astronomers This is followed by a long and detailed description of Hubble's system of classification, which the *Atlas* was designed to illustrate, with frequent references to individual photographs. Besides this, each photograph is provided with its own commentary, usually about 100 words but sometimes considerably more.

If we exclude clusters of galaxies and the universe itself, galaxies are the largest physical structures known to man We believe them all to be aggregates of gas, dust and stars, of total masses mostly in the range 10^9–10^{11} Suns, and with rather ill-defined optical diameters of the order of 10^4–10^5 or more light years Hubble's system of classification, the simplest proposed so far and perhaps for most purposes the most useful, is a purely morphological one The outstanding characteristic of nearly all these objects is that their form strongly suggests rotation, and spectroscopic measurements confirm this Hubble arranged galaxies into an order which in general is one of increased flattening, increased development of spiral arms, diminishing nucleus, growing signs of interstellar dust, probably more interstellar gas, more resolution into stars and greater abundance of young, hot stars He was always careful to point out that his sequence is not necessarily one of evolution, in either direction Sandage follows him, but can add in the light of more recent work that, whether evolutionary or not, the sequence as just described appears to run from old to young

One criticism of Hubble's scheme has been that it may impose too simple an order on an essentially complicated problem. For other reasons too, having little to do with Hubble, most astronomers until fairly recently have tended to carry in their minds an altogether too regular and standardized picture of galaxies Fortunately, a better appreciation of the problem has been growing and now this *Atlas* makes it abundantly clear that galaxies, like most natural objects, are very far indeed from being simple, mass-produced structures Most of our respectable old friends are here, for example, *M*31, *M*33, *M*81, but so also are some much more awkward customers What of *NGC*520, 2685, 2859, 5128, or even the Large Magellanic Cloud ? What are we to make of the nuclei of four *Sc* spirals, here reproduced separately on a large scale, on page 31 ?

Hubble's original scheme, apart from a bifurcation between ordinary and barred spirals, is essentially one-dimensional But even on a purely morphological basis the quite limited sample of galaxies here cannot be fitted in neatly without qualification As Sandage remarks, "the classification sequence is not infinitely narrow perpendicular to the $Sa \rightarrow Sb \rightarrow Sc$ direction" Among *Sb* spirals, for example, he directs attention to variations in the size of the nucleus, in the thickness and surface brightness of the arms, and in the distribution of dust Some *Sb* galaxies have two sets of arms, of different pitch Among the *Sc* galaxies "at least six major sub-groups can be distinguished" Nevertheless, as a broad generality, a first approximation, Hubble's scheme still impresses one as valid

Although a mine of information, *The Hubble Atlas of Galaxies* is not a text-book Possibly the author should have emphasized this more in his preface It makes no pretence of dealing with the general problems of galaxies, of their dynamical structure, of their physical composition as revealed by the spectroscope, of their motions, of pairs of galaxies, multiple galaxies, colliding galaxies, linked galaxies, clusters of galaxies There is nothing of radio-astronomy, its results and its perplexities

There is also no discussion of the technical problems of photographing galaxies, the outer parts of which are much fainter than the natural sky background itself. (In these pictures the sky is reproduced nearly perfectly black, æsthetically satisfying but scientifically a little misleading) We are limited not by photographic speed but by sky brightness Equally, we cannot photograph detail less than about one second of arc, not because of the imperfections of telescopes and photographic emulsions, or their limited resolving power, but because of the optical irregularities of the Earth's atmosphere I think all this needs to be said clearly Observational astronomers take these familiar limitations for granted, but they are imperfectly understood by many others who may be users of this volume

What it comes to, of course, is that we need a full text-book on galaxies to accompany the *Atlas* Could Dr Sandage provide such a companion volume to give us instruction and pleasure in the same generous measure as does the *Atlas* itself ?

R. O REDMAN

THE HIDDEN FACE OF THE MOON

Atlas of the Other Side of the Moon

Edited by N P. Barabashov, A A. Mikhailov and Yu. N Lipskiy. Translated into English by Leon Ter-Oganian First published by the Academy of Sciences of the U S S R. Pp 141+30 photographs. (London and New York Pergamon Press, 1961) 50*s*

EVERYONE is familiar with the fact that the tidal friction operative in the Earth–Moon system has gradually slowed down the axial rotation of our satellite to the rate with which it revolves around Earth (the periods of these two motions being now synchronized within at least 0 1 sec)—which makes the Moon show us always the same face Lunar librations (both physical and optical) permit us, to be sure, to see from Earth somewhat more than one-half of the entire surface of the lunar globe—in fact, about 59 per cent of it—but the rest remained completely hidden to us, until the memorable flight of the third Russian space rocket in October 1959, which circumnavigated our satellite and by means of its photographic equipment carried aboard took at last the veil off its invisible face

The details of this feat are still sufficiently well remembered to require little repetition A preliminary report on the photographic data obtained in the course of this flight and televised afterwards to Earth was released by the U S S R Academy of Sciences in December 1959 (*First Photographs of the Far Side of the Moon,* the English translation of which was published by the Pergamon Press, London, in 1960), while a definitive discussion of the entire material followed a year later in a volume the English edition of which is under review

This volume summarizes the results of an exhaustive study of the topographic as well as photometric features of all available negatives, carried out independently at the Pulkovo Observatory, Sternberg State Astronomical Institute, Moscow, and the Kharkov Observatory, under the respective guidance of Profs A A Mikhailov, Yu N Lipskiy and N P Barabashov—all well-known students of the Moon The outcome of their impressive study (the methods of which are described in the first part of their book) resulted in the identification of 498 details on the lunar far side, the catalogue of which (classified according to their degree of reliability) constitutes the major part of the whole volume The formations discovered on the far side of the Moon do not, in general, differ from those we know on the visible face in their photometric or topographic characteristics—though even a cursory glance at the first photographs of the far side revealed a much greater preponderance there of mountainous areas (and a corresponding scarcity of the maria) than is the case on the side that is familiar to us

The last, and most valuable, part of the present volume consists of the reproductions of 30 frames of the individual photographs secured by the Russian space station Needless to stress, no one frame reveals all the details ascertained for that particular region from the ensemble of the material, but the best single frames appear to attain a ground resolution of some 10 miles

A map of the far side of the Moon, showing the distribution of topographic details listed in the catalogue, concludes the whole volume It is the first map of its kind, and comparable in amount of detail with maps of the visible face of the Moon available by the first half of the eighteenth century

All considered, the scientific outcome of the flight of the Russian third space station must be regarded as a splendid success, and a wonderful achievement in which the Russian nation and all their fellow inhabitants of this Earth can justly rejoice It does not mean that the days of discovery on the Moon are completely over, for a small part of its surface (approximately 40° in longitude) was not visible from the Russian space station at the time of photography and remains, therefore, still uncharted It will, however, certainly not be long before even this remaining *terra incognita* of the Earth–Moon system will reveal its secrets to the inquisitive eye of the next lunar circumnavigating device, and the pioneer days of lunar mapping will irretrievably belong to the past

The volume under review constitutes without doubt an important landmark in selenographic literature, and Pergamon Press is to be commended for the speed with which it introduced it to the English reader This speed entailed, however, also some obvious drawbacks, for by its external appearance the English edition (printed in Poland) does not come up to the quality of the Russian original, and the translation is decidedly weak—in marked contrast with the professional fluency and manner in which the preliminary report was translated, the year before, by Sykes

ZDENĚK KOPAL

IONOSPHERIC RESEARCH DURING THE INTERNATIONAL GEOPHYSICAL YEAR

Some Ionospheric Results obtained during the International Geophysical Year

Proceedings of a Symposium organized by the U R S I /A G I Committee, at Brussels, Belgium, September, 1959 Edited by W J G Beynon (U R S I Monographs) Pp xi + 401 (Amsterdam Elsevier Publishing Company , London D Van Nostrand Company, Ltd , 1960) 72*s*

DURING the International Geophysical Year a vast mass of data was accumulated by investigators of the ionosphere all over the world and some considerable time must elapse before these data have been analysed and fully interpreted The purpose of the symposium with which the present volume deals was to discuss the preliminary results derived from the data

The book contains sixty papers contributed by many of the leading ionospheric workers All papers are written in English and some of them have summaries in French They are grouped in approximately equal numbers under the headings (1) F-2 layer phenomena, (2) high-latitude studies, (3) disturbance phenomena, (4) ionospheric irregularities, (5) $N(h)$ profiles, (6) absorption, (7) drifts, and (8) noise, whistlers, rockets and satellites The first section contains papers on such topics as the equatorial anomaly in the F2-layer, the world distribution of ionization in this layer, and on the forecasting of world values of ionization density Section (2) is mainly concerned with the morphology of sporadic-E ionization and of black-out in polar regions In section (3) there are eight papers, most of them very brief, on ionospheric storm phenomena Two other papers in this section deal with the effects of the two Johnston Island nuclear explosions in the ionosphere and on radio signals traversing it The phenomena of spread-F and sporadic-E ionization are considered in section (4) where some interesting observations of both phenomena in equatorial regions are described Some of the early results of calculation of the variation of ionospheric electron density with height above Earth are given in section (5) Of particular interest in this section are contour charts, for four separate times of day, showing the variation with height of the plasma frequency along the 75° W meridian

Section (6) deals with investigations of ionospheric absorption by the pulse reflexion method and by measurement of cosmic noise Section (7) is noteworthy for three Russian papers on movements of ionospheric irregularities and for a preliminary report on the results of world-wide measurements of horizontal drifts in the F-layer The papers on atmospheric noise in section (8) include a short summary of the measurements made throughout the world , no results are given in this paper but the section includes other papers giving results obtained in both the U S S R and Japan Rockets and satellites are dealt with by American authors one paper is a very concise record of the results of ionospheric measurements by American rockets, the other paper deals with the determination of total ionospheric electron content below a satellite by Faraday rotation observations

As all these papers were written very soon after the end of the International Geophysical Year it is not surprising that many of them contain information of a very tentative character, and others are little more than brief reports on phenomena in a very early state of investigation Indeed, some of the papers have been made obsolete by later publication by the same author before the appearance of the present volume It is, nevertheless, a useful record of the symposium and is well edited and well produced It is a pity that its high price will deter many workers from adding it to their private bookshelves

A F WILKINS

PRANDTL'S COLLECTED PAPERS

Ludwig Prandtl

Gesammelte Abhandlungen zur Angewandten Mechanik, Hydro- und Aerodynamik Herausgegeben von Walter Tollmien, Hermann Schlichting und Henry Gortler, Schriftlietung F W Riegels Erster Teil Pp xix + 1–574 Zweiter Teil Pp xiii + 575–1071 Dritter Teil Pp xiv + 1071–1620 (Berlin Springer-Verlag, 1961) 273 D M , Ganzleinen 288 D M

THE monumental work of leaders of nineteenth-century physical science, such as Helmholtz, Kirchhoff, Kelvin, Maxwell, Boltzmann and Rayleigh, in creating a structure of concepts by means of which physical and engineering phenomena could be understood and predicted, was of such comprehensive character that the task of those who had to follow them and lead the way to the explanation of the mysteries that remained was formidable indeed No hope lay in continued logical building on the structure of their predecessors On the contrary, completely new intuitions were required

The scientific public knows well how these were introduced into the physics of atoms, molecules and radiation by Planck, Einstein, Rutherford, Bohr and the rest The revolution in mechanics is less well known No better grasp of it can be attained than by a study of these three admirably produced volumes containing the collected papers of the leader in that revolution, Ludwig Prandtl

The mystery in this field at the turn of the century was thickest in the mechanics of liquids and gases Three of the eight main groups of papers into which these volumes divide Prandtl's work are devoted to the three outstanding new intuitions by means of which light was to be shed on that mysterious subject

The first of these, chronologically speaking, a joint achievement of Stodola and Prandtl, was the elucidation of steady supersonic flow, stimulated at the time by the requirements of steam turbine nozzles, though later to become the foundation of supersonic aerodynamics It included a full description of the effect of varying the nozzle back-pressure, through and beyond those values which force supersonic flow to terminate at a normal shock-wave in a range of positions and strengths, to the values where a supersonic jet is produced, the structure of which was sketched by means of the ideas of oblique shock-waves and of what were later to be called Prandtl–Meyer expansions A preliminary theory of shock-wave structure was also obtained in these early years Prandtl's later work on gas dynamics, with Busemann, evolved a highly versatile and accurate theory of two-dimensional supersonic flows, using the geometry of their characteristics, and so derived jet shapes and flows about aerofoils with closer experimental agreement It is interesting to note too, in this group of papers, not only the detailed studies of the effect of compressibility in subsonic flow but also a short work of 1938 giving clear warning of supersonic bangs and considerable detail about their geometry

The famous elucidation of the true role of viscosity followed closely that of compressibility, the paper stating all the essential properties of laminar boundary layers being presented in 1904 The nature of turbulent boundary layers was appreciated soon afterwards, and a 1914 paper used the two concepts to explain the extraordinary vagaries in the drag of spheres Each of these well-known papers includes a lovely *experimentum crucis* Later investigations in the group of papers devoted to boundary layers and frictional drag relate to the law of turbulent resistance of smooth and rough pipes and plates

The third new branch of fluid dynamics created by Prandtl, perhaps the one of greatest technological importance, was the theory of lift and lift-induced drag It was a complete lifting surface theory that Prandtl, with his pupils Betz and Munk, derived and applied to monoplanes, biplanes, propellers and other systems As might be expected, the largest section of Prandtl's collected papers is devoted to all its ramifications

The volumes are a reminder, nevertheless, of the outstanding achievements of Prandtl in fields quite different from those so far mentioned A long section devoted to elasticity, plasticity and rheology reminds us of Prandtl's striking contributions (followed up by his pupil von Kármán) to a reconciliation of theory and experiment in elastic stability We recognize, too, his early appreciation of the practical significance of 'perfect plasticity' theory, which he elaborated mathematically on lines parallel to those of the supersonic flow theory to illuminate hardness tests, extrusion and the like, and which, with his work on permanent set, rupture, and materials the stress-strain relations of which include a relaxation time, helped to create the mechanics of solids as we know it to-day

Another section is devoted to vortex development and turbulence, including the rolling-up of vortex sheets, flows past sharp edges, rotating cylinders, etc In turbulence theory proper, Prandtl's momentum transfer theory attached too little significance to the role of big eddies, but the theory and associated experiments illuminated to a substantial degree the effect of temperature gradients on turbulence and, conversely, of turbulence on heat transfer, as well as a host of complex phenomena such as the secondary flow in straight pipes of non-circular section.

The meteorological section includes Prandtl's computation of the distribution of wind velocity and direction near the ground (Ekman spiral) using a turbulent exchange coefficient It includes also his contribution to the understanding of why the west-east momentum in the general circulation of the atmosphere is transported on the average at a positive rate from the tropics to the temperate zones (even though any 'random' mechanism would cause it to flow the other way, down its gradient) Prandtl noted, in this context, that any loss of energy in the high-altitude west–east circulation (due to large-scale disturbances) would reduce the Coriolis force balancing the poleward pressure gradient, and so permit poleward movement down that pressure gradient until the energy had been restored, and he noted also that the geostrophic relation between poleward pressure gradient and west–east momentum implies that energy-restoring motions must also make the west–east momentum flow polewards Other illuminations in this field include work on the structure of fronts, and, remarkably enough, an original paper dated 1950 on the dynamics of the jet stream

The volumes close with a section covering many of Prandtl's significant contributions to experimental technique, which laid the foundations of modern low-speed and high-speed wind-tunnel practice (and, as a curiosity, included experimental studies in a rotating room), followed by a group of miscellaneous papers, among which stand out a beautiful piece of work on bearing lubrication and some eloquent advocacy of the use of vector analysis

Those who love theoretical and applied mechanics can be recommended, if necessary, to curtail some future holiday in order to be able to afford these absorbingly interesting volumes Such a drastic measure might also enable them peacefully to enjoy the pleasures of their first perusal!

M James Lighthill

THEORY OF BOUNDARY LAYERS

Boundary Layer Theory

By Dr. Hermann Schlichting Fourth edition. Translated by Dr J. Kestin. (McGraw-Hill Series in Mechanical Engineering) Pp xx+647. (New York McGraw-Hill Book Company, Inc ; London McGraw-Hill Publishing Company, Ltd, 1960) 128*s*

New Methods in Laminar Boundary-Layer Theory

By Dr D Meksyn Pp xxiv+294. (London and New York Pergamon Press, 1961) 70*s* net.

THE concept of the boundary layer was discovered by Prandtl in the first years of the present century, and since then it has occupied a central position in fluid mechanics The boundary layer is essentially a skin effect, which occurs in the flow of a fluid with small viscosity, but the behaviour of the layer may have a profound influence on the general character of the whole flow For this reason, the mechanics of the boundary layer is of great importance to engineers and mathematicians interested in the problems of fluid flow. In recent years the study of boundary-layer flow has attracted increasing attention, both theoretically and experimentally, and a great number of papers have appeared on this subject

Prof Schlichting has been one of the leading contributors in this field, and his book, which was originally published in German ten years ago, has now appeared in its fourth English edition This follows the same general plan as the first German edition, but the accretion of new material has made it considerably bigger The first part provides the basic physical ideas and the fundamental equations for the flow of viscous fluids Some of the exact solutions of these equations are discussed, and there is a chapter on the approximate solutions which are valid when the viscous forces are dominant over the inertia of the fluid Laminar boundary layers are treated in the second, and longest, part of the book This side of the subject is the most fully understood, and has received extensive mathematical treatment A full account of two-dimensional flow is given, including chapters on heat transfer and compressibility Axisymmetric and three-dimensional boundary layers are treated more briefly, and there is a chapter on boundary layer control

The third part is on the transition from laminar to turbulent flow It contains a description of the theory of hydrodynamic stability, its experimental verification and its relation to the formation of turbulence The factors influencing stability are considered, with applications to the flow over aerofoils The last section of the book deals with fully developed turbulent flow Here the treatment is of necessity largely empirical, and based on the analysis of experimental results Separate chapters deal with flow in pipes, over flat plates, with the calculation of turbulent boundary layers, and with free turbulence in jets and wakes

Schlichting's book is written for engineers, and emphasis is placed on those methods and results which are likely to prove most useful Topics which are not yet amenable to routine calculation, such as the flow over a general three-dimensional surface, are dealt with only briefly Each chapter has an extensive list of references, and there are many figures illustrating theoretical results and experimental observations In a book on such a quickly growing subject there is inevitably scope for improvement and addition in detail, but it is likely to remain a standard work for several years. The legend of Fig 5 15, on page 90, has an error, and a corresponding one is made in the description, on page 91, of the effect of Reynolds number on the flow in a divergent channel. The book is well produced and clearly printed

The scope of Dr. Meksyn's book is more limited, as its title indicates It is concerned with mathematical methods, and deals mainly with steady laminar flow and with stability theory The author has written several papers on these subjects since the War, and his book presents a systematic account of his work Other aspects of laminar boundary layers are mentioned rather cursorily. The reader who is already familiar with boundary-layer theory may find Meksyn's methods of interest, but he would be well advised to check the formulæ carefully The original papers contained several errors, of which most have been corrected, but some still survive There are a number of minor slips

E J Watson

MEASUREMENT OF WATER-FLOW

Hydrometry

Theory and Practice of Hydraulic Measurements. By Prof Adam T Troskolanski Pp xix+684 (London and New York Pergamon Press, Warszawa Panstwowe Wydawnictwa Techniczne, 1960) 120*s*. net.

WHEREAS in most other countries 'hydrometry' means the science of water-flow measurement, in Britain it has been connected with measuring the density of liquids The title of this book is therefore yet another example of a distinguished foreigner teaching us our own language

Although water-flow measurement is one of the oldest sciences, it has not been well documented and we are therefore indebted to Prof Troskolanski for providing a comprehensive survey of the subject Before entering the Technical University of Wrocław, the author was chief engineer of the Polish National Bureau of Measures and therefore writes with authority, both as engineer and scientist He has confined himself mainly to water-supply engineering, although water-flow measurements for the hydro-electric industry are mentioned briefly

This new book is an enlarged version, in English, of Volume 3 of the author's text-books *Hydromechanika Techniczna* and includes new chapters on recent advances, such as magnetic flow-meters, radioisotope techniques and water-level measurements by γ-ray probes

The three main sections are (1) units, and principles of measuring length, area, volume, time, pressure, velocity, flow-rate and total flow , (2) instruments and techniques for measuring these physical quantities ; (3) laboratories for calibrating instruments and absolute standards of flow measurement

The subject is dealt with logically and in considerable detail All equations are dimensionally consistent, but in addition both British and metric units are used throughout. The author's philosophy is that measurement of the flow is a special branch of metrology, depending fundamentally on measurements of length, time, etc Such basic aspects are covered in detail in the first section, leading up to the measurement of flow-rates and total flow

It is difficult to understand why the translator preferred "intensity of flow" to the more widely used term "flow-rate" Similarly, "potential-head" is used rather narrowly It is not generally the same as the height of filling of a tank, unless the bottom of the tank is the zero potential datum However, despite such minor criticisms, the translated text is lucid and much careful revision has gone into the notation, where even the mnemonic subscripts have been translated into English British standards have been widely used and quoted

The second section gives a full account of measuring instruments and recorders, including several not usually described in English text-books, such as the Andersson travelling screen, measuring cascades, Danaïdes, and the detailed analysis of current meter performance and calibration Instruments used in water-supply practice, such as level gauges, construction and mechanical flow-meters, are particularly well described

The final section, on hydraulic laboratories and meter calibration, provides the sound foundation which is so often missing in books on measuring instruments Accurate calibration is essential, and it is valuable to have detailed descriptions and recommendations for reliable test facilities

The descriptive material is excellent and is well illustrated, together with many physical tables and a comprehensive index in English, French and German Some of the information about fields other than water supply is a little out of date For example, both the Allen and Gibson methods have now been accepted internationally for the latest International Electrotechnical Commission code for water turbine testing Sodium chloride has been largely superseded by other chemicals in the salt titration technique

The Allen salt-velocity method is reviewed more unfavourably than it deserves in view of its successful use in the United States and elsewhere On comparative flow measurement tests no more recent references than the Walchensee tests of 1926 are quoted, nor *is it made sufficiently clear that the weir is a secondary* method of flow-measurement and for highest accuracy must always be calibrated

However, in general this is a most valuable collection of detailed information, set out clearly and logically, on a subject which until now has not been well documented It can be recommended to all hydraulic engineers interested in flow measurement

S. P HUTTON

A HISTORY OF METALLOGRAPHY

A History of Metallography

The Development of Ideas on the Structure of Metals before 1890 By Prof Cyril Stanley Smith Pp xxi + 291. (Chicago University of Chicago Press, London Cambridge University Press, 1960) 8 50 dollars, 68*s*

THIS volume, which has been eagerly awaited by those aware of its preparation, was worth waiting for In the author's own words, "Perhaps the study even has a certain timeliness, for it is abundantly clear that the proper balance between 'applied' and 'pure' science that is necessary for technological and cultural well-being has yet to be achieved"

Broadly the book is divided into three main sections, first the background of metallography in ancient works of art, then the philosophical background and the observations of structure before the use of the microscope, and finally, the period of increasingly active observation and theory from the time of Sorby onwards It is a story that involves the history of art, of 'natural philosophy' and of technology, and ends about 1890, by which date metallography had begun to be recognized as a definite discipline in its own right, though Chapter 16 does carry the story down to recent years Two appendixes give in full a paper of Sven Rinman of 1774 on "Experiments of Etching upon Iron and Steel", and Sorby's paper of 1885 to the Iron and Steel Institute "On the Microscopical Structure of Iron and Steel" which differs considerably from the version published in 1887 and of which few are probably aware A long list of bibliographical notes bears testimony to the extent of the sea over which the author has cast his net, the catch being a work of real scholarship as well as a first-rate contribution to the history both of science in general and of metallurgical technology in particular

The earlier portion of the book dealing with the relation between æsthetic appearance and structure is concerned with the fact that, long before it was appreciated by the philosopher that metals are aggregates of innumerable small crystals randomly packed together, this existence of 'structure' was used by the artisan to produce his effects "Differential chemical attack was used in Europe to give a decorative mark of quality to swords as early as the second century A D , but the technique passed out of use" though in the East surface texture did continue in use for this end "The superiority of the swords of Damascus and Japan stemmed directly from the fact that the smith related a certain visible texture to the serviceability of the weapon and adjusted his manufacturing process to produce it " That metal workers have been on occasion intuitively sensitive to the inner structure of their materials and have empirically developed ways of revealing it there can be no doubt, and such observations clearly led to the deliberate choice of manufacturing methods as well as of a kind of metallographic control over the working and heat-treatment processes required in order to obtain the surface structure desired both for beauty and service

The section which describes the gradual development of the idea of a metal as an aggregate of crystals is the most substantial and, in many ways, the most interesting of the whole book The anti-Aristotelian trend of the early seventeenth century paved the way for renewed consideration of the inner nature of metals, and Descartes himself must have been a close observer of the operations of the iron-smelters and of the smith In 1639 he was in correspondence with Mersenne regarding the reason for the hardening of steel, and it is quite clear that he had watched the production of wrought-iron during its manufacture in the finery hearth "The whole bath of molten metal becomes divided into many small lumps or drops, the surfaces of which become smooth" Although these early ideas were necessarily crude, often inaccurate, and, from a modern point of view, often bordering on the fantastic, Rohault, in 1671, in his *Traité de physique*, when discussing the properties of ductility and hardness, could say "we cannot but think that the component Parts of Metals are long, otherwise we cannot understand how Metals should be so ductile as they are, whether they be forged upon an Anvil or drawn through a wire-drawing Iron, whereas if we suppose them to be somewhat long, it is easy to conceive, that when they are pressed on one Side, they slip sideways of each other without quite separating Further—we cannot but think that they must necessarily so order themselves as to place themselves by each other's Side, and correspond Length-ways to the Length of the whole Piece, which will make it easier to bend that Way than any other—And we find Strings in them, as in the Slip of an Ozier These Strings ought not to be in a Metal that is cast and has not been forged And so we find that cast Metal is as easy to break one Way as another" It was, however, left to de Réaumur to develop such corpuscular theories into something useful, and a century before the 'particles' of such theories had developed into the chemical atom on one hand, and the stacking of polyhedra had given rise to mathematical crystallography on the other, Réaumur was using the idea of 'parts' aggregated together on different scales to develop theories regarding the nature of iron and steel and to explain the structures which he saw on fractured metals

The final section, essentially the work of Sorby and his successors, brings the story down to men known personally to the older generations still alive Sorby himself, Arnold, Stead, Sauveur and evidently to the author Martens and Osmond in particular all play their parts The bitter controversy which raged regarding the relative importance of carbon or a hard allotrope of iron is recalled, and it is perhaps not without a feeling of justice to those on both sides to remember that, to the best of our present-day knowledge, the true explanation contains an element of both theories As McCance was probably the first to suggest explicitly, a highly distorted iron lattice the distortion being due not to allotropy but to the high state of supersaturation of carbon in the quenched steel, is as near as the ordinary metallographer probably needs to go

Beautifully produced and illustrated, this is a book which both Prof Smith and the publishers will look on with real pride F C THOMPSON

THE MECHANICS OF VIBRATION

The Mechanics of Vibration

By Prof R E D Bishop and Prof D C Johnson Pp xii+592 (Cambridge At the University Press, 1960) 120*s* net

THIS is a book written with painstaking care on the subject of the basic principles of mechanical vibration and their application to the study of specific problems Its title and contents direct attention to the diversity of meanings that may be attached to the word "mechanics" To some engineers this book, being largely algebraic, will appear to be abstrusely mathematical, to a mathematician the mathematical operations in it are elementary The engineer may be appalled by the authors' insistence (on p 96) that they are restricting their attention to the mechanics of vibration and that the mathematics is a separate subject that they hope to present in another volume He may clutch at a straw floating on p 114 because it suggests that the mathematical part of at least one problem may be "reduced to arithmetic" The quantity of arithmetic involved in some solutions, however, implies a generous interpretation of the word 'reduced'

A good deal of space is rightly used in demonstrating applications of the concept called 'receptance' in 1954 after earlier use of the name 'mechanical admittance' for it by Prof W J Duncan in *Reports and Memoranda* No 2000 (1947) of the Aeronautical Research Committee, mentioned in several places in this book It may be added that the application of this concept to mechanical problems is much older than either of these names for it

A chapter on "The Analysis of Real Systems" begins with a quotation from Routh and refers to Rayleigh's principle, Dunkerley's method and Southwell's theorem in a succession that directs attention to nice distinctions in nouns

It is remarkable to find, in this book, a suggestion that for the purpose of calculating its natural frequencies, a system of rotors and shafts in series may be replaced by one composed of distributed masses, merely to avoid the Holzer tabulation which is laborious for a system of more than 3 or 4 masses The Holzer procedure is in fact a good way of calculating relative amplitudes of vibration for a known natural frequency, but as a means of determining frequencies it is at least thirty years out of date and ought never to be used for that purpose either with or without electronic 'brains' The rational procedure is a routine well within slide-rule range

In Chapter 7, reference is made to the method of estimating the lowest natural frequency of flexural vibration of a small non-uniform beam and the calculated value is compared with that determined by actual trial The smallness of the discrepancy inspires confidence in the mathematical procedure but without weakening the impression that where instruments for exciting and measuring vibration are available, testing of a model is a quicker and more reliable alternative to calculation as a means of determining natural frequencies of certain types of systems

The chapters on damping of vibration show that even when the usual drastic assumptions are made about the quantitative characteristics of damping, it is a seriously complicating circumstance in analysing any but the simplest vibrating systems Abandonment of those assumptions leaves difficulties that cause the authors to remark that "The assessment of damping is a subject in which research is needed" This describes with great delicacy the present state of knowledge of this particular subject

In the last chapter the authors introduce the subjects of "Non-harmonic Vibration and Transient Vibration" and show them to present much more formidable analytical problems than any solved by the authors or others in the subject of harmonic vibration Reflexion on this helps one to understand why some practical problems in vibration are analytically refractory and perhaps analytically insoluble

Any mechanical science graduate who studies this book and works through its numerous and excellent examples ensures for himself a thorough grounding in the theory of mechanical vibrations

W A Tuplin

INDUCTANCE COILS

The Theory and Design of Inductance Coils

By Dr V G Welsby Second edition Pp 232 (London Macdonald and Co (Publishers), Ltd , 1960) 30*s* net

DR WELSBY'S book on the design of inductance coils, first published in 1950, has now reappeared as a revised second edition Those who are familiar with the first edition will know that the book contains very extensive formulæ for the calculation not only of the inductance of coils but also of the effective resistance and the effective shunt capacitance The coils considered are air cored with solid and stranded wire, and those with laminated ferro-magnetic cores, with dust cores, and with ferrite cores The theoretical treatment given is not always as detailed as in the original papers but references are given for those who wish to study the theory fully, while the theory and formulæ given in the book are fully adequate for all whose main concern is to design and make efficient coils

The author is careful to explain the limitations of theoretical formulæ for coils with magnetic cores, but a slight amplification of the parts of the book covering experimental results and methods of correlating theoretical formulæ with these results would add to the practical value of the book

The two principal changes in the present edition are the use of M K S units and the use of the principles of electromagnetic wave propagation to derive some of the formulæ Both changes represent an improvement, but in both cases the new material is not entirely happily welded into the old It is difficult to justify the author's use of the symbol η in place of μ_0 for the magnetic constant of space, the use of μ in place of μ_r for relative permeability, and the use of μ_0 for initial permeability, except on the basis of preserving inviolate some of the formulæ given in the first edition The factor 10^{-9} is also to be seen in many of the formulæ instead of the factor 10^{-7} more usually associated with M K S formulæ

The theoretical treatment tends to be most detailed for the simpler parts and to be uncomfortably condensed for the difficult parts In particular, clearer and more detailed explanations of the method of applying field theory to the solution of coil problems would be advantageous It is rather surprising also, in view of the author's liking for field theory, that capacitance is treated as if it were in shunt across the coil terminals and not as a distributed effect

These minor defects should not be allowed to obscure the real value of the book, covering in the space of 230 small pages a great quantity of most useful information not readily found elsewhere

A H M Arnold

DIFFUSION AND HEAT FLOW IN LIQUIDS

Diffusion and Heat Flow in Liquids

By H J V Tyrrell Pp xii+329 (London Butterworth and Co (Publishers), Ltd , 1961) 65*s*

THOUGH somewhat uneven in its treatment of various themes, this book is a valuable and scholarly addition to texts dealing with certain irreversible phenomena in liquids Chapters on the principles of non-equilibrium thermodynamics, on concentration diffusion, and on thermal diffusion are informative and well balanced Descriptions of experimental methods for investigating various kinds of diffusion processes cover present practice in a very useful way, by comparison, a chapter on thermal conductivity measurements seems much less extensive Interpretation of the phenomena observed is presented in the form of discussions This part of the book will probably need revision soonest, since experimental information in the fields examined is growing rapidly

One chief difficulty in discussing the interpretation of transport processes in liquids stems from their correlation with viscosity At present, there seems to be no widely applicable treatment for linking viscosity with diffusion, despite the great convenience of viscosity coefficients for describing certain types of relaxation in liquids For liquids of very simple structure, viscosity coefficients for relaxation of simple macroscopic shear have been related theoretically with mass migration, for example in the theories of Eyring However, Eyring's models do not deal with the variety of specialized micro-relaxation processes which can occur in liquids containing polyatomic molecules, and in ionic solutions and melts Viscosity coefficients tend to be injected quite generally into interpretations of transport processes without adequate recognition or description of their limitations This book does not avoid the difficulty The author's discussion of liquid viscosities is very incomplete This unavoidably restricts the assessment of various molecular models devised to represent transport processes in liquids, in terms of their structure

Despite this gap in the discussion of transport processes in liquids, the present book can be commended to research workers and libraries, particularly those concerned with diffusion phenomena Scientific interest in diffusion is widening The author's discussions of work on the separation of components by thermal diffusion should help to promote technical advances in this field

A R. Ubbelohde

RADIOACTIVE WASTE DISPOSAL

Atomic Energy Waste

Its Nature, Use and Disposal Edited by E Glueckauf Pp xi + 420 + 4 plates (New York Interscience Publishers, Inc ; London Butterworth and Co (Publishers), Ltd , 1961) 95s

THE days are long past when industrial wastes could be discharged uncontrolled to the environment, and the new atomic energy industry has developed in a period which has seen new legislation introduced generally for the control of environmental pollution and specifically for radioactive discharges Strict legislation (described in one of the articles in the book), a very sensitive public awareness and an exceptional concern with health hazards within the industry have stimulated a considerable amount of research on radioactive wastes

A considerable part of the research is reviewed in this book It is concerned primarily with the fission products separated as waste in the processing of nuclear fuels, which are regarded as the source of ionizing radiations to be used for man's benefit. It develops in more detail, and indeed in considerable detail, some of the views presented by the editor in a paper to the Second International Conference on the Peaceful Uses of Atomic Energy, wherein the fission products were seen as a source of radiations, of rare and precious elements and of specific radionuclides available for peaceful uses of atomic energy

The book concentrates on the utilization of the liquid fission product waste as a source of ionizing radiations It is not a general text on radioactive waste disposal The small-scale use of radionuclides and the wastes arising are deliberately excluded , there is no account of the wastes arising at nuclear power stations , and although one good chapter is devoted to dispersion of activity from chimney stacks, there is no account of the treatment of solid and gaseous radioactive wastes

The book consists of seventeen separate articles The first, nearly a quarter of the book, describes the fission process and the fission products, nearly half of it in tabular and graphical form This article and one on radiation chemistry, though good in themselves, go into far more detail than is required for the discussion of radioactive waste disposal By contrast, the articles on α-emitters in reactor wastes, effects of radiation on living cells, movement of radioactive substances in food chains and radiation protection present the broad aspects and concentrate on the essentials

While the first half of the book, the introductory or background part, is somewhat uneven in its treatment of the topics covered, the second half contains good review articles of the processes and operations used in the treatment of liquid radioactive wastes, the design of irradiation units using packaged sources and the uses of ionizing radiations for agricultural research, food preservation and induction of chemical reactions

The treatment, perhaps naturally, leans towards British practices Space might have been found for a more detailed discussion of wastes from fuel processing in other countries and of wastes from the processing of fuel elements made from stainless steels and zirconium alloys The work carried out in the United States on disposal in salt formations and by deep-well injection might also have been described The therapeutic use of ionizing radiations is a less easily understood omission Some comparison of radiations from nuclides and radiations from machines, particularly in food preservation, would have been valuable

Only a few errors were noted The last entry on p 251, referring to the allowed discharge from the Atomic Energy Research Establishment, Harwell, should be 20, not "2" On p 227, what is referred to as slow sand-filtration, a method of treatment of drinking water, is really the trickling filter process for sewage purification In the glossary, the 'rad', a unit of absorbed dose, is defined as a unit of amount of radiation

The book is authoritatively and well written and well produced It should, as the editor intends, illuminate "the background from which future developments can emerge" A W KENNY

AN AMERICAN TAPESTRY OF STATISTICS

Contributions to Probability and Statistics

Essays in Honor of Harold Hotelling Edited by Ingram Olkin, S G Ghurye, Wassily Hoeffding, William G Madow and Henry B Mann. (Stanford Studies in Mathematics and Statistics, No 2) Pp x + 517. (Stanford, Calif Stanford University Press, London Oxford University Press, 1960) 52s. net

THE name of Prof Harold Hotelling is chiefly familiar to English students of statistics as one of the founding fathers of that branch of their subject known as multivariate analysis His share in the foundations was laid in half a dozen outstanding papers of the early 1930's, and the bibliography given here shows that this period saw his major contribution to theoretical statistics and that later he has been more concerned with applications, chiefly to economics It might seem strange, on the face of it, therefore, to find a volume of 38 papers offered to him on his sixty-fifth birthday by statisticians for the most part very much of the mathematical variety But Prof Jerzy Neyman's introductory article shows clearly that Hotelling has been a powerful formative influence in American statistics and provided it with much of the dynamic which got it really under way Not only did his papers help, at a crucial time, to demonstrate to the American mathematical and statistical worlds that statistics could be, simultaneously, both scientifically useful and mathematically rigorous but, over and above this, he seems also to have had a talent, akin to genius, for picking students and staff of outstanding ability and firing their enthusiasm for statistics The list of thirty such men (some no longer living) which Neyman gives is extremely impressive and of these about a score contribute to the *Festschrift*

The papers included are all on topical problems of theoretical statistics, and it is not untoward that only a minority refer to Hotelling's own work, since, as has been said, his major theoretical contributions are now embodied in the corpus of statistical classics There is no unifying theme here The topic treated by any given author could have been predicted from his own previous publications with some accuracy The only obvious difference between this collection and, say, the last volume of the *J Roy Stat. Soc* (B) is that the papers here are slightly shorter on average This reflects the fact that though the papers here tend to be more fluent and essay-like than a typical journal article, they are, by and large, slighter As a consequence, they are for the most part easy to read But it would be a hopeless task to attempt to review all of them, or even to try to pick out objectively the most important All I can do is to mention some that caught my fancy These include the two offerings from England, by M G Kendall and M S Bartlett, the latter discussing his concept of quasi-stationarity which promises to be, in one form or another, a key idea in the future development of stochastic process theory Bradley and Prendergast extend modern ranking theory which already owes much to the former author Block and Marshak have an intriguing paper on a cognate topic. Roy and Bhapkar expound and develop the Pearson–Roy logical analysis of multiway contingency tables. But perhaps the tributes which do Prof Hotelling most honour are the papers by Bowker and Wilks developing classical multivariate analysis, and that of Stein, who looks at it from the most recent Californian point of view D E. BARTON

ANALYSIS ON RIEMANN SURFACES

Riemann Surfaces

By Prof Lars V. Ahlfors and Prof Leo Sario (Princeton Mathematical Series, Vol. 26) Pp x+382 (Princeton, N J. Princeton University Press, London· Oxford University Press, 1960) 80*s*. net

THE modern era of Riemann surfaces was ushered in by H Weyl's book *Die Idee der Riemannschen Fläche* Here we find for the first time the systematic development of the idea of an abstract Riemann surface as an analytic manifold of one complex dimension, essentially a space with local parameters related in such a way that two distinct parameters are regular functions of each other in their common region of definition. Since the Second World War there has been a tremendous development in the field and books have appeared by Nevanlinna, Pfluger, Schiffer and Spencer, and Springer, for example

Ahlfors and Sario's *Riemann Surfaces* goes further than its predecessors The first chapter contains all the necessary topology which plays a fundamental part in the theory The next chapter introduces Riemann surfaces and some of the basic methods for treating them, subharmonic functions, and the problem and integral of Dirichlet, Rado's theorem about the separability of Riemann surfaces which follows in a particularly simple and elegant way, and the fact that a smooth Riemannian metric on a surface induces an essentially unique conformal structure, a result which is frequently stated but seldom proved The third chapter treats functions with singularities on a surface by Sario's elegant method of normal operators and deduces the principal mapping theorems for planar surfaces, that is, those topologically equivalent to plane domains Such surfaces can be mapped on a plane domain bounded by various kinds of slits In the simply connected case, we obtain the closed or open plane or the unit disk

The second half of the book consisting of the last two chapters is more for experts. The fourth chapter introduces a complete exposé of the modern theory of degenerate surfaces, that is, those not admitting functions of various kinds and their inter-relations. This subject was opened by Ahlfors in the late 'forties and the solutions of the problems raised by him have now been almost completely obtained by the authors of the present book and others The last chapter treats differentials on arbitrary Riemann surfaces and finishes with a welcome section on the classical theory of integrals on closed surfaces and the Riemann–Roch theorem

This is an authoritative and long-awaited treatise by two of the greatest experts in the field The elegant formulation of many of the ideas and proofs have made it possible to include more material than there is in any other book on the subject The presentation is fairly clear but the subject is difficult, and particularly in the latter chapters the approach is very abstract If one wishes to have complete generality and at the same time prove the classical theorems, it is most economical in time and space to deduce the latter from the former, but it is not necessarily the easiest way for the reader Although this book is completely self-contained, I should recommend it rather to those readers who already know something of the subject—having looked, for example, at G. Springer's *Introduction to Riemann Surfaces*—than to the complete tyro. As a reference book, however, and a complete survey of all techniques right up to the frontiers of the subject, it is unequalled, and every serious mathematical library should contain it W K HAYMAN

MODERN GEOMETRY

Lectures on Modern Geometry

By Beniamino Segre (Consiglio Nazionale delle Ricerche—Monografie Matematiche, 7) Pp xv+479 (Roma Edizioni Cremonese, 1961) 7,000 lire

MODERN geometry is a term which has seen service in many campaigns and under many commanders However, in the present instance, the use of the title is not only justified at the moment but also stands a good chance of remaining so for some time to come, for this book, which is a greatly enlarged version of an Italian original of 1948, completes in various respects a generalization which has been under way for centuries

Algebraic geometry as we know it to-day stems from two main sources the invention of co-ordinates, and the analysis of axiomatic structure The latter process has given a whole series of shocks to intuition; these, once absorbed, have led to the creation of new geometries which find their natural expression in terms of suitable co-ordinate systems. In a definitive account this process may well be reversed; in Prof Segre's work we have examples of both procedures.

Part I of the book is a lucid exposition of the algebraic concepts and results which are required later A useful innovation here is the term corpus to denote a general field, the latter name being reserved for commutative corpora. Part 2 is devoted to the foundations of projective geometry over an arbitrary corpus. The first chapter deals with linear spaces, that is, geometry based on homogeneous point co-ordinates In the second chapter, which is concerned with graphic spaces, the approach is axiomatic a graphic space is any set of abstract elements ('points') which satisfy the incidence relations of projective geometry On this basis one proceeds to erect a co-ordinate system

At this point Desargues' theorem on triangles in perspective comes into play. It can be shown that the theorem holds in every linear space, but that there are graphic planes in which it is false. In contrast with the situation in higher space, Desargues' theorem in the plane is not a consequence of the axioms of projective geometry A second group of results concerns Pappus' theorem (that is, Pascal's theorem for a line-pair) it may be proved that this theorem holds in a plane if and only if the corpus defining the co-ordinate system is commutative Also the theorem cannot be established on the sole basis of the incidence postulates and Desargues' theorem, but Desargues' theorem can be deduced from these postulates together with Pappus' theorem

There follows a very interesting chapter on finite linear spaces, a subject to which the author has made fundamental contributions during the past few years It is here, in the geometry of a finite number of points, that intuition receives its gravest shocks and that seemingly unshakeable theorems tumble like ninepins

A substantial appendix by L Lombardo-Radice on non-Desarguesian finite graphic planes takes the theory further, but much has happened in the two years since the book went to press The style and content of this work are such as we have come to expect of the distinguished author, whom all geometers will thank for this valuable addition to the literature

L ROTH

"OPINIONS OF THE LATE AND BEST PHISITIANS"

Tobacco

Experimental and Clinical Studies—a Comprehensive Account of the World Literature By Prof P S Larson, Prof H B Haag and Prof H Silvette Pp xii + 932 (Baltimore, Md The Williams and Wilkins Company, London Baillière, Tindall and Cox, Ltd , 1961) 160*s*

IN 1595, an anti-smoking tract appeared in England entitled "Opinions of the Late and Best Phisitians concerning Tobacco", establishing a tradition of criticism which has persisted to the present day This reference does not appear in the book under review, but there can be few of scientific interest which are not included The authors' stated intention is "to be deliberately non-selective, printing good work and bad, sound and uninformed opinion, alike, in the same type", for they discovered that "there was scarcely to be found consistency of data in any area of observation, and unanimity of interpretation was even more rare" The result is an enormous work with more than 6,000 references It is not, however, an unevaluated mass of contrapuntal observations, echoing the disagreements of a century and a half, for the authors have decided views expressed by length of quotation, inclusion of critical reviews, and their own comments on methods and principles of research

The authors are pharmacologists, and the first part is devoted to the pharmacology of tobacco in many species

The human race has experimented on itself with tobacco so persistently that short-term effects due to nicotine must be relatively unimportant It has been said that nicotine can be regarded as a naturally occurring hormone in regular smokers It is not significant that it can be toxic in large doses, so, after all, is adrenaline Since nicotine is the major constituent it has pride of place, and our ignorance of the other 150 constituents of tobacco smoke, 10 of which are carcinogenic, is startlingly apparent In such chronic habits as smoking these 'tracer' substances must be as worthy of study as nicotine

A large part of the volume is clinical One feels that in referring to particular diseases the aid of specialist clinicians might have been an advantage For example, the statement is made, correctly, that most authorities forbid smoking in thromboangiitis obliterans, but the patients observed by these authorities were almost certainly arteriosclerotic, and their conclusions will not stand up to critical appraisal

This is a minor criticism The authors' general critical approach could be imitated with benefit by many clinicians who are in the chase after chronic influences on chronic diseases

In most cases one must agree with the authors that the correct verdict on tobacco is "Remanded for further evidence" The greater part of the literature is critical of tobacco, but in this instance quantity does not count There is no disease from piles to coronary thrombosis for which it has not been blamed, but it has also been used to treat most of them It would be a mistake to condemn it from puritanical tendencies, when there may be beneficial effects as difficult to demonstrate convincingly as its alleged disadvantages One supposes that the habit is of some value to those who use it

KENNETH BLOOR

ÆTIOLOGY OF ALCOHOLISM: CONTROLLED OBSERVATIONS

Origins of Alcoholism

By William McCord and Joan McCord, with Jon Gudeman (Stanford Studies in Sociology, 1) Pp xi + 193 (London Tavistock Publications (1959), Ltd , 1960) 35*s* net

THIS is an important work It is a well-controlled study of a large group, of both 'normal' and 'pre-delinquent' boys, of which the original purpose was to consider the value in prophylaxis of criminality of guidance by trained social workers A vast amount of information was obtained In due course the boys grew up, and some of them became alcoholics Thus for the first time we have unprejudiced information about the alcoholic, obtained before the onset of the disease In other very numerous investigations the information necessary for evaluating pathogenesis has been elicited retrospectively and liable to many sorts of bias In the work presented in this book, bias seems to have been eliminated—partly by the multiplicity of observers and observations, partly by the absence of any design, in the original scheme, of studying alcoholism, and partly by careful coding and rating techniques

They find no support for some widely held views for example, glandular diseases, malnutrition, oral tendencies, latent homosexuality, do not correlate with alcoholism They list seventeen variables in the childhood background that did correlate with alcoholism Six were highly important (1) maternal alternation between affectionate and rejecting attitudes, (2) maternal deviance, for example, alcoholism, promiscuity, (3) paternal antagonism, (4) paternal escapism, (5) an 'outsider' in conflict with parental values, (6) absence of high demands on the child by the parents

They believe that their results indicate that by such forces the alcoholic has had developed strong desires to be dependent, but these are incompatible with the self-image in the stereotype of the American male—independent, courageous, dependable and resolute Drinking satisfies this by being a 'manly' pursuit, which yet satisfied dependency desires (I found this less cogently argued than the rest of the book)

They note differences between the personality of the pre-alcoholic and that of the developed alcoholic, and between factors that precede criminality in one, and alcoholism in another

It would perhaps be dangerous to generalize from this investigation about the genesis of alcoholism generally—there are some apparent differences between the alcoholics that the McCords describe and those with whom I am familiar Nevertheless it gives what appears to be valid and reliable information on an area in which hitherto we have had little but speculation W McAdam

THE BRITISH NATIONAL HEALTH SERVICE

The Genesis of the British National Health Service

By John Jewkes and Sylvia Jewkes Pp xi+67 (Oxford Basil Blackwell, 1961) 6*s* 6*d* net

The human and financial implications of Britain's National Health Service are so tremendous that the significance of a survey by Prof and Mrs Jewkes cannot be overrated They question the assumptions on which the National Health Service was established, challenge the validity of comparisons between Britain's centrally directed system and those of countries where the health services owe less to central direction and more to local enterprise, and have considerable reservations about whether or not the Health Service is as effective as its protagonists would proclaim

In their essay, the authors make a critical examination of the belief that, before the War, the British medical system was seriously defective and that nothing short of a centrally controlled free system could provide appropriate remedies, further inquiries are to be made into the economic and social consequences of the National Health Service As a basis for their inquiry, the Jewkes's have investigated the numbers and distribution patterns of doctors, dentists and nurses, the numbers of general practitioners in relation to the number of consultants, comparative mortality statistics and the way in which a health service might have developed if there had been no nationalized direction and control

Their findings are disturbing Although the statistics are not always revealing, it would appear that, even if the number of doctors has continued to increase under the National Health Service, the rate of increase has been no greater than that which obtained during 1921–39 The same can be said of nurses and dentists, and although, apart from our lesser number of dentists, British medical services compared favourably with those of the United States in 1939, the average American to-day is much better served, the gap between us continues to widen

With the introduction of the National Health Service it was hoped that the distribution of doctors throughout Britain would become more even Between 1911 and 1951 the geographical pattern remained largely unchanged and, although there was a slight gain in the number of doctors in more unpopular areas during 1951–59, the ratio still varies widely between north and south, spas and areas of heavy industry Specialist services have improved under the National Health Service, treatment by medical consultants being now much more widely available Nevertheless, the percentage of consultants to be found in the more attractive southern parts of the country has varied little between 1938 and 1957 Prof and Mrs Jewkes also indicate that the increase in the number of consultants owes much to the up-grading of men who, before the introduction of the National Health Service, were called general practitioners and whose titles alone have changed

A frequent complaint before the Second World War was that too many doctors carried too many patients on their panels Some improvements have taken place, but, since 1954, the position has deteriorated Between 1952 and 1959, for example, the average number of patients per principal in under-doctored areas fell only from 2,851 to 2,745 The charge, too, that older people have received far less medical attention since the introduction of the Service is also refuted by the figures

Two other points emerge from the survey. One is that, since the inception of the National Health Service, mortality-rates in England and Wales have not fallen more rapidly than in other Western countries The second is concerned with the administrative pattern of medical services and suggests that "the more sweeping criticisms of the pre-war organization of British medical services are not to be trusted and that the view that the pre-war weaknesses could summarily be swept away by a centralized organization was over-sanguine"

The findings of this inquiry raise so many issues of national importance that many people will wish to see the promised surveys into the social and economic consequences of the National Health Service Others will see that this inquiry is important not only for its own sake but also as an exercise in social science In many parts of this essay, Prof and Mrs Jewkes take exception to statements made by Prof R M Titmuss and others which were adduced to support the principle of a centrally organized health service In so doing they themselves, however unintentionally, have raised the question of whether social scientists should begin with a view and look for information to support it, or whether more profitable discussion might ensue if the inductive method of physical science were applied more faithfully Is this one of the reasons why so much of social science is still looking for its basic concepts ? T H Hawkins

A NEW MEDICAL DICTIONARY

The British Medical Dictionary

Edited by Sir Arthur Salusbury MacNalty Pp xix+1680 (London and New York The Caxton Publishing Co , Ltd , 1961) 210*s*

The appearance of this truly monumental work will be received with delight by everyone who has, systematically or occasionally, the need to consult medical journals Coinage of new words and changed meanings for old ones have led to the emergence of an English medical vocabulary which has become almost the equivalent of a foreign language to many old and new members of the profession Now, following the devoted efforts of Sir Arthur MacNalty and a team of colleagues over more than a decade, here is the very book for which we have been waiting

The production is of the highest quality, an important feature in a work which is bound to receive rough treatment The binding is stout and arranged so that, when an entry is found, the pages do not curl back in that maddening way so typical of bigger books (This one weighs 7½ lb) The paper will stand up to thumbing and the layout and type make reading easy

The quality of the 110,000 entries is in keeping with the standard of the appearance Definitions are so clear and precise that room has been found to include many variations on a single theme The term 'abscess', for example, is plainly described as a "collection of pus circumscribed in a cavity produced by tissue disintegration and displacement" The general entry, however, is followed by succinct explanations of more than 200 different kinds of abscess ranging from "atheromatous abscess" "bicameral abscess" and "collar-stud abscess" to "shirt-stud abscess" and "wandering abscess"

Each main entry is followed by its pronunciation, meaning and derivation, although readers are left to find out for themselves the pronunciation of secondary entries Where there is doubt, the simplest form of spelling is used, 'hæmospermia', for example, being preferred to 'hæmatospermia' for the condition in which "blood is discharged in the semen" Where shortening might lead to confusion, each spelling is included 'Hæmoblast', for example, is also listed as 'hæmatoblast' and 'hæmocytoblast', the actual definition appearing under the last-mentioned entry

For those who like to know at least a little about men and women whose names have left their mark on medicine, the treatment of eponyms is satisfying Baron von Munchhausen's connexion with his syndrome owes much to Richard Asher's historical knowledge and makes not only Munchhausen but also the entry 'come alive' The note about Dimitri Ivanovich Mendeléeff and, then, his Periodic Law, makes light work of mendelevium, a new transuranic element announced by Seaborg in 1955 The latter, incidentally, shows how recently some of the words in this dictionary have been coined and how wide-ranging has been the search for them

To complete this word-picture, words are included if they have any significance in medicine at all Thus, 'circuminsular' and 'circumvolute' are defined with precision along with more obvious medical words like 'circumocular' and 'circumoral' The only deliberate omissions are words which are obsolete or valueless, even here eponymous terms are included if they are still occasionally used in medical literature or have some historical interest Finally, to ensure that nothing is omitted or causes obscurity, three useful appendixes provide a firm base with a list of anatomical nomenclature, a number of physicochemical tables and specialized words used by pharmacists. The total result is a book of some 1,700 well-packed pages which will be kept up to date by cumulative annual supplements

English-speaking readers who have close or remote connexion with medicine will wish to thank the publishers for their vision and courage in producing such a magnificent dictionary They should see to it that all the editors of medical journals in countries in Europe and Asia who have been struggling to present articles in the English language should be informed of what could be a great aid to them These, and all English-speaking readers, have cause to be grateful to the editor and his distinguished colleagues for labouring so long to produce such a distinguished work Lexicographers, as Dr Johnson knew, are held high in national esteem With all his outstanding contributions to medicine, Sir Arthur MacNalty may have little thought that he is now most likely to be remembered as the editor of *The British Medical Dictionary.* K M HAWKINS

APPLICATIONS OF SCIENCE TO PEACEFUL USES

Current Trends in Scientific Research

Survey of the Main Trends of Enquiry in the Field of the Natural Sciences, the Dissemination of Scientific Knowledge and the Application of such Knowledge for Peaceful Ends By Pierre Auger Pp 245. (New York United Nations, Paris Unesco, London· H.M S.O , 1961) 33*s*

PROF AUGER'S ambitious survey of the main trends of inquiry in the natural sciences and the dissemination and application for peaceful ends of such knowledge, and on the steps which the United Nations, its specialized Agencies and the International Atomic Energy Agency could take to encourage the concentration of such efforts on the most urgent problems having regard to the needs of the various countries was undertaken at the request of the General Assembly of the United Nations This survey was carried out with the assistance of a special advisory committee and on the basis of texts prepared and supplied by the several agencies and other bodies Nevertheless, the task remains formidable and indeed forbidding, for the selection or omission of particular features must remain a matter of personal judgment which is liable to challenge, and if it is not easy to discern the precise purpose which the survey will serve, Prof Auger is to be congratulated on the courage and judgment with which he has discharged his mandate rather than criticized for particular omissions

The survey is in three parts The first, which occupies some 180 out of the total 229 pages, surveys the trends of scientific research, by functional divisions rather than academic disciplines, and is preceded by an excellent introduction on the development of research and the transition from discovery to application Here, and also in some of the separate sections, as in those on mathematics, the physical sciences, the chemical sciences and the biological sciences, Prof Auger attempts to set forth his conclusions as to the main trends in research, but in the applied sciences this is less frequently done and more open to criticism In the second part of the report Prof. Auger reviews very briefly the main factors which affect the organization of scientific research and the dissemination of its results, while in the third are set forth the recommendations concerning the practical steps which might be taken by the United Nations and the Agencies to concentrate effort on the most urgent problems from a general point of view By and large these two parts may well prove the most useful feature of the survey, the second part in particular should stimulate useful discussions, though here the absence of references is something of a drawback R BRIGHTMAN

SHORT REVIEWS

A Bibliography of the Honourable Robert Boyle, Fellow of the Royal Society

By Prof. John F Fulton. Second edition. Pp xxvi + 217 (Oxford Clarendon Press, London· Oxford University Press, 1961) 50*s* net

THE late Prof John Fulton's *Bibliography of the Honourable Robert Boyle* has now appeared in a second edition, almost thirty years after the first edition was published in the *Proceedings of the Oxford Bibliographical Society* Addenda to the first edition were also published by this Society in 1933 and 1947. In its revised form this exhaustive bibliography, with its scholarly annotations, has now been issued in one volume by the Clarendon Press, Oxford To this volume Prof Fulton has contributed a new preface, in which attention has been directed to changes rendered necessary as the result of newly acquired information. The preface to the first edition has been reprinted with "some omissions, several corrections, and a few added notes" This preface also contains some interesting notes on the dispersal of Boyle's library Major changes are few. The discovery by Miss M E Rowbottom (Wellcome Historical Medical Museum) in 1950 of a new and, incidentally, the earliest published writing of Boyle has necessitated an addition to the list of his works, and references to the large number of papers and monographs dealing with various aspects of Boyle's life and work that have appeared since the first edition have also been added It is a great convenience to have all this information incorporated in a single volume, and historians of science, librarians, bibliographers, book collectors and antiquarian booksellers will all be permanently indebted to Prof. Fulton for his work

V A EYLES

The Annual of the British School at Athens

No 55, 1960 Pp x + 238 + 59 plates (London British School at Athens, 1961) 63*s* net.

THIS volume from the British School at Athens includes a variety of interesting material It begins with a report on the rest of the pottery found at Karphi during 1937–39 with some observations on the relationship between the designs which appear on the painted wares and the patterns used for textiles Then there is Part 1 of a survey of prehistoric Laconia from the Neolithic period into the Bronze Age and even, in some cases, continuing on to Hellenistic and Roman times This is a little-known area, the scene of many incidents in the Homeric poems and other writings, and few excavations have taken place there It is followed by a consideration of Minoan trade relations as reflected by the Minoan objects found in Cyprus Four white 'lekythoi' in the National Museum at Athens are also described and some Euboean floral black-figured vases in the Museum at Chalcis There is also a discussion of the shrine of Kodros, Neleus and Basile at Athens

Work at Knossos is represented by reports on the excavation of a well containing Protogeometric and Geometric pottery and also of Protogeometric graves at Agios Ioannis Several inscriptions at Chios provide the material for a review of the local early tribal organization, and a fragment in the Piraeus Museum forms the basis for a brief study of the cult of Apollo Delios It is followed by interesting discussions of the Sibylline tradition at Delphi and of the connexion between the small clay nodules stamped with seal impressions, known as *cratulae*, and the Linear *A* accounting system The volume ends with some further consideration of architectural questions concerned with the Temple of Poseidon at Sunium. Numismatics and later work are both represented in a survey of the influence of the base silver deniers made at Tours in the thirteenth century A D on the coinage of Frankish Greece Altogether a useful volume full of good things

JOAN LIVERSIDGE

Observations and Experiments in Natural History

By Alan Dale Pp. viii + 154 + 8 plates (London William Heinemann, Ltd., 1960) 12*s* 6*d* net

THE aim of this book is to appeal to young scientists It is designed to encourage their own observations and experiments—two essentials for anyone taking up a scientific career

The book consists of notes of observations and experiments carried out by the author and others, and also suggestions for the reader's own activities Sometimes a problem is posed, and a line of experiment suggested, leaving the reader to discover the answer Wherever appropriate, broad or detailed biological facts are included

There are six chapters The first is an introduction The others cover invertebrate animals, excluding insects, insects, vertebrate animals, lower plants, higher plants As these titles suggest, a wide field is covered and, therefore, a little is learned about a lot This prevents the book from being a specialist or text-book, and, instead, acts as a stimulus This also accounts for the lack of continuity in style Each chapter is written in note form, with titles and sub-titles There are many small black-and-white figures, and eight black-and-white full-page plates

The book should interest all young people with a natural curiosity, not only those with an eye on their future

B. J G MAITLAND

Contributions to the Systematics of Oriental Termites

By M L Roonwal and P K Sen-Sarma (Entomological Monographs No 1) Pp xiv + 407 (New Delhi Indian Council of Agricultural Research, 1960.) Rs 12, 19*s*

FIVE papers dealing with the systematics of termites from the oriental region are presented here in one volume, the publication and distribution of which would appear to have suffered a delay of more than three years Related contributions by Dr Roonwal and his colleagues have continued meanwhile to appear in a variety of scientific journals, resulting in some confusion in the chronological sequence of their series

Four papers are short and deal with a variety of genera in several sub-families of the Termitidae The fifth paper, comprising 181 pages and 46 plates, is a revision of two genera of Kalotermitidae, *Kalotermes* and *Neotermes*, in which 29 species are re-described, measured in considerable detail and fully illustrated, and a further six species described as new In addition a number of lectotypes have been designated, since it was formerly the more usual practice to give a series the status of co-types It is noted that in some instances the lectotype selected is the only specimen at the authors' disposal, and would not appear to have been chosen from a series of co-types

This revision of two important genera of oriental dry-wood termites is a valuable contribution to the literature of the Isoptera, reflecting credit on the authors and the Indian Council of Agricultural Research, which financed the work It will be of much practical assistance to entomologists working in the region, and beyond, on the biology and control of these insects W V Harris

Ornamental Trees for Garden and Roadside Planting

By S A Pearce Chapters on Diseases by Derek A Reid, and Insect Pests by Joyce van Konynenburg Pp 195+94 photographs (London. W H and L Collingridge, Ltd , 1961) 35*s* net

THE author, who is on the staff of the Royal Botanic Gardens, Kew, in writing this book enjoyed the advantages of being able to refer to one of the finest collections of living trees

He has succeeded in passing on to his readers his expert knowledge, for this well-produced book contains in its sixteen chapters most that nearly all those interested in trees for ornamental purposes should require to know when selecting the right tree to plant in the right place. Having made this selection, advice is given on the correct planting of a tree and its after-treatment, including an excellent chapter on dangerous trees and how to deal with these, with some useful information on a tree owner's liability.

Ninety-four good black-and-white illustrations and nine helpful line drawings are included The student, however, who refers to the book may be a little disappointed in the absence of a good annotated diagram of a cross-section of a complete tree-trunk to illustrate the chapter on "Life of a Tree" and the omission of a paragraph on the *p*H of soils in the chapter entitled "Knowing your Soil". In keeping with the high standard reached by the author when dealing with the practical aspects of growing trees are the six chapters devoted to descriptive lists of these.

He is to be commended in arranging for the specialists Derek A Reid and Joyce van Konynenburg to contribute the authoritative chapters on "Diseases" and "Insect Pests of Trees" F P Knight

Cork and the Cork Tree

By Dr Giles B Cooke (International Series of Monographs on Pure and Applied Biology Division Botany, Vol 4) Pp. xii+121 (London and New York Pergamon Press, 1961) 50*s* net

CORK from the bark of the cork oak (*Quercus suber*) of southern Europe has been used by man from very early times and has been of importance commercially in Europe over a very long period In spite of the advance of synthetic materials the demand for cork does not lessen, probably because natural cork has a combination of unique properties not to be found in other materials A good idea of the manifold uses of cork in the modern world may be obtained from a perusal of this book The author was previously director of research of a large crown cork and seal company in the United States and has obviously made a close study of cork and its many modern applications

Last century, the cork oak was introduced to cultivation in many other parts of the world where the climate seemed suitable, often through the agency of Kew No lasting success or commercial development resulted from this, however, and southern Europe and north Africa continue to meet the world demand for cork Interesting details are given in the book of early planting of the cork oak in the southern United States and of a recent project to stimulate further planting there There is also up-to-date and useful information on the cultivation and treatment of the cork oak in Portugal and on production and trade in cork in that country—the world's largest producer The book contains chapters on the physical properties, chemical composition, and the manufacture of cork articles, and the many forms of composition cork so much used to-day F N Howes

Studies in Paleobotany

By Prof Henry N Andrews, Jr With a chapter on Palynology by Charles J Felix Pp xii+487 (New York and London John Wiley and Sons, Inc , 1961) 94*s*

THE appearance of a new and up-to-date book about fossil plants is of rare occurrence In the event, a special welcome must be accorded to *Studies in Palaeobotany*, for Prof Andrews writes with great clarity and his text is accompanied by numerous excellent illustrations, many of which are completely new or reproduced from publications not easily available to the majority of readers The larger part of the volume is devoted to a systematic survey of the major groups of vascular plants, but there are separate sections dealing with more general aspects such as the evolution of the seed, Palæozoic and Mesozoic floras, fossil plants of the polar regions, and palæobotanical techniques There is also a section on bryophytes, and Charles J Felix has contributed a most useful chapter on the specialized topic of palynology

Prof Andrews argues in favour of a polyphyletic classification of the vascular plants, but many would not agree that all the evidence on which his arguments are based is sufficiently convincing As one example, there may be noted the importance attached to *Aldanophyton*, the supposed lycopsid of Cambrian age Very little is really known about this fossil, and there is no proof of its affinities, still less that it was a genuine vascular plant It is true that the Lycopsida and Psilopsida were already co-existent during the Silurian, but there is no evidence as to which appeared first or whether they represent quite separate lines of evolution as polyphyletism demands To me, it seems that the more conventional outlook, which regards the *Rhynia*-type as having provided the ancestral stock for the other groups of vascular plants, is still not seriously challenged

Two errors need to be noted Fig 9–2*A* is not a reconstruction of *Calamophyton bicephalum* but of *C primaevum*, and on p 416 the Cathaysian flora is incorrectly stated to have overlapped the Angara province when, in actual fact, the reverse was the case Alan Wesley

Introduction to a Submolecular Biology

By Albert Szent-Gyorgyi Pp xi+135 (New York Academic Press, Inc , London Academic Press, Inc (London), Ltd , 1960) 5 dollars

FOR some twenty years Prof Szent-Gyorgyi's interest has been in possible mechanisms by which the reactions in cells are co-ordinated in metabolism and in the transfer of energy He has successively viewed this central problem from various physical points of view, first, the common-energy level or continuum theory, then the triplet state (in *Bioenergetics*), and now, in this new little book, the theory of charge-transfer complexes Once again, the possible catalytic effect of water is emphasized, and the author's lucid and enthusiastic prose is reinforced by photographs of the colourful events which happen when various solutions of donors and acceptors are mixed and frozen The results of extensive molecular orbital calculations by the Pullmanns are included, and serve as the backbone of the discussion, which considers the topics of drug action, adenosine triphosphate, the thymus gland and the living state There are growing numbers of scientists interested in electron mechanisms in biology who will find this book most stimulating, and a source-book of problems for some years to come

D D ELEY

The Falkland Islands

By M B R Cawkell, Dr D H Maling and E M Cawkell Pp xii+252+16 plates (London Macmillan and Co , Ltd , New York St Martin's Press, Inc , 1960) 18*s* net

THE relatively little-known Falkland Islands have had a most eventful history since human occupation reached them Their initial discovery is still somewhat obscure, in spite of active research by the more politically minded writers of South America In the present work, which is a worthy successor to that of the late Miss V E Boyson, the many facets of the islands are carefully described, although sometimes with less enthusiasm than others This volume proceeds systematically from 'discovery', through the early and later developments to administration and the role of the church and the school The more scientific aspects of the islands, the climate, vegetation, geology and structure, and bird life are admirably discussed and give the serious reader a very clear insight into the many problems of the islands The selected bibliography which appears at the end of the book is of great assistance in following up any specific subject more closely This volume should be read by those who are interested in the far-flung parts of the British Commonwealth, since it is clearly shown that the Falkland Islands is a well-developed colony R J ADIE

Water Supply

Economics, Technology, and Policy. By Jack Hurshleifer, James C de Haven, and Jerome W Milliman Pp xii+378 (Chicago University of Chicago Press, London Cambridge University Press, 1960) 7 50 dollars, 60*s*

THIS is an interesting but highly critical account of the problems of water supply in the United States approached through the eyes of two economists, Prof J Hurshleifer of the University of California and Prof J W Milliman of Indiana University, aided by a physical chemist, J C de Haven The work has been made possible through the Rand Corporation as part of a research programme

The authors believe that many water supply professionals are living in an artificial world of their own creation without respect for economic principles and rationality The present and approaching shortage of water and the proposed remedies are examined in the light of this belief Many of the conclusions reached are as a result highly controversial Much of the substance of the book will, however, be of considerable interest to those concerned with problems of the supply of water in other parts of the world Questions of over- and under-investment, financial returns on outlay, pricing, water in relation to other economic commodities, local regional and national organization and control, technological advances, and the legal position so far as water is concerned are all critically considered

A great deal of blame is laid by the authors on the imperfections in water law in the United States, where water rights are not clearly defined and cannot be transferred with ease as are rights in land and minerals, etc As a result it is argued that the economic market processes that ordinarily direct resources to uses that maximize their productivity do not operate

The establishment of clear property rights in water poses, of course, difficult problems Water is sometimes a transient resource, sometimes in store, sometimes above and at other times below ground, sometimes even unwanted But increasingly it is becoming a scarce resource in both semi-arid and humid areas, and capital will be increasingly invested to satisfy the public demand for more water The plea of this book is that this capital should be wisely invested with due forethought and on sound economic principles W G V BALCHIN

Population Redistribution and Economic Growth, United States, 1870–1950

By Simon Kuznets, Ann Ratner Miller, and Richard A Easterlin (Memoirs of the American Philosophical Society, Vol 51) Pp xiv+289 (Philadelphia American Philosophical Society, 1960) 5 dollars

THIS is the second volume of a three-volume project designed to link various indexes of economic growth to the redistribution of American population, under the direction of Prof Dorothy Thomas and Prof Simon Kuznets In the first volume, which appeared four years ago, the difficulties of collecting and adjusting comparable time series of economic data for individual years since 1870 were described, and the validity of the estimates discussed. The present volume consists of three monographs, dealing respectively with the labour force in different States, with the regional growth of income and with the changing distribution and structure of economic activity

In each of the three monographs there is noted a process of convergence of inequalities between individual States Labour force participation-rates (that is, the proportion of the population in gainful employment), income per head, and structure of manufacturing all show this trend The authors are content to describe what has happened, and do not attempt to theorize about the future, nor do they suggest that the process of convergence that they describe was a necessary condition of growth in the past 80 years

The third volume will deal with the role of migration and the interrelations between economic and demographic variables The whole project promises to be an important contribution to the study of American economic history and development E GREBENIK

Advances in Enzymology and Related Subjects of Biochemistry

Vol 22 Edited by F F Nord Pp v+567 (New York Interscience Publishers, Inc , London Interscience Publishers, Ltd , 1960) 105*s*

THE twenty-second volume of this useful series contains three articles on metabolic topics, two about the mechanism of action of enzymes, one on practical techniques, two on more biological topics, and one on pure chemistry

The sections of metabolism reviewed are the synthesis and hydrolysis of sulphate esters (by A B Roy), the biochemistry of sulphonium compounds, particularly with regard to transmethylation (by S K Shapiro and F Schlenk), and the biosynthesis of cholesterol (by G Popjak and J W. Cornforth) The last-mentioned review follows appropriately the review on carotenoid synthesis in the previous volume

An important article by D E Koshland on "The Active Site and Enzyme Action" summarizes the methods which may be used to label and identify the active centre of an enzyme, as well as putting forward some conclusions about the chemical mechanism of enzymatic catalysis S. Shifrin and N O Kaplan discuss the binding of coenzymes by enzymes, principally in cases where this may be studied by spectroscopic methods

The genetic control of enzyme activity, and the induced synthesis of enzymes, are considered in articles by J. R. S Fincham and H O Halvorson respectively These two articles emphasize the increasing interest in 'enzyme biology'

Two articles are of great practical importance to the practising enzymologist F Turba gives a valuable account of the purification of enzymes by column chromatography, with many well-documented examples J Baddiley and N A. Hughes give an up-to-date account of the chemical methods now available for the synthesis of nucleotide coenzymes and their analogues E. C. WEBB

Röntgenstrahlinterferenzen

Von Max von Laue Unter mitarbeit von E. H Wagner Dritte, neubearbeitete und erweiterte auflage Pp x+476 (Frankfurt am Main: Akademische Verlagsgesellschaft 1960) 75 D M

IT is always interesting and instructive to read a book written by the founder of a subject and to see how he considers that it has developed since it began Laue's book has, of course, been a standard work for many years and most other text-books have been based on it, either directly or indirectly Modern treatment of diffraction theory, for example, is much nearer to Laue's treatment than to the more vital and productive, but less clearly rigorous, treatment through Bragg's law

It is interesting, and not a little salutary, to see the difference between Laue's treatment of X-ray diffraction and that of British authors He justifies this third edition by stating that a great deal of progress has recently been made in research on the structure of crystals , yet this section of the book looks curiously old-fashioned, with its diagrams of diamond, graphite and fluorspar. The author's interests lie much more in diffraction by imperfect structures—liquid crystals, temperature-affected lattices and deformed crystals—than in the structures in which workers in Britain are mainly interested The names that appear frequently in our text-books—Bernal, Lonsdale, Hodgkin, etc —are scarcely mentioned in this book, but the elegant diffraction work of Hosemann and Renninger is given considerable prominence

It is sad to think that Laue's death in an accident has withdrawn the founder of the subject from among us and that he will not be present next year at the fiftieth anniversary celebrations of his great idea in Munich This book will serve as a constant memorial to him H LIPSON

Modern Physics

By M. S Smith Pp ix+254. (London Longmans, Green and Co , Ltd , 1960) 15*s* net.

THIS is a lively and well-written account of the way physics has developed in the past sixty or seventy years The author has a refreshingly original style and has attempted to convey the spirit of the investigations he describes as well as the essential detail , he has avoided the rather dreary repetition of standard diagrams and tables that so many text-books adopt

He is, however, rather unrealistic in claiming that it will serve as an introduction to the subject for sixth formers and undergraduates , it can scarcely yet be claimed, for example, that a detailed consideration of Schroedinger's equation is merely introductory The book is much more likely to be useful to the more mature physicist who wishes to have a readable survey of atomic and nuclear physics and who may wish to have some help in straightening out his ideas for presentation to others

Moreover, the book scarcely justifies its title , it contains nothing of solid-state physics, biophysics or modern developments in optics Admittedly a book of this size and modest cost could not be expected to be so general, but the title ought to have been more specific

The book is attractively produced and misprints and mistakes seem to be few But it was copper sulphate, not zinc sulphide, that was first used to diffract X-rays (p 40) ; the field of a dipole is inverse cube, not inverse fourth-power (p. 72) , and the word 'anticipate' is incorrectly used on p 80 H LIPSON

Laboratory Management and Techniques

By J. A. Edwards Pp xvi+207. (London · Butterworth and Co (Publishers), Ltd , 1960) 35*s*.

THE author makes clear in his introduction that this book does not pretend to deal with the higher problems of laboratory administration as this subject has been so adequately covered by E. S Hiscocks in his excellent book (*Laboratory Administration*, London, 1956) A reading of Mr Edwards' book, however, reveals that he too is dealing largely with administrative matters The essential difference appears to be one of point of view Mr Edwards' conception of laboratory management is that of a laboratory steward This conception is, in my opinion, better suited to the organization of teaching laboratories than to industrial and research laboratories The value of the contents of the main part of the book to all laboratories is nevertheless indisputable and quite independent of the organization-level from which it is viewed There are six parts to the book, dealing with the new laboratory, laboratory organization and maintenance, accident precautions, special and auxiliary units, purchases and records, and staff problems and human relations These parts are subdivided and filled with information and advice on design, organization, equipment and documentation This information and advice is of a kind which is obviously based on a wide experience of practical laboratory management and has rarely been recorded in an accessible form

Stores organization and fire precautions are particularly well dealt with The special and auxiliary units include the radioactive materials laboratory, lecture theatre, photographic processes, workshops and library

Those who occasionally import apparatus or chemicals will welcome the chapter on Customs and Excise procedures. This chapter deals also with applications for industrial methylated spirit, duty-free spirit and permission to use stills It is admirably concise and informative

The book is well produced and illustrated It will be of great value to all whose duties include the provision of the ways and means of scientific endeavour E I JOHNSON

Lehrbuch der Organischen Chemie

13, neubearbeitete und erweiterte Auflage Von Prof Paul Karrer Pp xx+1080 (Stuttgart Georg Thieme Verlag, 1959) 60 D M

ORGANIC chemistry has undergone a great transformation both in extent and depth since Prof Karrer's classic text-book first appeared in 1927, and much of this change has come about during the ten years that separate the last English from the new German edition of this work Prof Karrer has given recognition to this fact by inviting a group of authorities to revise and enlarge the existing text and to contribute a number of new chapters The general layout and the major portion of the contents, however, remain unaltered

While some of the newly written chapters, as, for example, those on conformational analysis, steroids, and amino-acids and polypeptides, are excellent, the shorter sections inserted into the old text are generally not well assimilated so that a consistent presentation fails to emerge In particular the treatment of reaction mechanisms leaves an unsatisfactory impression Throughout the book they are presented without formal consistency and the basis for their inclusion or omission is not readily apparent, alternative mechanisms are listed uncritically (Cannizzaro reaction, pinacol rearrangement, aldol condensation) and some are of an unorthodox nature (ester saponification, hemiacetal formation) Two further points of criticism force themselves on the reader's attention A good deal of up-to-date information has been included, but at times abbreviated to an extent which obscures its significance (chemistry of caryophyllene, biogenetic role of phenol dehydrogenations) On the other hand, there are some surprising omissions (mechanism of halogen and halogen acid addition to olefins, Michael addition, Bredt's rule)

Prof Karrer's book has for many years occupied a leading place among the text-books of organic chemistry Its principal virtue still lies in the generous amount of factual information to be found within its covers, but one wonders whether its value as a pedagogic text can be effectively maintained by the method that has been adopted in this latest edition K H OVERTON

Radiation Use and Control in Industrial Application

By Charles Wesley Shilling (Modern Monographs in Industrial Medicine, Vol 5) Pp viii+223 (New York and London Grune and Stratton, Inc , 1960) 6 75 dollars

IN his preface, Dr Shilling emphasizes that this book is non-technical and is intended for the person who needs only a working knowledge of the subject; in particular "the physician who must handle radiation exposure and injury cases and who must answer questions concerning the mysteries of radiation" In consequence the book has been written with a medical bias, but the author's aims have not been achieved since the treatment of the physical aspects of protection, such as shielding, instrumentation and monitoring techniques, is meagre and rather inadequate

The first section of the book covers the origin and nature of the radiations and the dose to man due to natural sources, and is followed by sections on the prevention and treatment of radiation injury, the control of radiation and the use of radioisotopes Although the treatment of each topic is, of necessity, concise, the subject-matter on the medical aspects is sufficient to acquaint the industrial physician with the various problems and to provide a basis for further reading It should be noted, however, that the control procedures are considered from the United States point of view, and British readers should acquaint themselves with the national legislation and recommendations

Some of the illustrations appear unnecessarily elementary and there are one or two errors in the text, for example, the maximum permissible concentration of radionuclides in air and water for the exposure of the entire population is given as one-tenth of the occupational exposure-level, whereas for individuals the International Commission on Radiological Protection recommends a reduction factor of one hundred However, these errors are not serious provided the book is used for general reading rather than as a text-book B E JONES

Commutative Algebra

Vol 2 By Prof Oscar Zariski and Prof Pierre Samuel (The University Series in Higher Mathematics) Pp x+414 (Princeton, N J . D Van Nostrand Company, Inc , London. D. Van Nostrand Company, Ltd , 1960) 58*s* 6*d*

THE first volume of this work was published in 1958 Now that the second volume has appeared it is possible to be much clearer on the motivation behind the subject as a whole This is buried in the history of algebraic geometry, and the authors have been careful to indicate what parts of geometry have led to the immense algebraic developments of recent years The book is very carefully written and will be an essential reference, and even a text-book, for the pure mathematician who moves or wishes to move in this field

There are three long chapters, given to valuation theory, polynomial and power series rings and local algebra In addition there is an appendix of about eighty pages containing a good deal of previously unpublished material. The contents of the chapter on valuation theory are illustrated largely by examples from the theory of algebraic function fields, and the notions of normal and derived normal models, of great importance in geometry, are introduced Geometry is seriously introduced in the chapter on rings, and includes an account of algebraic varieties in affine and projective space, together with a treatment of dimension theory, the Hilbert characteristic function and chains of syzygies The chapter on local algebra gives an account of local rings and their properties, and then makes use of these rings in the local study of algebraic and analytical varieties This study culminates in a section on analytical irreducibility and analytical normality of normal varieties

This volume, together with its predecessor, is abstract from cover to cover But the exposition is not condensed, and the work is likely to become the standard introduction to the arithmetic foundations of algebraic geometry For this reason it seems a pity that, apart from a few scattered references, there is no bibliography L S GODDARD

Reciprocals of the Integers from 1000 to 9999, with an Appendix on Mechanical Barrel-setting Calculators

By T H Redding Pp iv+42+2 plates (London Taylor and Francis, Ltd , 1960) 18s 6d net

THE actual entries are the reciprocals to six figures, multiplied by 10^6, so that each entry is divided into two blocks of three digits by the decimal point, thus facilitating transfer from table to machine The author does not say much about how the calculations have been checked, but some small sampling has revealed no error There are no horizontal rules except in the frame, so the page is fairly clean, with 1,000 entries at an opening and a thumb-index A clear modern 'equal height' fount is used, though it is scarcely as effective as the old-style 'head and tail' fount used by Comrie and Peters There is an appendix of some twenty pages, in which the nature and use of barrel-setting desk machines are described in simple terms for the novice The book should be of most value to those who are not expert at computation but have need now and then to make desk calculations. T A A BROADBENT

An Introduction to the Theory of Numbers

By Prof Ivan Niven and Prof Herbert S Zuckerman Pp viii+250 (New York and London John Wiley and Sons, Inc , 1960) 6 25 dollars, 50s

THIS compact and lucid text could be used by good sixth-form specialists or first-year honours students In the first part of the book the basic concepts and theorems are presented divisibility, primes, congruences, quadratic reciprocity, number-theoretic functions, diophantine equations, Farey series and continued fractions, the simpler properties of the distribution of the primes The last three chapters introduce more specialized topics algebraic numbers and number fields, the partition functions with the formulæ of Euler and Jacobi, and the fairly recent study of the density functions of a sequence of integers The proofs are clear and concise, but they rightly demand a certain sophistication of outlook without which the reader is unlikely to be able to see why a particular line of argument is chosen An excellent feature is the supply of exercises for the student; these range from simple corollaries on the main text to quite searching extensions of it, answers are given where appropriate A young student with a taste for number theory and a firm grasp of elementary algebra would find the book profitable reading

Elementary Logic of Science and Mathematics

By Dr. P H Nidditch Pp vii+371 (London University Tutorial Press, Ltd , 1960) 18s

THIS book has two aims to provide a detailed but elementary course in modern logic and to present basic material in the philosophy of science, covering probability and measurement, the scientific method and the construction of theories The author has succeeded well in writing a book that is both accurate, informative and easy to read About one-third of the book deals with formal logic, sentence logic is studied by means of a valuation theory (truth tables), and in terms of axiom systems The predicate calculus is developed both as an axiomatic system and as a Gentzen-type system of natural inference; some attempt is made to wed the two codifications but a full equivalence proof is wanting Among other axiom systems presented are Boolean algebra and a theory of probability Definitions are given with great care, there are numerous historical references and a good collection of examples, with solutions or hints for solution

The method used in the second part of the book is largely that of judicious quotation, the great scientists are allowed to speak for themselves and there is no attempt at criticism or comparative study, but there is a valuable selection from original sources

R L GOODSTEIN

Health in Industry

A Guide for Engineers, Executives and Doctors

By Prof R C Browne Pp. vii+157 (London Edward Arnold (Publishers), Ltd , 1961) 18s net

OVER the years the Nuffield professor of industrial health at King's College, Newcastle upon Tyne, has delivered courses of lectures to science, medical and technological students at the University on various aspects of occupational health The subjects have been varied and topical, and have shown concern for the health of the executive, the heavy cost of sickness absence, the importance of good design, the causes and consequences of accidents and fatigue, automatic processes and their effects on the worker and the part played by good working conditions on output Particular attention is directed to the essential precautions which must be taken in using ionizing radiations, while Prof Browne also analyses the influence of various chemical substances on the factory worker

The author has now addressed his lecture-material to all employers and managers who are, in some way or other, responsible for the industrial environment Much of it will be of interest and value, and the book should appeal to many who bear responsibility for people in industry The book could also be used in industrial health sessions during courses on industrial administration In such a book a surprising feature is the absence of all reference to skin diseases and the use of barrier creams which may cause more complaints than they cure In the section on the health of the manager, the author might also have given some practical advice about diet Many industrial executives become ill because they are unaware of the simple rules of health, although they sometimes call their complaint 'pressure of work' T H HAWKINS

LETTERS TO THE EDITORS

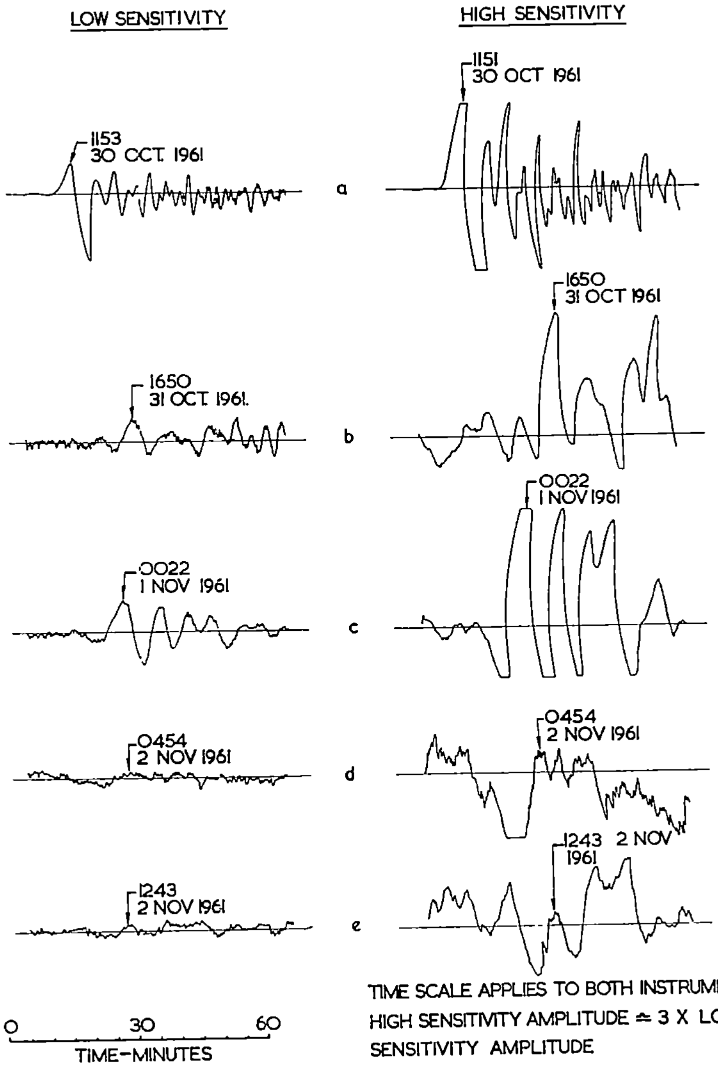
SEISMOLOGY

Microbarograph Records from the Russian Large Nuclear Explosions

DURING the recent series of Russian atomic weapon tests, microbarographs were operated at this Establishment The instruments used were purely mechanical devices having limited frequency response Excellent records were obtained from several of the larger firings at Novaya Zemlya and particularly from the 50-megaton firing of October 30. Taking the origin time as 08 33 hr from a study of the seismic signal, we saw the start of the first characteristic microbarograph record (Fig. 1*a*) 3 hr. 11 min later at 11 44 hr on October 30, subsequent to this, we obtained clear signals at approximately 16 40 hr on October 31 (Fig 1*b*), 00.10 hr on November 1 (Fig 1*c*); and 12 40 hr on November 2 (Fig 1*e*). We looked for a signal at around 05.10 hr on November 2 but could not see one against the background (Fig 1*d*)

It would appear that the waves are being successively reflected at the antipodes of Novaya Zemlya and at Novaya Zemlya, with relatively little absorption or scattering. We have re-examined our records of the 30 megaton firing on October 23 and they show similar evidence of reflexions corresponding relatively in time to those shown in Figs 1*b* and *c* for the 50-megaton firing, but with the signal to noise ratio much smaller.

Observations made on the phenomena with more refined instruments or arrays and a compilation of results on a world-wide scale could yield extremely interesting data about the Earth's atmosphere We have noticed a number of points of interest already in our brief examination of the records For example, there is a good agreement between the wave shape observed and the form one would expect from a study of the paper by Hunt *et al* (ref 1, particularly Fig 10). Brune *et al.*[2] have recently treated the problem of an elastic surface wave on a sphere and found a $\pi/2$ phase shift at each polar reflexion, our observations, although difficult of interpretation because of dispersion, do suggest a phase reversal after two such reflexions The waves diverging from the origin (Figs. 1*a*, *c* and *e*) also appear to have a simpler, more compact, waveform than the waves converging on to the origin (Fig 1*b*).



TIME SCALE APPLIES TO BOTH INSTRUMENTS
HIGH SENSITIVITY AMPLITUDE ≏ 3 X LOW SENSITIVITY AMPLITUDE

Fig 1 Microbarograph records taken at the Atomic Weapons Research Establishment, Foulness

An interesting comparison can also be made with the records obtained following the eruption of Krakatoa in 1883[3] The average time along several paths for waves to pass around Earth following Krakatoa was 36 hr. 20 min ± 10 min (*S E* 35 readings), the corresponding figure from our records being 36 hr 27 min with an accuracy of a few minutes

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ASTRONOMY

The Galactic Red Shift

The recent development of the technique of resonant scattering of γ-rays, using the Mossbauer effect, appears to raise the possibility of a terrestrial test of one theory of the red shift observed by Hubble in the light coming from distant galaxies

The usual interpretation of the galactic red shift is that it is a Doppler effect due to mutual recession of the centres of the galaxies, there are, however, other possible interpretations, and among these is the suggestion by Finlay-Freundlich[1] that it is due to loss of energy by light in passage through a radiation field Finlay-Freundlich was led to this conclusion from a study of stellar red shifts and put forward the empirical relation that the fractional energy loss is proportional to the path-length and the fourth power of the temperature (energy density) of the radiation field With this simple empirical relation and using the observed stellar and galactic red shifts, he deduced a value for the average intergalactic (radiation) temperature which does not seem unreasonable on general thermodynamic grounds

Born[2] pointed out that Finlay-Freundlich's empirical relation might correspond to an otherwise unknown photon–photon interaction in which loss of energy occurs without change in direction of the photon Born showed that there was some evidence in the empirical relation for a fundamental length which ought perhaps to be part of a general description of the radiation field, and suggested that the energy apparently lost might reappear as radio emission

These suggestions by Finlay-Freundlich and Born if correct, would have very far-reaching consequences in cosmology and in the field theory of elementary particles However, several authors[3] have directed attention to certain difficulties Among other points it is asserted that the observed stellar radio emission and the change in red shift of binary stars in eclipse are too small to be consistent with the proposed theory Some of these difficulties may be very real, but bearing in mind the experimental difficulties in measuring stellar red shifts, the variety of causes which can result in both red and blue shifts, and that an otherwise unknown photon–photon interaction could result in the appearance of an entirely unknown particle, it seems worth considering the possibility of a terrestrial experiment under controlled conditions

At present, attempts are being made at Harwell and elsewhere to observe the gravitational red shift using γ-rays from iron-57 and the Mossbauer effect The resolution using iron-57 is $\sim 6 \times 10^{-13}$ It is easy to show that if the γ-rays in these experiments were allowed to pass through a radiation field of similar quality and the same order in energy density as on the surface of the Sun, extending over a distance of about a metre, the red shift expected from Finlay-Freundlich's relation is about 1 per cent of the resolution 1 per cent is not far from the expected gravitational red shifts in the present experiments in which the photons fall considerably larger distances In a practical attempt to test Finlay-Freundlich's relation, it would probably be necessary to have a radiation density times path-length such that the expected shift would be of the same order as the resolution, and although this would require a considerable effort, it does not seem entirely beyond present pulse techniques such as are used in thermonuclear work, but in this case involving lower plasma temperatures It should be a reasonably fair test if most of the energy in the radiation field lies in or near the visible region, and if the dependence on energy of the incident photon in Hubble's law can be regarded as sufficiently well established

A negative result for this proposed experiment would serve the useful purpose of removing one theory of the galactic red shift At present it would be idle to pursue in detail the various possible interpretations of an unambiguous positive result, but it may be of interest to outline one of these briefly and simply here

If we can regard our Sun as typical of about 10^{11} stars in a typical galaxy and use a value of $\sim 2 \times 10^{6}$ light years as the average intergalactic distance, it is easily shown that the average rate R_1 of release of radiation into unit volume of intergalactic space is $\sim 3 \times 10^{-29}$ erg/c c /sec The average rate R_2 of apparent loss of energy by photons in the galactic red shift is $K\rho$, where K is Hubble's empirical constant and ρ is the average energy density of radiation in intergalactic space Using a value for K of 5×10^{-18} sec $^{-1}$ and for ρ of $\sim 6 \times 10^{-12}$ erg/c c., it is clear that $R_1 \sim R_2$ Confirmation of Finlay-Freundlich's relation would justify this value of ρ and also the qualitative interpretation $R_2 = K\rho$ In these circumstances the galactic red shift becomes a vast sink which receives the radiation from the stars and transforms it into some other form, the processes involved being in approximate equilibrium at present It does not seem entirely inconceivable that at least some of the energy thus transformed might reappear eventually in space as neutrons by a net reaction involving unknown particles The hydrogen from the neutron decay would provide fuel for new stars as in Hoyle's theory of continuous creation The net effect of the entire process would be to slow up what seems at present an inevitable decline towards a universe of black dwarfs, photons and neutrinos as the hydrogen in the stars is steadily transformed to helium photons and neutrinos The amount of recycling of hydrogen by such a hypothetical process could be quite large, as most of the energy released in the net reactions in the stars goes to the photons which escape from the stellar surfaces Approximate equilibrium at present need not conflict, at least qualitatively, with the recent results of Ryle, if a 'beginning' consisting of a vast hydrogenous cloud is assumed, for in the early stages of condensation the low value of ρ would correspond now to an apparent increase in the frequency of observation of low-intensity (assumed distant) sources

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OCEANOGRAPHY

Ultra-violet Absorption of Sea Water

It has been shown that at wave-lengths less than 230 mμ , the absorbency of sea water is about double that of a solution containing the same concentration of inorganic salts[1,2] We ascribe the difference to the presence of organic matter which may be the same as the blue-absorbent substances named "Gelbstoff" by Kalle[3]. We have found regional differences in the east Atlantic and English Channel, high ultra-violet absorbencies usually being characteristic of coastal water[2]. This property may be used in hydrographic surveys, and for this purpose we have measured the absorbencies of filtered samples at 220 mμ, using a 10-cm. cuvette in a Unicam *S.P.*500 spectrophotometer.

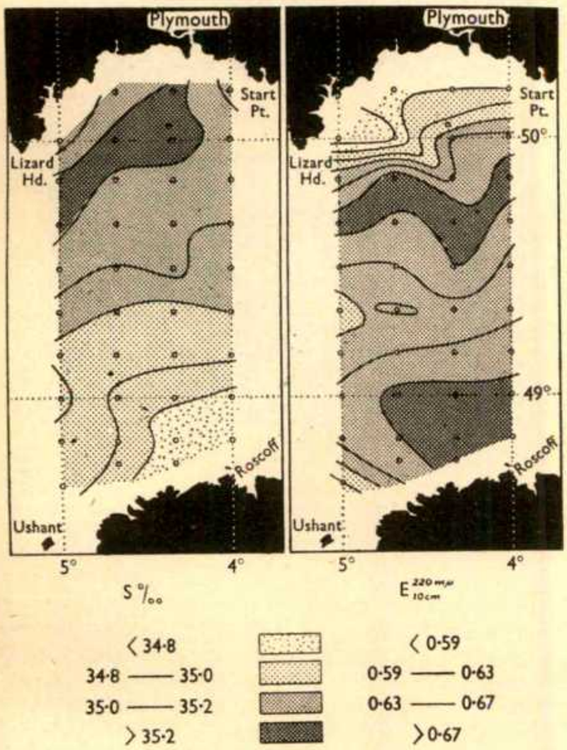

Fig. 1

Ultra-violet absorbencies of samples from 10 m. depth from a survey across the English Channel in April, 1961, are compared with salinities in Fig. 1. Water of low salinity and high ultra-violet absorption was found in the south-east corner of the area, and has presumably come down Channel on the French side, being diluted with land drainage. On the English side there is an intrusion from the west of higher salinity water of low ultra-violet absorption which we think is of Atlantic origin. This water appears to have displaced water of salinity 35·1–35·2 per mille but of higher ultra-violet absorption (*E*(200 mμ, 10 cm.) approx. 0·7) which was found off Plymouth in February, 1961 [2]. This displaced water, though indistinguishable by its salinity, is made evident as a band of high ultra-violet absorption in mid-Channel.

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PHYSICS

Proposed Harmonic Generator or Mixer for Optical Frequencies

In a recent communication[1], Lainé has suggested the use of my latest form of 'cold cathode' arc harmonic generator as a mixer for obtaining beats at high microwave frequencies (that is, frequencies corresponding to submillimetre waves) between the outputs from two optical masers. I would like to comment on this proposal and suggest a simpler alternative device.

If one accepts the principle of field emission as the source of electrons from the cathode of the arc harmonic generator, then Lainé's suggestion is indeed feasible. On this basis one calculates that for the liquid platinum cathode used at present, the electron emission current density must be about 10^8 amp./cm.^{2} for a cathode spot diameter of about 1μ. The positive ion field required to produce this emission must be of the order of 10^8 volts/cm., extending over a region of only 10 Å. outwards from the cathode surface. The time-constant of the electron emission process should thus not exceed 10^{-15} sec. If the outputs from the two optical masers were first plane-polarized with the electric vectors perpendicular to the cathode surface and then focused into a region one optical wave-length in diameter (at glancing incidence) at the cathode spot then the positive ion space-charge field would have a superimposed field at optical frequencies and the electron emission should vary at the beat frequency. To produce a reasonable effect, optical fields in excess of 3×10^7 V./cm. would be required—implying maser powers of tens of kilowatts. A second disadvantage is that microwave conduction along the arc plasma (the only means of communication with the cathode spot) is shunted by diffusion spreading of the plasma in the region of the cathode, thereby imposing an upper limit to the beat frequency which can be detected. For the argon plasma at 300 atmospheres pressure this limit is about 700 Gc./s.

However, a possible field emission mixer can be made very simply. All that is required is a small drop of liquid cæsium in a glass vacuum enclosure. The energy from the optical masers should be plane polarized with the electric field perpendicular to the cæsium surface and focused into a spot about 1μ diameter striking the cæsium at glancing incidence. Strong field emission would take place for field strengths of 10^7 V./cm. which could be obtained with 3 kW. of maser power. The optical waves would penetrate the liquid surface to a depth more than an order of magnitude greater than that needed to overcome the potential barrier. During the positive going half-cycle of the optical electric field, electrons would thus be emitted in a highly 'non-linear' manner, travelling outwards for about 20 Å. in 10^{-15} sec. This oscillating charge would radiate at the beat frequency and at harmonics of the optical frequencies. As an optical frequency doubler, efficiencies approaching 1 per cent might be attained.

Further reduction of the maser power required for beat production should be possible if one makes use of the field emission studies of Martin, Trolan and Dyke[2]. They have drawn electron currents of several m.amp. continuously from a tungsten point of 0·1μ radius. If the maser output could be focused on to such an emitting point, the electron current would be modulated at the beat frequency. Some sort of magnetic focusing of the electron beam would be necessary to prevent space-charge demodulation and to enable the energy in the electron bunches to be coupled to a microwave waveguide circuit. Beat frequencies in excess of 1,000 Gc./s. should be practicable by such a method.

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Detection of Spin Magnetization Variation in a Two-level Maser Oscillator

THE phenomenon of amplitude modulation in the spontaneous emission of two-level masers has been observed by several investigators[1–3], and has been the subject of a number of theoretical discussions[3–5]. The basic mechanism underlying most of the semi-classical treatments is an oscillatory transfer of energy between the spin system, in the working substance, and the cavity radiation field. In such a process the spin magnetization vector M should undergo damped, pendulum-like nutations, correlated to the modulation of the emission, between the inverted state, M anti-parallel to the steady magnetic field in the z direction, and the thermal equilibrium state, M parallel to the steady field. The quantum mechanical treatment[6] of the interaction between the spin system and the radiation field in the cavity also suggests the oscillatory behaviour of M_z. Stevens[6] further suggested that the direct observation of the variation of M_z, during an oscillation pulse, would be a more instructive test of the theories mentioned than monitoring the modulated cavity energy. Evidence confirming the suggested behaviour of M has been obtained in an induction experiment designed to detect the variations in the 'dipole' field of M_z.

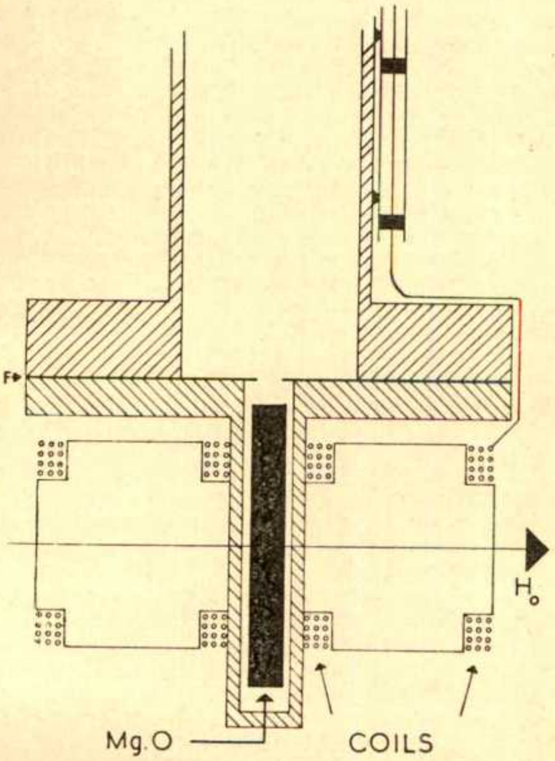

Fig. 1. Cavity with sample, pick-up coils and co-axial lead. Cavity, 0·9 × 0·1 × 0·8 in., sample, 17·5 × 2·0 mm. *F*, Silver-plated copper foil with iris to wave-guide

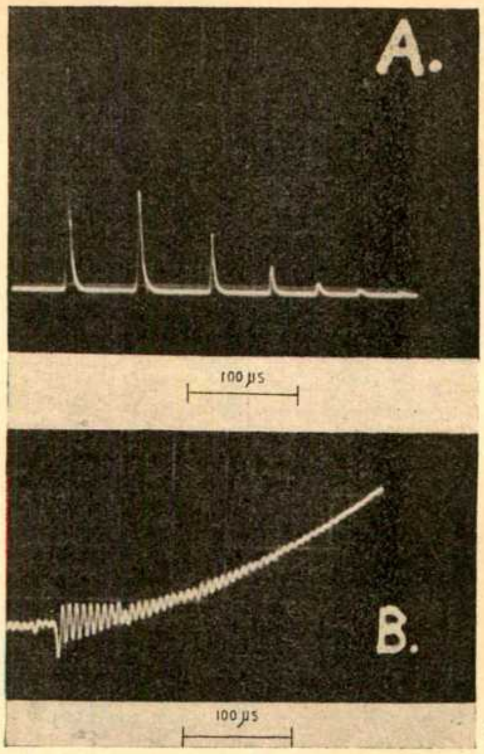

Fig. 2. *A*, Modulated emission from field-swept two-level maser: maximum power, 17 mW. *B*, Shock excitation of pick-up coils due to nutation of magnetic moment of the spin system; maximum pick-up voltage, 0·5 mV.

The maser operated in this laboratory[7] is of the field-swept type. The sample, neutron-irradiated magnesium oxide containing about 5×10^{18} spins, was mounted in a rectangular 'TE_{102}' plastic cavity resonator[8]. Oscillation pulses have been observed in which the amplitude modulation frequency was dependent upon the rate of field sweep. It was estimated that the skin-depth at these frequencies was greater, by orders of magnitude, than the cavity silver plating, so that variations of the field of M_z would not be totally confined within the cavity. Furthermore, calculations predicted that the voltage induced by this flux in an external coil, of 800 turns, surrounding the sample would be of the order of a millivolt.

Such a coil system should be balanced against pick-up from the swept field. The coil assembly used in this experiment satisfies this requirement, to a sufficient extent, and is shown in Fig. 1. The field of M_z would couple only to the inner coils, whereas the outer coils compensate for the pick-up of the swept field in the inner coils. At 1·2° K. the coils had a resonant frequency of 150 kc./s.; $Q = 4$ and a damping time constant of 60 μsec.

Maser oscillations and the corresponding pick-up voltage in the coils were simultaneously recorded on two oscilloscopes. Typical oscillograms of these recordings are shown in Figs. 2*A* and 2*B* respectively.

The oscillation envelope, shown in Fig 2*A*, is not continuous so that variations of M_z should occur only during the intermittent pulses, leading to shock excitation of the pick-up coils This was found to be advantageous because the response time of the system is too long to record completely the variations of M_z In Fig 2*B* there are shock excitations coincident with the first three oscillation pulses This coincidence was checked in a special experiment The rising base-line is due to the residual pick-up from the swept field This evidence, in support of the nutating character of *M*, is further substantiated by the fact that no effect was observed when the inverting microwave power was reduced below the level necessary to sustain maser oscillations so that only the residual pick-up was observed.

This experiment establishes the existence of variations of M_z and therefore of the nutations of *M* during emission. Further measurements of M_z will, very likely, provide useful information on the mode of operation of maser oscillators The technique described could be improved, to give detailed measurements of M_z, either by using critical damping in the pick-up coils, or by modifying the system to respond to the nutations of *M* as modulations of a high-frequency carrier wave fed to the coils

We are indebted to Prof J F Allen for placing low-temperature facilities at our disposal and to the Royal Naval Scientific Service for the interest shown in this work

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GEOLOGY

Occurrence of Magnesite–Dolomite Sediments in South Australia

In November 1960 samples of precipitating sediment were taken from a lagoon of saline water near Salt Creek in the south-east province of South Australia These were found to consist of magnesite and dolomite in about equal proportions The salinity of the water was 3 2 per cent, and analysis showed that calcium constituted a much smaller proportion of the total salts than in normal sea-water The proportion of magnesium was also lower. The *p*H was about 10

Samples were taken from the bed of the lagoon after it had become dry during the summer At the surface the proportions of magnesite and dolomite were approximately equal. At a depth of 6 in. the approximate composition was magnesite 20 per cent, dolomite 80 per cent. At 10 in , magnesite was no longer detectable and the sediment consisted entirely of dolomite This persisted to a depth of 20 in , where it graded sharply into black sulphide-bearing sands

X-ray examination of the magnesite showed that the lattice was expanded when compared with normal magnesite The lattice constants were estimated as $a_0 = 4\ 66$ and $c_0 = 15{\cdot}23$ This may be compared with the magnesium carbonate described by D L Graf, A J Eardley and N F. Shimp[1] from Lake Bonneville, U S A , which has a significantly expanded unit cell with lattice constants $a_0 = 4\ 669$ and $c_0 = 15\ 21$

Last year we[2] recorded the occurrence of hydromagnesite–aragonite sediments in a similarly situated lagoon which is about 8 miles south of the lagoon in which the magnesite–dolomite is forming This new discovery may throw more light on the origin of the magnesite–dolomite sediments of Proterozoic age in South Australia.

We believe this to be the first recorded observation of naturally precipitating magnesite–dolomite sediment.

This work is supported by grants from the American Petroleum Institute.

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CRYSTALLOGRAPHY

X-ray Data for the Chrome-Urania System, $Cr_2O_3\,2UO_3$

A new binary compound in the chrome-urania system has been briefly described by Borchardt[1] This compound has been prepared by mechanically mixing 78 weight per cent uranium oxide (UO_2) and 22 weight per cent chromium oxide (Cr_2O_3), pressing the mixture into pellets and firing to 1,250° C in platinum dishes The samples were reground and refired and then examined by chemical, optical, and X-ray techniques. A trace of free chrome was observed optically in mixtures where the chemical analysis indicated excess chromium over a Cr U atom ratio of 1. The X-ray powder patterns indicated only one phase

The preparations were black, with grain sizes less than 50µ Two small crystals which were recovered from the preparations showed external morphology with 6/*m* symmetry Single-crystal precession photographs, however, showed the crystals to be truly orthorhombic with three-fold interpenetrant twinning producing the pseudo-hexagonal habit Fig 1 shows the true orthorhombic lattice and its relation to the twin in the 0*kl* zone Of particular interest is the superposition of the 002 and 041 reflexions Table 1 lists the X-ray powder pattern along with the indexing based on the cell determined from the single-crystal patterns Table 2 lists the cell data and optical properties of the compound

The presence of the pseudo-hexagonal twinning suggested the possibility of a high-temperature inversion to a truly hexagonal phase Using a hot-stage furnace (Materials Research Corporation), samples were examined in air up to 1,400° C , but no evidence for the expected phase change was found. However, samples left at 1,250° C. for several days developed clusters of multiple and single crystals of the compound on the surface of the sample The crystals were yellow-brown tabular crystals with a rectangular outline Most of the crystals were twinned, producing

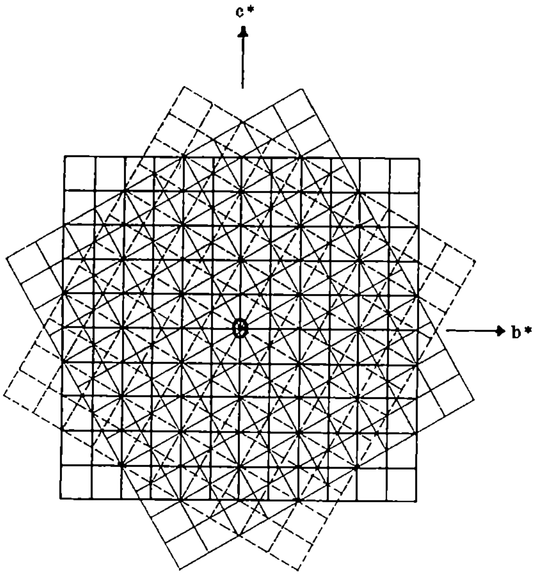

Fig 1 Relationship of the true cell in the 0*kl* zone with respect to the twinned lattices The true cell is shown as heavy lines Only points with *k* = 2*n* are included, since reflexions with *k* = 2*n* + 1 are extinct Notice particularly the superposition of 041 and 002 in different lattices

a 'cockscomb' effect around the periphery of the plates

Several crystals were examined with the precession camera The twinning was the same as mentioned above Untwinned crystals showed extinctions in *hk*0 when *h* + *k* = 2*n* + 1, *h*0*l* when *l* = 2*n* + 1, and 0*kl* when *k* = 2*n* + 1. Very weak reflexions are noted for *hkl* when *h* + *k* = 2*n* + 1, and *h*0*l* when *l* = 2*n* + 1 The extinctions indicate the unique space group *Pbcn* with a pseudo symmetry *Cmcm* The specific gravity of the crystals is greater than 3 3, which suggests either one molecule per unit cell with a calculated specific gravity of 4 18 or two molecules with a specific gravity of 8 36 One molecule would produce a problem with respect to the position of the ninth oxygen in the unit, because the space group does not allow any odd-fold special positions This inconsistency can indicate either that the oxygen is statistically distributed over 2-, 4-, or 8-fold sites, or the chemical analyses are incorrect in indicating a fully oxidized uranium The specific gravity of 8 36 is reasonable when compared with specific gravities of the components Using Vegard's law and values of 5 24 and 8 24 for Cr_2O_3 and UO_3 respectively, the calculated specific gravity for 80 weight per cent UO_3 and 20 weight per cent Cr_2O_3 is 7 64. Because deviations from Vegard's law would tend toward smaller unit-cell dimensions and high specific gravities, the values of 8 36 for two molecules per unit cell is acceptable

Table 1 X-RAY POWDER PATTERN FOR Cr_2O_3 2UO_3

hkl	$\sin^2\theta_{calc}$	$\sin^2\theta_{obs}$	*I*	*hkl*	$\sin^2\theta_{calc}$	$\sin^2\theta_{obs}$	*I*
020	0 0174	0 0174	vw	240	0 1700	0 1686	w
110	0 0295	0 0294	w	202	0 1931	0 1930	s
021	0 0405	0 0408	vw				
111	0 0526	0 0532	vs	241	0 1932		
130	0 0642	0 0643	s	222	0 2105	0 2105	vw
040	0 0694	0 0687	w	023	0 2255	0 2259	m
131	0 0873	0 0874	vw	310	0 2307	0 2308	vw
041	0 0926	0 0925	m	?		0 2347	w
				113	0 2377	0 2384	m
002	0 0925						
200	0 1006	0 1006	mw	170	0 2378		
022	0 1099	0 1108	w	062	0 2488	0 2474	w
220	0 1180	0 1179	w	311	0 2538	0 2535	m
112	0 1220	0 1225	w	260	0 2568	0 2579	m
150	0 1337	0 1323	m	330	0 2654	0 2640	m
221	0 1411	0 1412	ms	133	0 2724	0 2735	vw
060	0 1562	0 1567	s	043	0 2776	0 2778	m
132	0 1567						
151	0 1568			080	0 2778		
042	0 1620	0 1614	w	081	0 3009	0 2974	m

Table 2 X-RAY AND OPTICAL DATA FOR Cr_2O_3 2UO_3

a_0 = 4 857 ± 10 Å
b_0 = 11 692 ± 10 Å
c_0 = 5 074 ± 10 Å
V = 288 0 Å^3
Z = 2
ρ_{calc} = 8 36 gm /c c
Colour = golden brown
N_x > 2 11
N_y > 2 11
N_z > 2 11

We thank Dr J S Kahn, of this Laboratory, for the optical data

Single-crystal intensity data are now being collected for determination of the crystal structure

This work was carried out under the auspices of the U S Atomic Energy Commission

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Structure of Asparagine Monohydrate

THE structure of asparagine has been discussed several times during recent years[1-5] A number of properties would be better explained by a ring structure than by the conventional straight-chain structure[1,2], but on the other hand, the carboxylic group had to be free[3] The structure of crystalline asparagine monohydrate has now been determined by us with X-rays, and although this does not give definite information about the structure of the molecule in solution, it does give some indications about it

Table 1 ATOMIC CO-ORDINATES (Å)*

	y	*z*	*x*
C_1	4 888	9 247	0 421
C_2	3 867	9 549	1 529
C_3	4 404	0 563	2 679
C_4	0 097	9 795	3 294
N_1	3 257	8 272	2 029
N_2	0 584	0 629	4 344
O_1	5 554	0 428	0 008
O_2	4 916	8 091	11 747
O_3	0 621	8 773	2 818
O_4	1 695	2 258	1 319

* With the origin halfway between three pairs of non-intersecting screw axes

The molecules in the crystal appear to be held lightly together by several hydrogen bonds, as could be expected (Fig 1 and Table 2) First of all, there is a continuous spiral of hydrogen bonds, in the direction of the *a* axis, involving the water molecules (O_4) and the double-bonded oxygens of the carboxylic groups (O_1) Then there are three hydrogen bonds linking the nitrogen of the amino group (N_1) with different oxygen atoms O_3 (in another molecule), O_4 and O_2 The nitrogen of the amido group (N_2) forms only one good hydrogen bond with O_1, the other one, with O_2, is weaker

Of interest in connexion with the discussion whether the structure of asparagine is a ring or an

Table 2 INTERATOMIC DISTANCES (Å)

Covalent bonds		Hydrogen bonds	
$C_1 — O_1$	1 27	$O_4 — O_1$	2 84
$C_1 — O_2$	1 34	$O_4 — O'_1$	2 80
$C_1 — C_2$	1 54	$N_1 - O'_3$	2 80
$C_2 — N_1$	1 50	$N_1 — O'_2$	2 81
$C_2 — C_3$	1 51	$N_1 - O'_4$	2 85
$C_3 — C_4$	1 53	$N_2 — O'_1$	2 92
$C_4 — O_3$	1 24		
$C_4 — N_2$	1 33		

A prime indicates an atom from a different molecule

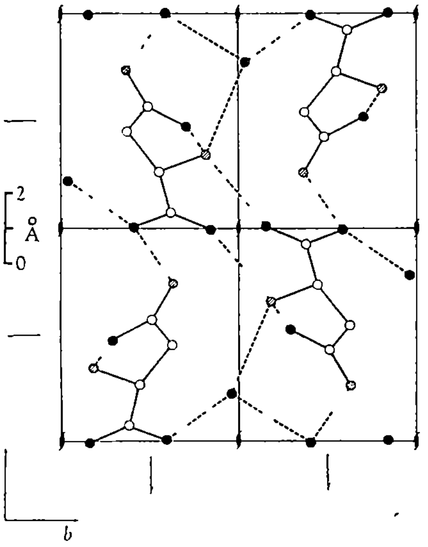

Fig. 1. Arrangement of the molecules of asparagine and water in the unit cell, as seen along the *a* axis, origin on two-fold screw axis parallel to the *a* axis. Covalent bonds and the hydrogen bonds ≤ 3.0 Å are shown. ○, Carbon; ⊘, nitrogen; ●, oxygen

open chain is the question whether there is some kind of a bond between N_1 and O_3 atoms of the same molecule. The distance between N_1 and O_3 within the same molecule is rather short (3.09 Å), but the distances between N_1 and three other oxygen atoms are still shorter (Table 2). Moreover, the difference Fourier shows hydrogen atoms in correct positions for hydrogen bonds between those atoms, but nothing between N_1 and O_3 atoms of the same molecule. Therefore, it can be concluded that there is no intramolecular hydrogen bond between N_1 and O_3 in crystalline asparagine. However, this does not rule out the possibility of a hydrogen bond in this position in solution. The structure would then be a kind of intermediate between a ring and a chain and so could account for all properties. The ring, for example, looks like the ring in proline and could therefore explain why asparagine reacts with ninhydrin in the same way as proline, and not as the other amino-acids, including glutamine[1,2].

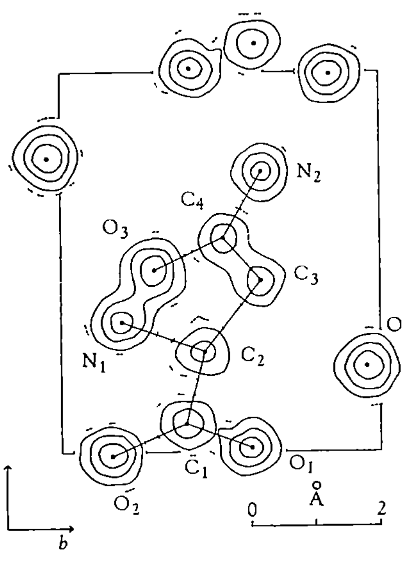

Fig. 2. Fourier projection of asparagine monohydrate on the (0*yz*) plane, after two cycles of refinement of the *y* and *z* co-ordinates. Origin as in Fig. 1

Crystallographical data about the structure are: space group $P2_12_12_1$, $a = 5.582$ Å, $b = 9.812$ Å, $c = 11.796$ Å, unit cell volume = 646.1 Å^3, four molecules per unit cell. D_0 = 1.543 gm./cm.^{3} (ref. 6). D_x = 1.543 gm./cm.^{3}.

The structure was solved using the three-dimensional sharpened Patterson. The *y* and *z* co-ordinates were first refined using the Fourier projection on (0*yz*), after two cycles giving an *R* factor of 16.2 per cent and the Fourier projection represented in Fig. 2. Then all the co-ordinates and the anisotropic temperature factors were refined using least squares programmes on the *IBM* 704. The final *R* value was 7.4 per cent.

The final atomic co-ordinates are shown in Table 1, the intra-molecular bond-lengths and the hydrogen bond-lengths ≤ 3.0 Å are shown in Table 2; these hydrogen bonds are also shown in Fig. 1. Other distances between nitrogen and oxygen shorter than 3.25 Å are N_2–O_2 = 3.02 Å and N_1–O_3 (intramolecular) = 3.09 Å. There were no intermolecular distances involving carbon atoms shorter than 3.3 Å.

This work was supported by the United States National Institutes of Health.

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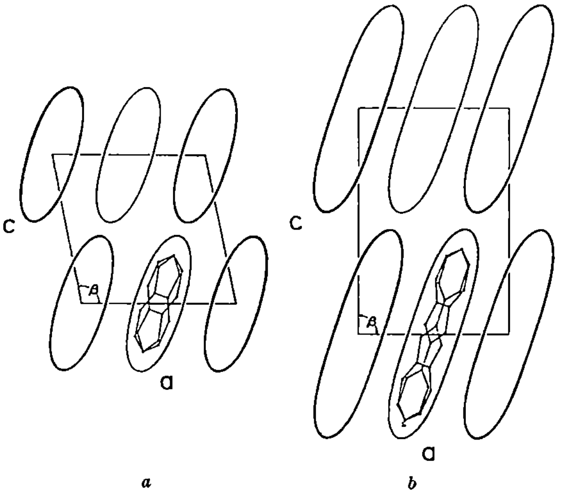
Crystal Structure of 2-Phenylazulene

AFTER a long controversy, the space group of azulene is now apparently established as the centrosymmetric $P2_1/a$, with two non-centro-symmetric molecules in the unit cell[1,2], a result which leads to a disordered structure in which successive azulene molecules in the crystal are reversed in direction at random. Two previously observed cases of random positioning, namely, between chlorine and bromine in *p*-chlorobromobenzene[3] and between methyl and chlorine in 2-amino-4-methyl-6-chloropyrimidine[4], involve atoms or groups of comparable size, and are not as surprising as is the case of azulene, where there is a rather large difference in size between the five- and seven-membered rings.

The disorder in the azulene structure complicates the precise determination of the bond-lengths in this interesting molecule. Accordingly we initiated an X-ray study of a derivative, 2-phenylazulene. Crystals of this substance were found to be monoclinic, with a = 7.92 ± 0.02, b = 5.79 ± 0.03, c = 11.81 ± 0.02 Å, β = 90°. The observed density is 1.24$_8$ gm./cm.^{3}, the density calculated for two molecules per unit cells is 1.252 gm./cm.^{3}. Weissenberg photographs taken about the three principal axes showed the absences ($h0l$) with h odd and ($0k0$) with k odd. The space group is therefore also $P2_1/a$. The lattice constants of azulene itself are[1] a = 7.91, b = 6.00, c = 7.84 Å, β = 101° 30′, and it may be noted that the values of a and b are quite similar for



a *b*

Fig 1 The unit cells of azulene (*a*) and 2-phenylazulene (*b*)

both crystals In the azulene structure, the long axis of the molecule lies very close to the direction [102] If the molecule is now lengthened by the substitution of a phenyl group in the 2 position while at the same time preserving the relation between adjacent molecules as found in azulene, then the unit cell of 2-phenylazulene is reproduced very closely In the broad sense, then, the two structures are isotypic, as may be seen in Fig 1, which shows the close relationship between the two unit cells

A trial structure for 2-phenylazulene was formulated on the basis of the above assumptions, and after trial and error adjustments the Fourier transform of two superimposed molecules yielded satisfying agreement with the observed values of F_{h0l} The electron density projected down *b*, as calculated with 48 of the 72 observed F_{h0l} together with the signs obtained from the transform, is shown in Fig 2

These considerations strongly imply that the structure of 2-phenylazulene, like that of azulene, is disordered, with the direction of the long molecular axis reversed at random A disordered structure for azulene is unexpected, but a similar structure for the 2-phenyl derivative seems even more unexpected since it suggests that the two ends of the molecule are essentially indistinguishable during the crystallization process Additional refinement of the structure is proceeding

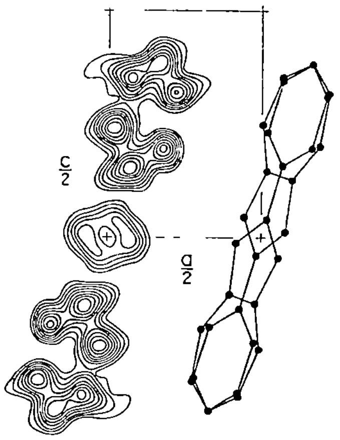


Fig. 2 Projection of the 2-phenylazulene structure on (010)

We wish to thank Prof E. Heilbronner for excellent crystals of pure 2-phenylazulene This work was supported by the U S National Science Foundation

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CHEMISTRY

Preparation of Single-Crystal Graphite from Melts

Single-crystal graphite demonstrates a very high degree of anisotropy between the properties obtained in directions parallel and normal to the basal plane This has generated a great deal of scientific curiosity The closest approach to single-crystal graphite so far has been in the purification and recovery of flakes of natural graphite Unfortunately, the flakes of natural graphite are imperfect, well-developed single crystals are rather scarce, especially in the larger sizes It is felt that the following technique for producing single-crystal graphite will provide basic material for work on carbon and graphite and for optical work requiring single-crystal lamellæ

We have been successful in producing single-crystal graphite by heating in a graphite crucible some titanium boride powder at 2,500° C under vacuum for 2 hr A thin, highly reflecting, flexible film forms at the surface of the melt, which may then be carefully removed from the substrate (Fig 1) Alternatively, single-crystal graphite films can be produced in graphite crucibles on the surface of melts of tungsten, zirconium carbide, nickel, chromium rhenium and molybdenum, heated at the proper temperatures

Results from X-ray diffraction of the films indicate that the material possesses the highest degree of orientation, having lattice spacings identical with the classical values given in the literature Electron diffraction observations (Fig 2) and electron optical investigations confirm the single crystallinity of the material (ref 1 and personal communication between T H Johnson and H Fernandez-Moran)

The mechanism of the technique described for graphite single-crystal growth is undoubtedly different from that in the decomposition of aluminium carbide[2],

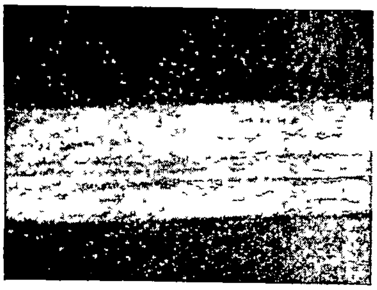

Fig 1. Cross-section of single-crystal graphite (× 500)

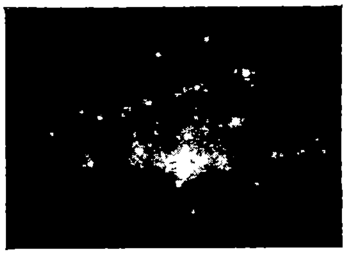

Fig 2 Reflexion electron diffraction pattern of single-crystal graphite (Radio Corporation of America *EML*-1 electron microscope, at 50 kV electrons and 50 cm distance)

in the present instance, the growth may be attributed to the formation of supersaturated solutions of carbon in the melt A detailed investigation of the mechanism in the present technique for graphite growth has begun

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Definition of Rate Constant and Activation Energy in Solid State Reactions

DISLOCATIONS in alkali halide crystals doped with silver ions can be 'decorated' by a hydrogen treatment, which causes reduction and subsequent precipitation of silver[1] In the course of work on the kinetics of this solid state reaction, the problem arose of deducing the reaction constant and the activation energy without exact knowledge of the reaction mechanism, which is rarely discussed in the literature.

The definition of a rate constant k and an activation energy E for solid state reactions in analogy with reactions taking place in gases or solutions gives rise to several difficulties In the latter reactions, the rate constant is the proportionality factor between the reaction velocity and the concentration of the starting products Thus, it can be defined as the velocity for unit concentrations The dimensions of k are sec^{-1} for first-order, and $l\ mol^{-1}\ sec^{-1}$ for second-order, reactions The activation energy E is derived from the dependence on temperature of k according to Arrhenius's law

In solid-state reactions, the concepts of concentration and order of reaction generally have no significance. The reaction velocity is defined here as the change with time of the thickness of the layer of product formed, or of the weight of this layer, or of the number of gram equivalents of product formed.

Consequently, a rate constant cannot be defined in the same way as for reactions in gases or solutions If we accept the same conditions as for gas reactions, k must be independent of time and have the factor sec^{-1} in its dimensional formula, and increase with temperature according to an exponential law, from which an activation energy for the reaction could be derived It will be difficult to predict whether the latter condition will be fulfilled, since the well-known assumption for gas reactions of an equilibrium between activated and non-activated molecules according to the Boltzmann distribution is not valid here. In certain cases, other circumstances will lead to the same exponential dependence on temperature of k

This is generally the case for many reactions such as tarnishing reactions (for example, oxidations), substitution reactions and double decompositions which follow the parabolic reaction law In these cases, the velocity is governed by a diffusion process through a growing layer The velocity dx/dt is proportional to the reciprocal of x, the thickness of the layer, hence $dx/dt = k_1/x$, which leads to $x = (2k_1t)^{1/2}$ The rate constant k_1 is expressed in $cm^2\ sec^{-1}$

If we consider the weight of the layer per unit area instead of its thickness, a rate constant k_2 having as dimensions $gm^2\ cm^{-4}\ sec^{-1}$ is defined If we consider the number of gram equivalents of product formed, a new constant k_3 can be defined from the differential equation of reaction-rate with dimensions $gm\ equiv.\ cm^{-1}\ sec^{-1}$

In tarnishing reactions, the rate-constant is generally proportional to the diffusion coefficient D of one of the reaction partners in the product formed. The exponential dependence on temperature of D leads to the validity of the Arrhenius relation for the rate constant:

$$k_1 = k_0 \exp(-E/RT)$$

where E is the activation energy for self-diffusion Nevertheless, Jost[2] emphasizes that, on account of the complexity of the tarnishing process, a simple dependence on temperature of the net process often cannot be expected

The theory of Wagner[3] for the velocity of substitution reactions and double decompositions, based on the model of the closed galvanic cell, leads to an expression for k which is proportional to the specific conductivity σ of the products formed For the reaction $AgCl + Cu \rightarrow Ag + CuCl$, for example, k is proportional to σ_{CuCl} The exponential dependence on temperature of k will be a consequence of the exponential dependence on temperature of σ in solids The activation energy of the reaction will be that of electrical conduction (which is the same as for self-diffusion)

Thus, for reaction laws of the parabolic type, a rate constant can be defined in a similar way as for gas reactions, its exponential dependence on temperature included Dimensionally it is dependent on the way the reaction velocity is defined.

Consider now a reaction following the linear law, for example, tarnishing reactions when the formed layer is porous or incoherent Here, the reaction is governed by phase-boundary processes, and the velocity is independent on time

$$dx/dt = k_4 \text{ or } x = k_4t$$

The constant k_4, which is expressed in $cm\ sec^{-1}$, is the rate of the phase-boundary process (for example, chemisorption) which will generally not increase exponentially with temperature Consequently the Arrhenius relation will not be valid

In solid state reactions governed by logarithmic laws, new difficulties arise on account of the presence of several constants independent of time and dependent on temperature

Hence for solid state reaction kinetics, rate constant and activatom energy can only be clearly defined when a suitable hypothesis for the mechanism of the given reaction is available If the rate constant is defined from the experimental relation between the quantity of product formed and time, it is difficult to predict whether it will show an exponential dependence on temperature Even if this is approximately the case, the interpretation of the activation energy thus found remains dubious (see, for example, ref 4)

Suppose that no hypothesis for the parabolic law was available, and that the rate constant k_5 had been defined by $x = k_5 t^{1/2}$ The activation energy deduced from the temperature dependence of k_5 would not be E, the activation energy for diffusion, but $\frac{1}{2} E$

$$k_5 = (2k_1)^{1/2} = (2k_0)^{1/2} \exp(-\tfrac{1}{2} E/RT)$$

Moreover, k_5 has not the right dimensions (cm sec $^{-1/2}$ instead of cm ^{2} sec $^{-1}$)

This work is part of a research scheme supported by the Institut pour l'encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, Comité d'étude de l'Etat Solide I thank Prof W Dekeyser for many valuable discussions

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Heat of Formation of the Difluoromethylene Radical

ATKINSON[1] has accounted for the products of the mercury photosensitized decomposition of tetrafluoroethylene by supposing that the excited mercury atoms (Hg $^3P_1$) are able to decompose tetrafluoroethylene to give two difluoromethylene radicals This requires that the dissociation energy of the carbon–carbon bond in tetrafluoroethylene is equal to, or less than, 112 kcal /mole Since this value is significantly smaller than the lower (125–30 kcal /mole)[2] of many values suggested for the dissociation energy of the carbon–carbon bond in ethylene, it is of some interest to obtain independent estimates of the heat of formation of the difluoromethylene radical

The fluorobenzene ion ($C_6H_5F^+$) is an abundant species in the mass spectrum of benzotrifluoride. Similarly, difluorobenzene ions are abundant in the mass spectra of fluorobenzotrifluorides This appears to be a specific rearrangement similar to that observed by Beynon[3] in the case of oxygen-containing compounds Energy considerations suggest that the most probable process in these cases involves the expulsion of a difluoromethylene radical from the trifluoromethyl group, as for example

$$C_6H_5CF_3 + e \rightarrow C_6H_5F^+ + CF_2 + 2e$$

In this case, the appearance potential ($A(C_6H_5F^+)$) of the fluorobenzene ion (13 35 eV.) taken together with the ionization potential ($I(C_6H_5F)$) of fluorobenzene (9 30 eV , cf the value of 9 20 eV from photoionization work[4]), and the heats of formation of fluorobenzene[5] (−26 5 kcal /mole) and of benzotrifluoride[6] (−138 9 kcal /mole), the heat of formation of the difluoromethylene radical is found to be equal to or less than −17 kcal /mole Thus, using the value for the heat of formation of tetrafluoroethylene[7] (−152 kcal /mole), the dissociation energy of the carbon–carbon bond in this compound is found to be 118 kcal /mole Within the uncertainties in the experimental quantities (which amount to about ± 5 kcal /mole) this is a maximum value for this quantity In a similar way the appearance potential (13 15 eV) of the *p*-difluorobenzene ion from *p*-fluorobenzotrifluoride together with the ionization potential of *p*-difluorobenzene (9 15 eV), the heat of formation of *p*-difluorobenzene (−72 2 kcal /mole, calculated from $\Delta H_f(p-C_6H_4F_2)$ (l) = −79 5 (Scott, D W, private communication), taking ΔH_v (estimated) = 7 3 kcal /mole), and the heat of formation of *p*-fluorobenzotrifluoride (assumed to be close to that of *m*-fluorobenzotrifluoride, −184 2 kcal /mole (Scott, D W, private communication)), give a further estimate for the heat of formation of difluoromethylene (−21 kcal /mole) consistent with the first value Since there is no value available for the ionization potential of *m*-difluorobenzene it is impossible to make any calculation based on the appearance potential (13 75 eV) of the ion of this molecule from *m*-fluorobenzotrifluoride

A less-certain but independent estimate of the heat of formation of the difluoromethylene radical may be obtained from some kinetic data of Atkinson and Atkinson[9], who have shown that octafluorocyclobutane is able to decompose by the process

$$c-C_4F_8 \rightarrow CF_3—CF=CF_2 + CF_2$$

The energy of activation for this process must fulfil the condition

$$E \gg \Delta H_f(CF_3CF=CF_2) + \Delta H_f(CF_2) - \Delta H_f(c\text{-}C_4F_8)$$

Atkinson and Atkinson[9] give for the rate coefficient for this reaction

$$k = 3{\cdot}9 \times 10^{16} \exp -79{,}000/RT\ s^{-1}$$

Taking the energy of activation together with the heats of formation of octafluorocyclobutane (−352 kcal /mole from the heat of dimerization[10] and the heat of formation[6] of tetrafluoroethylene) and of hexafluoropropene (−259 from Duus's results[11], and the heat of formation of tetrafluoroethylene) requires that the heat of formation of difluoromethylene is equal to, or less than, −13 kcal /mole The value quoted for the rate coefficient, however, was obtained from an analysis of the data that supposed that the quasi-stationary concentration of octafluorocyclobutane was controlled by its decomposition into two molecules of tetrafluoroethylene A fuller analysis would probably require a slightly larger energy of activation and a larger pre-exponential factor for the rate coefficient, and this in turn would require a slightly more positive limiting value for the heat of formation of difluoromethylene

Our value for the heat of formation of difluoromethylene (about −17 kcal /mole) supports the values proposed by Reed and Rabinowitch[12], and by Mann and Potocki[13]. It suggests that the radical is more stable than does the value (about −5 kcal / mole) implied by the results reported by Reed[14] from a study of electron impact processes with carbon tetrafluoride Our conclusions about the bond dissociation energy in tetrafluoroethylene are similar to those of Margrave[15], but there remain the problems of the discrepancy between the value assumed by Margrave[15] for the ionization potential of the difluoromethylene radical (11 eV) and that found by Reed[14] (13 35 eV), and of the nature of the process by which the difluoromethylene ion is formed from tetrafluoroethylene Our values for the heat of formation of difluoromethylene and the heat of formation of the trifluoromethyl radical (−115 kcal/mole) give for the carbon–fluorine bond dissociation energy in the latter radical the value 117 kcal/mole. This is a little less than that (121 kcal/mole) of the carbon fluorine bond in carbon tetrafluoride[14]

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Dislocations as Active Centres in Heterogeneous Catalysis

Much work has been carried out in recent years on the relation of heterogeneous catalysis to point defects in solids or to semiconductivity We have found an example of catalysis in which dislocations play the part of the active centre in the case of copper for the decomposition of diazonium salt (Gattermann's reaction) A sample of copper, free from point defects and containing dislocations only, is obtained by annealing the cold-worked sample in an appropriate temperature-range The necessary data on annealing, a twisted sample, as given by Boas[1], are summarized in Fig 1

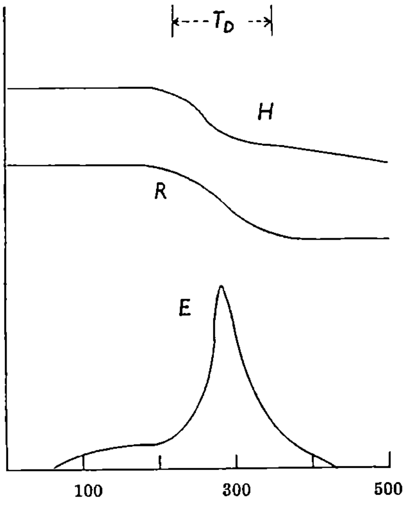

Fig 1 Changes of hardness (*H*) and electrical resistivity (*R*), and the release of defect energy (*E*) during annealing of copper samples (99 96 per cent) deformed in torsion to $nd/l = 1\ 8$, where *n* is the number of turns, *d* the diameter and *l* the length (see ref 1)

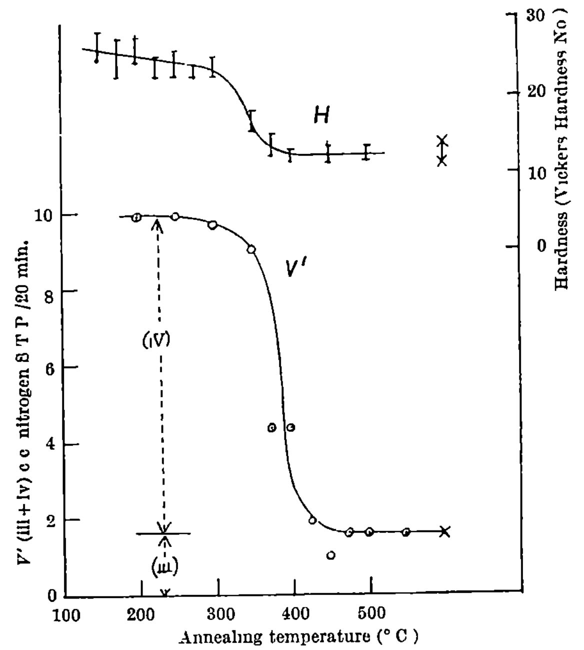

Fig 2 The heterogeneous catalytic activity at 25° (*V*′) and hardness (*H*) of copper cold-worked and annealed at various temperatures ×, Values for samples not cold-worked

In a temperature-range T_D, a sudden change takes place in hardness, in electric resistivity and in density, with the rapid release of defect energy and recrystallization[2] These phenomena have been attributed to the disappearance of dislocation in the deformed metal, because point defects produced in copper by deformation or irradiation at sub-zero temperatures disappear by annealing below room temperature

In our experiments pieces of copper wire (99 96 per cent pure) were twisted to $nd/l = 1\ 0$ after complete annealing at 550°, and then were annealed again at various temperatures The samples were washed with ammonia water and thrown into a solution of diazonium chloride, the reaction velocity was measured by the total volume of nitrogen (*V*) evolved in 20 min, the part of *V* to be ascribed to heterogeneous catalysis (*V*′) is shown in Fig 2 together with hardness of the samples Though the value of T_D is considerably influenced by impurities as well as by the degree of cold-working of different samples, the sudden and concurrent changes in hardness and in catalytic activity in the annealing temperature-range, 300–430° C, are ascribed to the disappearance of dislocations Consequently, it may be concluded that the reaction corresponding to (iv) in Fig 2 is a catalytic effect due to dislocations, amounting to 84 per cent of heterogeneous catalysis in the case shown The value of *V* consists of the rate of (i) natural decomposition, (ii) homogeneous decomposition catalysed by cuprous ion dissolved from the metal, (iii) heterogeneous decomposition catalysed by the normal surface of copper and (iv) heterogeneous decomposition catalysed by the end of the dislocation at the surface The change of surface area on annealing is considered to be negligible, because it was found that catalytically active copper powder deposited from copper sulphate solution by means of the reaction with zinc dust has almost the same adsorption capacity for methylene blue even after it is annealed at 500° and loses most of the activity

(3 2 × 10^{-7} and 3 1 × 10^{-7} mole/gm , respectively) The volume of nitrogen evolved, V, for a sample without dislocations (obtained by annealing at temperatures higher than T_D) is the sum of (i), (ii) and (iii), and the decrease in V on removing copper from the solution is the sum of (iii) and (iv) when reduced to the initial concentration of the solution, hence each value can be obtained separately For example, (i) is 4 6, (ii) 1 5, (iii) 1 6 and (iv) 8 3 c c for samples annealed below T_D as shown in Fig 2, (ii) as well as (iii) being almost constant irrespective of the annealing temperature

In the case of nickel, the catalytic activity for the hydrogenation of ethylene is greatly enhanced by rolling the metal foil, but this effect is eliminated by annealing at a temperature between 200° and 300° (ref 3). From the results of annealing experiments for deformed nickel[1,4], the evolution of energy and the change of physical properties between 90° and 320° and those between 520° and 660° were concluded to be due to the disappearance of vacancies and dislocations, respectively. Consequently, the greater proportion of the enhanced activity found by us may be attributed to point defects at the surface formed by cold-working (although the value of T_D may not be exactly the same as that with vacancies in the bulk crystal), contrary to Cratly and Granato's postulate[5] ascribing the activity to dislocations. By means of the method described, we hope to obtain some knowledge of the physical nature of the active centres in metallic catalysts

We wish to thank Dr M Kikkawa and the late Mr H Ushio for supplying us with copper samples, and Prof. H. Wakeshima for his advice

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BIOCHEMISTRY

Polysaccharide Content of the Prolapsed Nucleus Pulposus of the Human Intervertebral Disk

The nucleus pulposus of normal human intervertebral disk contains the two acidic mucopolysaccharides, chondroitin sulphate and keratosulphate[1-3] During ageing an increase of collagen occurs[4] leading to a decreased ratio of mucopolysaccharide to collagen, although the absolute amount of mucopolysaccharide remains constant[4].

A recent paper by Hendry[5] has shown that the prolapsed nucleus pulposus is in fact relatively desiccated *in situ* He postulated that this was due to a loss or deterioration of the protein–polysaccharide complexes present. We have analysed a series of nuclei pulposi, from prolapsed disks, for ester-sulphate and have found that without exception these contain very low amounts compared with our measured normal specimens

The values obtained for the prolapsed nuclei pulposi are considerably lower (in most cases less than half) than values in the literature for the normal nucleus The decrease in ester-sulphate content on ageing shown in Table 1 is apparent rather than absolute, due to increase of collagen increasing the total percentage dry weight It is unlikely that all these low ester-sulphate values are only due to an abnormal increase in the collagen content of the nucleus, in fact, percentage dry weights of up to 75 per cent would be required to explain these values It seems more likely that a genuine decrease in the absolute quantity of mucopolysaccharides has occurred before or during prolapse

Table 1 Ester-Sulphate Analyses of Prolapsed Human Nucleus Pulposi at Various Ages

Age (yr.)	Ester-sulphate sulphur as percentage of dry weight
18 *f*	1 1
19 *f*	1 2
26 *m*	1 0
39 *m*	1 0
49 *m*	0 6
53 *f*	0 6

m, Male, *f*, female (free sulphate in all cases was found to be negligible)

It is seen that these results appear to favour Hendry's theory of relative dehydration of the nucleus leading to prolapse An alternative hypothesis arises which involves the possibility that the normal deposition of collagen fibrils, which with age tends to make an intervertebral disk more rigid and less likely to prolapse, might not occur so completely with a genuine deficiency of mucopolysaccharide, thus allowing the nucleus pulposus to remain a relatively unstrengthened gel Such a disk would then be the one more liable to prolapse than a normal disk of the same age group The possibility arises that the mucopolysaccharides in the surrounding annulus fibrosus may also be reduced, giving rise to more friable wall structure

The cause of the loss in acidic mucopolysaccharides is unknown, although it is of interest that we have found that all samples of nuclei pulposi contain a hitherto unreported free monosubstituted guanidine compound (Sakaguchi test and electrophoretic isolation)

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Metabolism of Propionate by Homogenates of Normal Sheep Liver

Propionic acid, one of the major products of cellulose fermentation in the ruminant stomach, is of unique importance in the carbohydrate economy of the sheep[1], in addition to its presumed function as a direct source of energy Following injection of propionate into the blood-stream of a fasting sheep, there may be observed both a sharp rise in blood glucose[2], and an increase in the rate of consumption of oxygen of the animal

This communication deals with the metabolism of propionate by whole homogenates of sheep liver, prepared to retain mitochondrial respiration. Measurements were made of the rate of consumption of propionate and the rate of uptake of oxygen In addition, the nature of the products of propionate metabolism was examined, and a hypothesis is proposed which relates these findings to the mechanism of propionate metabolism by the sheep

The experimental conditions were designed to permit the measurement of oxygen consumption in an atmosphere of 95 per cent oxygen, 5 per cent carbon dioxide, by the indirect method in ref 3 The apparent solubility of carbon dioxide in the medium used was determined to be 1 57 µl carbon dioxide/µl fluid/atmosphere (37° C) Volumes of 6 ml. and 3 ml , containing 1 0 ml and 0 5 ml of a 1/7 homogenate in 0 25 *M* sucrose, were used, the high solubility of carbon dioxide in the medium permitting standard Warburg flasks to be used despite the similar gas volumes of the pairs of flasks Replicate measurements indicate a precision of ±6 per cent for oxygen consumption-rates, and ± 9 per cent for rates of evolution of carbon dioxide

It was found that propionate was not consumed in the absence of oxygen, carbon dioxide, adenosine triphosphate (ATP) (or adenosine diphosphate), or of additional cytochrome *c* In the complete medium the mean rate of propionate consumption was 2 39 µequiv. / mgm. nitrogen / hr , with a standard error of 0 15 (eighteen observations) Gas-liquid chromatography[4] of reaction products failed to demonstrate either the accumulation of other volatile acids, or the disappearance of any volatile acid other than propionic

Fig 1 shows the relationship between the rate of propionate consumption and the rate of uptake of oxygen, only those experiments having been included in which it was demonstrated that the rate of consumption of propionate was identical in both 6-ml. and 3-ml volumes This criterion is necessary because only under these conditions is the measure of oxygen consumption-rate valid, and because the rate of propionate consumption was found to be extremely sensitive to loss of carbon dioxide from the medium during the initial manipulations The regression derived from the results shown in Fig 1 yields a value of 39 1 ± *SE* 1 0 µl. oxygen/µequiv of propionate consumed ; this is equivalent to the complete oxidation of half the propionate metabolized

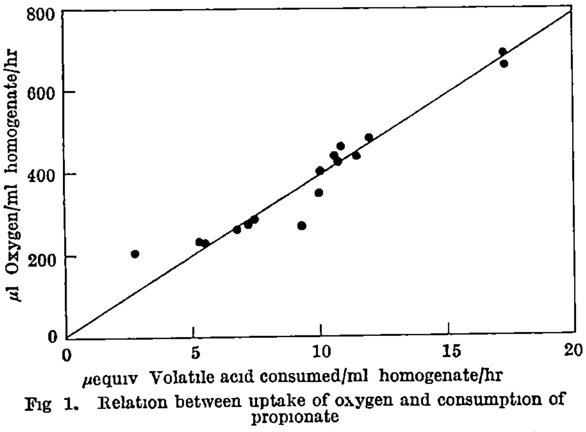

Fig 1. Relation between uptake of oxygen and consumption of propionate

Experimental conditions were as described in the legend to Table 1, but with varying homogenate concentrations Propionic acid consumption was measured as steam-volatile acid consumed, volatile acid being distilled from constant volume and titrated under nitrogen

The only detectable products of propionate metabolism were carbon dioxide and the dicarboxylic acids succinic acid, fumaric acid and malic acid Lactic acid production, presumably from endogenous glycolysis, was identical in the presence and absence of propionate and no consistent effect was found on the small endogenous ketone production. The dicarboxylic acids were detected following exhaustive extraction with ether, by paper chromatography using four different solvent systems[5-8]. Methyl malonic acid could not be detected in these reaction mixtures, but has been found to accumulate when propionyl coenzyme A and labelled carbon dioxide are incubated with sheep liver homogenates in which mitochondrial structure has been disrupted by homogenizing in *p*H 8 *tris* buffer for 5 min in a Servall Omni-mixer

Table 1 shows the rates of uptake of oxygen by homogenates respiring in the presence of succinate, L-malate and propionate These rates are consistent with an oxidation of propionate via L-malate, and since it has also been found that the stimulation in the rates of oxygen consumption caused by propionate and L-malate is not additive, it is inferred that propionate oxidation proceeds via the complete oxidation of L-malate

Table 1 OXIDATION OF PROPIONATE, SUCCINATE AND MALATE BY SHEEP LIVER HOMOGENATES

Exp No	µl. oxygen/mgm nitrogen/hr.			
	No substrate	propionate	succinate	malate
25	48	100	168	103
26	27	60	125	83
27	28	76	125	85
35	50	110	173	120

Oxygen consumption was measured by the indirect method of Warburg in 6 0 ml and 3 0 ml volumes of a medium having the following composition KH_2PO_4 3 6 × 10^{-3} *M*, K_2HPO_4 1 64 × 10^{-3} *M*, $NaHCO_3$ 2 50 × 10^{-2} *M*, KCl 9 0 × 10^{-2} *M*, $MgCl_2$ 5 × 10^{-3} *M*, sucrose 4 0 × 10^{-2} *M*, ATP 1 3 × 10^{-3} *M*, cytochrome *c* 1 1 × 10^{-5} *M*, substrate 5 0 × 10^{-3} *M* and liver protein contributing 3–4 mgm nitrogen/6 0 ml After gassing with 95 per cent oxygen, 5 per cent carbon dioxide for 9 min in the bath at 37° C , flasks were equilibrated for 10 min and buffered isotonic substrate added from the side-arm to a final concentration of 5 0 × 10^{-3} *M* Manometric readings were taken at 6-min intervals for 36 min , oxygen consumption-rates being computed over the final 30 min Homogenates were prepared from the livers of freshly killed sheep in 0 25 *M* sucrose in a glass homogenizer immersed in ice water Nuclei and cell debris were removed by centrifuging at 600*g* for 10 min in a refrigerated centrifuge

In attempting to explain the quantitative link between the rates of consumption of propionate and uptake of oxygen, two possibilities have been considered, either of which may be expressed by the reaction sequence shown in Fig 2, in which it is proposed that the complete oxidation of 1 mole of L-malate leads to the activation of 2 moles of carbon dioxide.

One means by which this could be achieved is by the maintenance of a stable rate of oxidative phosphorylation Assuming the activation of propionate to release pyrophosphate[9], and the fixation of carbon dioxide to release orthophosphate[10], then the P/O ratio necessary to sustain the rate of propionate uptake found would be 6/7 (0 86 ± 0 02, cf Fig 1), a curious value, maintained with unusual precision

A more probable explanation may lie in the direct transfer to propionyl coenzyme A of carbon dioxide derived from oxidation of the malate carbon skeleton.

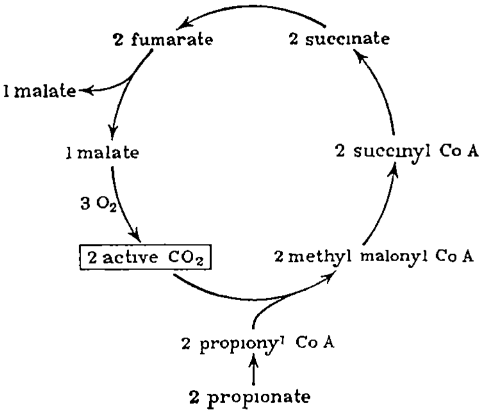

Fig 2 Proposed mechanism of propionate metabolism by sheep liver

The recent observation of Swick and Wood[11] of the direct transfer of the oxalacetate β carboxyl group to propionyl coenzyme A to form methyl malonyl coenzyme A in cell-free extracts of *Propionibacterium shermanii* supports this hypothesis Swick and Wood pointed out that such transferable carboxyl groups exist in methyl malonyl coenzyme A and malonyl coenzyme A in addition to oxalacetic acid, and that these molecules have a common structural feature in that the active carbon dioxide group is derived from a carboxyl group β to a carbonyl function which is in turn adjacent either to a second carboxyl group or to a coenzyme A moiety During complete oxidation of malate via pyruvate and the tricarboxylic acid cycle, both oxalacetic acid and oxalosuccinic acid lose carboxyl groups of this nature With this in mind, work on the mechanism of propionyl coenzyme A carboxylation in sheep liver mitochondria is being continued

The relationship between uptake of oxygen and consumption of propionate by liver homogenates is of considerable interest in relation to the *in vivo* synthesis of glucose from propionate The observations reported here demonstrate a mechanism whereby precursors of carbohydrate are derived from propionate at twice the rate at which propionate may be oxidized

We thank Dr H R Marston for suggesting this work, which was undertaken as part of a general investigation of cobalt deficiency in sheep

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Binding of *RP2-L*—a Nuclear Protein of Neoplastic Tissues—to Deoxyribonucleic Acid

Recent work in this laboratory has indicated that the incorporation of radioactive amino-acids into nuclear proteins, especially the acid-soluble nuclear proteins, represents an important metabolic pathway in transplantable rat tumours[1-3] The acid-soluble nuclear proteins obtained from rats injected with L-lysine-U-^{14}C were chromatographed on carboxymethyl-cellulose columns[4] after the acid extracts had been dialysed against 0 5 *N* acetic acid The cellulose columns (sodium form) had been equilibrated against 0 05 *M* sodium acetate buffer, *p*H 4 0 Gradient elution was used with *N* formic acid followed by 8 *N* formic acid All procedures were carried out at 4° C The resolving power of this method provided specific chromatographic patterns for protein distribution and distribution of radioactivity for the acid-soluble nuclear proteins of the Walker tumour and various tissues of the tumour-bearing rat The chromatograms of the acid-soluble nuclear proteins from the various non-tumour tissues contained one to three radioactive peaks with relatively low specific activities The chromatograms of the nuclear extracts of the Walker tumour, and a number of other malignant tumours, contained a radioactive peak which was not found in any of the growing or non-growing[4,5] non-tumour tissues studied

In the present work, deoxyribonucleoproteins were extracted with water from cell nuclei, isolated from the Walker tumour and liver of tumour-bearing rats[6-10] The deoxyribonucleoproteins were then precipitated by making the medium 0 15 *M* with respect to sodium chloride (ref 10) L-lysine-U-^{14}C (10 μc.) was injected 1 hr before the tissues were excised[3 4] The nuclei from 3–5 gm of tissue were blended in 50 ml distilled water 6 times for periods of 10 sec. The temperature was maintained at 4° C The sample was centrifuged at 2,000*g* for 30 min and the nuclear residue was washed twice with 50 ml distilled water. To the combined supernatant solutions containing the deoxyribonucleoproteins, solid sodium chloride was added to make a final concentration of 0 15 *M* and the sample was kept in the cold laboratory (4° C for 1 hr) and was again centrifuged at 2,000*g* for 30 min after the supernatant solution (the 'nuclear sap') was decanted The white deoxyribonucleoprotein precipitate was stirred with 0 25 *N* hydrochloric acid for 20 min and then was centrifuged at 100,000*g* for 30 min After centrifugation of the supernatant nuclear sap at 100,000*g* for 30 min, 2 *N* hydrochloric acid was added to the combined supernatant solutions to make a final concentration of 0 25 *N* hydrochloric acid Of the total deoxyribonucleic acid (DNA) found in the various fractions, 80–90 per cent was in the residue which was precipitated after the acid extraction of the nucleoproteins The remaining 10–20 per cent was in the water-insoluble residue No DNA was found in the supernatant solutions in which the concentration of sodium chloride was 0 15 *M* In this communication the DNA-bound protein is referred to as 'chromosomal protein' and proteins of the supernatant fractions are referred to as proteins of the nuclear sap

The optical densities at 280 mμ of a standard volume of 3 0 ml of extracts of chromosomal proteins of tumour and liver respectively were 0 30 and 0 20 per gm wet weight of tissue The optical densities of the acid-soluble proteins of the nuclear sap of tumour and liver respectively were 2 0 and 1 0 The

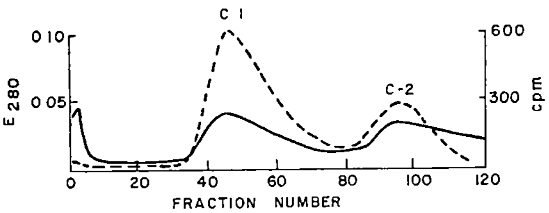

Fig 1 Chromatographic patterns of both radioactivity and protein-concentration for chromosomal proteins of the Walker 256 carcinosarcoma The data are averages of three experiments Gradient elution was employed with N formic acid followed by elution with 8 N formic acid at fraction 80 Solid line, optical density, broken line, radioactivity

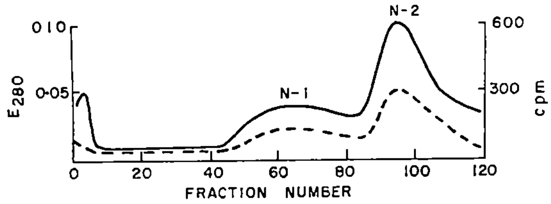

Fig 2 Chromatographic patterns of both radioactivity and protein-concentration for nuclear sap proteins of the Walker 256 carcinosarcoma The data are averages of three experiments Solid line, optical density, broken line, radioactivity

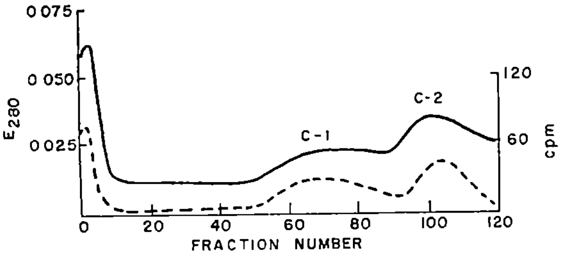

Fig 3 Chromatographic patterns of both radioactivity and protein-concentration for chromosomal proteins of the liver of the tumour-bearing rat The data are averages of three experiments Solid line, optical density, broken line, radioactivity

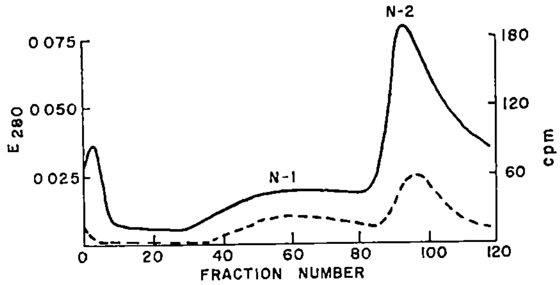

Fig 4 Chromatographic patterns of both radioactivity and protein-concentration for nuclear sap proteins of the liver of the tumour-bearing rat The data are averages of two experiments Solid line, optical density, broken line, radioactivity

extracts obtained were chromatographed on carboxymethylcellulose as described above, after dialysis against 0 5 N acetic acid

In order to maintain maximum resolution of the proteins, extracts of 'chromosomal proteins' from 4–6 gm of tissue and extracts of nuclear sap from 2–3 gm of tissue were chromatographed

The patterns for the protein distribution and radioactivity in the chromatograms of 'chromosomal' (DNA-bound) proteins and the cationic proteins of the nuclear sap of tumour and liver are presented in Figs 1–4 The specific activity of the chromosomal proteins of the tumour was much higher than that of the other extracts The most radioactive proteins, that is C–1 of the tumour (Fig 1), were eluted with N formic acid in the same position as $RP2$–L, the protein peak found in neoplastic tissues Like $RP2$–L, the specific activity of the protein of peak C–1 was high (10,050 c p m /E_{280}) In addition, the protein of peak C–1 of the tumour contained 35 per cent of the total counts of the total acid extract Since C–1 or $RP2$–L was concomitantly dissolved and precipitated with DNA, and was not found in the preparations which did not contain DNA, these data indicate that C–1 or $RP2$–L is a DNA-bound chromosomal protein

The finding that a protein, found thus far only in neoplastic tissues, is synthesized rapidly on the chromosomes suggests that the genetic change in tumour cells may be reflected in production of abnormal chromatin These findings may provide a tool for investigation of the nature of the abnormalities of the DNA in neoplastic cells

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Isolation and Identification of Rhamnose from Rabbit Skin

THE presence of neutral sugars such as galactose, mannose and L-fucose in several animal sources such as glycoproteins or blood group substances is well known[1] but L-rhamnose has not been previously reported as a constituent of mammalian tissues

Skin from rabbits (1–2 years old), injected with glucose-U-^{14}C, was treated with a depilatory, cut into strips, frozen in liquid nitrogen and ground into a fine powder in a Wiley mill The powder was homogenized with water in a Virtis-45 homogenizer at maximum speed for 30 min , the extract centrifuged at 5,000 r p m. for 30 min , the supernatant filtered through glass wool and lyophilized to yield 0 10–0 20 gm /10 gm of milled powder

Hydrolysis of the lyophilized material with 0 25 N hydrochloric acid for 30 min.–1 hr , followed by methyl pentose analysis[2] of the supernatant, indicated that the lyophilized material contained 1–3 mgm. methyl pentose/100 mgm of material The presence of rhamnose was suggested by the relative mobility of reducing substances in butanol/pyridine/water (6 4 3), butanol/acetic acid/water (4 1 5) and isopropanol/water (9 1)[3-6] The latter solvent gave the best separations from other methyl pentoses such as fucose

250 mgm of the water-soluble fraction were hydrolysed as above (1 hr) and salts removed on a 'Dowex 50 H^+–Dowex 1', HCO_3^- mixed-bed resin

The resulting solution was concentrated, banded on acetic acid washed Whatman *BMM* paper, and chromatographed for 17–20 hr at room temperature in a solvent system of 9 parts isopropanol : 1 part water Visualization of reducing substances with silver nitrate on a parallel strip showed three spots, one of which had a mobility corresponding to that of rhamnose. The appropriate area was eluted with water, and an aliquot assayed for radioactivity in a Packard 'Tri-Carb' liquid scintillation counter using dioxane/anisole/dimethoxyethane (6 1 : 1) as solvent and 2,5-diphenyl oxazole 1,4-*bis*-2-(5-phenyloxazolyl) benzene (40 . 1) This fraction was found to contain carbon-14. 50 0 mgm of crystalline L-rhamnose hydrate was added as carrier, the solution concentrated to a thick syrup and allowed to crystallize for 2 hr –3 days The crystalline rhamnose obtained in several experiments was identified by infra-red spectrometry and contained 85–98 per cent of the total radioactivity present in the water eluate of the chromatogram, the specific activity was not lowered on recrystallization

The fine structure of the rhamnose-containing compound is at present unknown and will be the subject of subsequent investigation

This work was supported by research grants *A*–2903(C2), *A*–4315 and *H*–3582 and *M*–2109, from the National Institutes of Health, U S Public Health Service, Bethesda, Maryland

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Formation of Histamine and Histamine-like Substances from Histidine by Quartz Dust

It has been shown[1] that quartz dust is capable of participating in a number of oxidative and hydroxylative reactions The present communication reports the production of histamine, from the interaction of quartz dust with histidine, and the chromatographic isolation of additional products of this reaction, namely, imidazole–carboxylic acids from the lungs of guinea pigs treated with quartz dust

Attention was first directed to a possibly harmful effect of histamine in silicosis by Uspensky *et al* [2], who in 1957 reported an overall increase of this material in the lungs of silicotic animals over that in the lungs of normal animals, and ascribed the toxic action of quartz to its release of histamine in excessive amounts into the tissue

It has now been shown in this laboratory that quartz dust, when treated with the amino-acid *dl*-histidine base, produces several imidazole-like substances, one of which has been identified as histamine both chromatographically (descending unidimensional system using Whatman No 1 paper, *n*-propanol / 0 2 *N* ammonia (3 1), spraying reagent· Pauly's sulphanilic acid reagent) and biologically (guinea pig uterus) Similar chromatographic products were obtained when hydrogen peroxide was treated with histidine under the same conditions. The reaction of quartz with histidine may be of the oxidation-reduction type due to the peroxidative properties of quartz reported earlier[1] Deamination and partial decarboxylation of histidine were obtained by Holtz[3] by treating it with ascorbic acid and glutathione in air or oxygen, or by passing oxygen and hydrogen through a medium containing histidine and metal catalysts

In the present work a yield of at least 5 μgm histamine (0 1 per cent) was obtained by alternatively shaking and incubating 1 gm of quartz dust with 5 mgm. histidine in 5 ml water at 37° for 5–10 days After centrifugation, the dust was washed with ethanol–hydrochloric acid and this washing was added to the supernatant mother liquor before concentrating to about 0 2–0·5 ml The reaction was accelerated and the yield was increased when the medium was made slightly alkaline with sodium carbonate This was also found when the contents of the vessel were allowed to dry out during shaking When carried out in acid solution (hydrochloric acid), which also stabilized any histamine formed, the reaction took place rapidly at 85–90° C. Iron ions in the medium either retarded or inhibited histamine formation· this finding agrees with that of Holtz[3]. Furthermore, acid-washed quartz produced more histamine from histidine than did untreated quartz with an iron content of 0 03 mgm /gm

The asbestos dusts, crocidolite and chrysotile, and mine dust (a dust collected from ventilation shafts in a Witwatersrand gold mine) were also able to produce histamine from histidine in the presence of sodium carbonate, but the relatively inert dusts, kaolin and talc, were without effect These latter have already been reported[1] to have low or negligible oxidizing or hydroxylating abilities.

Dusts producing histamine also gave rise to a second Pauly-positive material which was detected chromatographically (orange red, R_F value 0 1–0 14), and which was later identified by the use of other solvents as a complex spot consisting of a number of imidazole carboxylic acids Glutamic acid, a well-known degradation product of histidine[4], was also produced.

Quantitative assays of lung tissue for histamine indicated that this reaction between active dusts and histidine might occur *in vivo* The assays were carried out on the wet, unperfused lungs of two groups of four guinea pigs, the animals of one group were exposed for 140 days to a superfine quartz dust (80 per cent of particles less than 2μ) at a concentration of 25,000 particles/c c for about 7½ hr a day; the other group were controls. A mean value of 8 7 mgm /100 gm. dried tissue for histamine (as the dihydrochloride) was obtained for the lungs of the group treated with quartz dust compared with 5 4 mgm /100 gm. for the control group This represents a 29 per cent increase of histamine in the dusted groups of animals. In a further group of animals dusted for a period of 180 days, the increase was of the same order (namely, 31 per cent) Chromatographic analyses of the trichloroacetic acid extracts of lungs after adsorption with activated charcoal and elution with ethanol / hydrochloric acid (2 1) showed the presence in the dusted group of the Pauly-positive spot described above (R_F 0 1–0 14), but not in the control sample even when much higher concentrations of lung extracts were used This spot, however, was absent when lung extracts of animals treated with dust for only 70 days were chromatographed. On further resolution in other chromatographic solvent systems, the imidazole carboxylic acids listed in Table 1 were identified In addition, traces of urocanic acid were identified in lung extracts from

Table 1 R_F VALUES OF MATERIALS FOUND IN TWO SOLVENT SYSTEMS

Solvent system	Material	R_F value
n-Butanol/acetic acid/ water (50 12 50)	Imidazole pyruvic acid	0 24
	Imidazole lactic acid	0 27
	Imidazole acetic acid	0 34
	Hydroxymethyl imidazole	0 41
Isopropanol/ammonia (0 88)/ water (160 8 16)	Imidazole pyruvic acid	0 04
	Imidazole lactic acid	0 07
	Imidazole acetic acid	0 06

animals treated with dust, this might be a hydrolysis product of its choline ester, murexine, a substance producing pronounced pharmacological effects Very small amounts, or none, of these materials were detected in extracts from the lungs of the control group The enzymatic conversion of histidine to some of these degradation products has been reported by van Thoai[5], who treated L-amino-acid-oxidase with histidine

The evidence obtained from these experiments suggests that not only can quartz produce histamine from histidine *in vitro*, but also a number of other derivatives, some of which are biologically active The presence of these substances in the lungs of animals exposed to quartz dust but not in controls makes it seem likely that the *in vitro* course of events shown above may occur *in vivo*.

The possibility of histidine as a basic substance being adsorbed on quartz dust after this has entered the lung tissue, and its continual transformation into small amounts of histamine and other potentially harmful substances might over a long period of time be of importance in the pathogenesis of silicosis

I wish to thank the director of this Unit, Dr H S Gear, for facilities extended to me during this work, the senior biochemist, Dr J S Harington, for valuable assistance with the preparation of this manuscript, and Mrs Lippuner, of the Department of Pharmacology, University of the Witwatersrand Medical School, for carrying out the biological assays.

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Influence of *p*H and Urea on the Ultra-violet Fluorescence of Several Globular Proteins

RECENT work by Weber[1-3] has shown that the ultra-violet fluorescent spectra of globular proteins differ from those predicted for the summation of the independent contributions of their aromatic amino-acid constituents Those examples investigated which contained tyrosine, but no tryptophan, showed a tyrosine emission spectrum, but with a reduced quantum yield Those containing both tyrosine and tryptophan showed an emission spectrum predominantly resembling that of tryptophan alone, with an emission maximum in the range 320–350 mμ The present communication will be concerned solely with proteins of the second category

These include a protein showing a reversible structural transition at extremes of *p*H (bovine serum albumin), a protein the internal structure of which is probably invariant to *p*H (lysozyme), and a protein undergoing a simultaneous denaturation and unfolding into a random coil at neutral *p*H (pepsin) An Aminco-Bowman spectrofluorometer, equipped with a thermostatted cell-holder, was used for all measurements Unless otherwise specified, the temperature of measurement was 24° C The wave-lengths of excitation and of emission were 290 mμ and 340 mμ, respectively

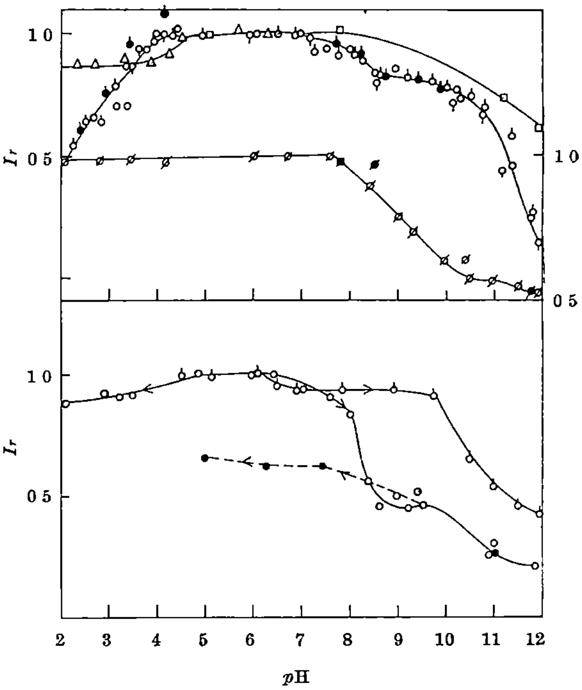

Fig 1 Relative fluorescence intensities Upper ⫯, Armour bovine plasma albumin in water, ○, bovine plasma albumin fraction from diethylaminoethyl cellulose column (containing 0 24 mole —SH per mole albumin in water, △, same, in 2 0 *M* potassium chloride, □, same, in 8 *M* urea, ⫰, bovine plasma albumin fraction from diethylaminoethyl cellulose column (containing 0 98 mole —SH per mole albumin) in water, o, Armour crystalline lysozyme in water The curve is displaced downward by 0 5 unit with respect to the albumin curve The right-hand ordinate applies Lower ○, Armour crystalline pepsin in water, ⫰, same, in 0 2 *M* potassium chloride The protein concentration is 0 1 gm /l in each case The filled circles represent reverse points The ordinate is the fluorescence intensity relative to that at *p*H 6, which is the starting point for either acid or alkaline titration The wave-lengths of activation and of emission are 290 mμ and 340 mμ, respectively

All the proteins under examination had an emission spectrum characteristic of tryptophan, with a maximum in the range 320–350 mμ The dependence of fluorescence intensity on *p*H was very different for the various proteins All these had, however, the common feature of undergoing a pronounced quenching in the *p*H region 9–12 (Fig 1)

In the cases of bovine serum albumin and lysozyme, the reverse curve obtained on back titration from alkaline *p*H showed no major deviation from the forward curve, in harmony with expectations arising from the reversible character of the alkaline titrations for these proteins

The presence of urea in high concentration (10 *M*) appears to reduce the magnitude of alkaline quenching at a given *p*H for bovine serum albumin considerably

At *p*H's acid to *p*H 5 the various proteins examined showed a wide divergence of behaviour Lysozyme and native pepsin, the macromolecular properties of which appear to be invariant to pH in this range, show a small decrease in intensity. However, bovine serum albumin, which is known to undergo a structural transition in this zone[6] of pH, shows a reversible quenching of fluorescence if the titration is carried out in the absence of added electrolyte. In 2·0 M potassium chloride the acid quenching is reduced somewhat, presumably because of the suppression under these conditions of the inflation of the molecular domain occurring at low ionic strengths[6].

In addition to the acid and alkaline quenchings bovine serum albumin showed a small but definite quenching in the region pH 7·0–9·0 (Fig. 1). It is of interest that spectral evidence exists for a minor structural change in this zone[7]. Katz and Klotz have reported changes in calcium ion binding properties in this region[8].

Pepsin, which is known to undergo an irreversible denaturation, accompanied by a gross molecular disorganization at a critical neutral pH zone[6–9], shows somewhat different behaviour[9]. The emission increases slightly between pH 2 and pH 5. The onset of irreversible denaturation, which occurs at pH 7·5–8·5 in the absence of added electrolyte, is reflected by a sharp drop in fluorescence intensity (Fig. 1). If 0·2 M potassium chloride is present the extent of the drop is greatly reduced. In either case a second region of decrease is noted in the zone of tyrosine ionization, pH 9–12.

A complete interpretation of this fairly complex behaviour will certainly require the disentangling of several effects in each of the examples considered. It is likely that the ionized form of tyrosine is the only group the absorption spectrum of which overlaps sufficiently with the tryptophan emission spectrum to permit an important degree of radiationless energy transfer[4,5]. From the behaviour of pepsin and bovine serum albumin, it is clear that the intensity of fluorescence of globular proteins is a function of their molecular organization and can be expected to be influenced by molecular events which alter the secondary or tertiary structure and thereby modify the environment of the fluorescent group.

Bovine plasma albumin fractions were obtained through the courtesy of Dr. R. Hartley.

Note added in proof. In subsequent work with another lysozyme preparation a minor (10 per cent) quenching was found between pH 6 and pH 2. The acid branch of the lysozyme curve of Fig. 1 may thus require revision.

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Retinene-1 in Insect Tissues

Attempts to detect vitamin A in insect tissues have been largely unsuccessful. One species of cockroach (*Blatella germanica*) has been raised for generations on a diet devoid of vitamin A and carotenoids[1]. As no vitamin A could be detected in oil extracted from these insects, it appeared that the vitamin played no part in their metabolic processes. Other workers[2] similarly have reported their inability to detect vitamin A in Blattidae. Acetone extracts of the locusts *Schistocerca gregaria* and *Locusta migratoria migratorioides* gave negative tests for the vitamin[3], while extracts of the heads of fruit-flies, grasshoppers, and dragonflies also have been reported to be lacking in vitamin A (ref. 4). No dietary requirement for vitamin A could be demonstrated for either *Drosophila melanogaster*[5,6] or *Anopheles labranchiae atroparvus*[7].

However, extracts of the pupæ of the silkworms *Bombyx mori* and *Antherea pernyi* have been reported[8,9] to contain vitamin A, while Goldsmith[10] recently has demonstrated that extracts of the heads of honey-bees contain retinene-1, and that a visual pigment of this insect is a rhodopsin compound analogous to vertebrate visual pigments. Wolken *et al.*, in a recent report[11], have announced the detection of retinene-1 in house-flies.

In the experiments reported here, the presence of retinene-1 in the honey-bee and the house-fly was confirmed, and the compound detected in extracts of the following insects, which represent many major orders of insects: *Paracolletes fulvescens* (Hymenoptera), *Locusta migratoria* (Orthoptera), *Uropetala carovei* (Odonata), *Cicindela tuberculata* (Coleoptera), *Nyctemera annulata* (Lepidoptera), *Cutelia sedilotti* (Orthoptera).

Adult specimens of these insects were collected locally, and in some cases were preserved by deep freezing, and in others were killed by decapitation and immediately experimented on. The heads were separated from the rest of the body and the two tissues extracted separately. In the case of the bees, the legs were removed to avoid excess carotenoids from pollen. The tissues were ground with anhydrous sodium sulphate and the mixture extracted with anhydrous acetone. This treatment releases carotenoids, vitamin A and retinenes from proteins. The resulting extracts were evaporated and the residues taken up in petroleum ether. On chromatography on aluminium oxide columns, yellow absorption bands developed, and were eluted with acetone–petroleum ether mixtures (4 per cent, 10 per cent and 40 per cent v/v). Each fraction was evaporated and the residues taken up in a small amount of chloroform. A Carr-Price reaction with saturated antimony trichloride solution in chloroform, and acetic anhydride, was conducted on each fraction. One fraction from insect heads of each species gave a noticeable blue coloration and an absorption maximum at 664 mμ.

These observations demonstrate the presence of retinene-1 in the heads, and its absence in the bodies, of each of the insect species investigated. It is a reasonable conclusion, considering the known functions of retinene in vertebrates and in other invertebrate phyla, that the retinene detected occurs in the insect eyes and is involved in their visual processes. It is of considerable interest that no vitamin A alcohol was detected in the above experiments. Moreover, the amounts of retinene present in the insect heads was very small and about 10 gm of tissue was necessary to give a satisfactory positive result

These findings indicate that the rhodopsin type of visual pigment occurs in many insects This being the case, the experiments[1,5-7] in which insects have been raised on diets devoid of vitamin A and carotenoids are difficult to understand Perhaps there is some other insect visual system that does not involve retinene, or alternatively, and more likely, perhaps the insects raised in these nutritional experiments were visually defective

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PHYSIOLOGY

Oscillatory Flow in an Air-filled Elastic System

A REPORT has been published by me[1] of the study of oscillatory flow in a fluid-filled elastic system, using an 'oscillator' It has now been discovered that the same device (Fig 1) will oscillate, in stationary wave motion, when there passes through it a stream of air, instead of water or blood

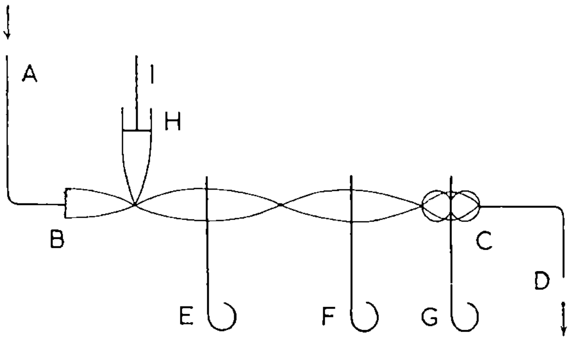

Fig 1 Diagram to show the component parts of the 'oscillator'

Air compressed to a pressure of about 0 7 kgm /cm^2 is delivered from a tank of which the outlet is controlled by a screw valve The air is led by the rigid tube (*A*) to the elastic tube (*BC*), which is composed of latex rubber, 0 7 cm in diameter and 97 cm long The other end of *BC* is connected to the rigid outlet tube (*D*) Extensions of the torsion springs (*E*) and (*F*) compress the elastic tube at the sites of antinodes, the extension of the torsion spring (*G*), at the site of a pseudo-stationary node, the loops being as shown The waves are both transverse and longitudinal, belonging to two systems of differing length, namely, 1–2 cm and approximately 75 cm As the wave-length approaches the diameter of the tube, the particles of air describe more or less circular orbits The correct position of the springs may be determined previously, by running water through the model from a reservoir, as already described At this stage, the quarter-wave matching stub (*H*) is useful in suppressing stationary waves at the proximal end of *BC* one end of the stub is connected to a node, as shown, the other closed by the piston (*I*)

Using compressed air, the model can be made to produce a great variety of noises and sounds, including musical notes Oscillations of low frequencies resemble purring noises at high frequencies, squealing noises result Harsh sounds occur, resembling those made by parrots or chirruping sounds such as those of other birds

Tuning is effected by altering the pressure of the springs on the elastic tube In the case of the spring (*G*), however, tuning is also effected by moving the point of application with relation to the junction of *BC* and *D* Tuning is particularly sensitive at this site The effect of transverse compression appears to be analogous to the application of a 'shunted inductance' the greater the pressure, the higher the frequency and conversely Movement of the point of the spring, in relation to the end of the tube, alters the length of the system of short stationary waves The segment of elastic tube involved can be regarded as a cavity resonator, so that the effect is analogous to that of altering the 'capacitance' The shorter the wave-length, the higher the frequency and conversely Increase of 'resistance', brought about by closing the screw valve which controls delivery of air, causes the pitch of the note, or noise, to rise and conversely

The spring (*G*), pressing on the elastic tube, produces a shape closely resembling that of one vocal cord It may be assumed that, in the larynx, the wave-lengths of the vibrating expired air approach the dimensions of this structure and of the related cavities The system of long waves of the tube (*BC*) is then related to the vibrating columns of air in the trachea and bronchi during speech and the apparatus can be regarded as an analogue for study of the mechanism of phonation

The model suggests that, in addition to approximation of the vocal cords, elevation and depression of the larynx when the pitch of the voice rises and falls, respectively, are also of significance in tuning In phonation, moreover, the action of the thorax and lungs is that of velocity modulation of the expelled air, whereas that of the larynx is to effect density modulation of this air

Acknowledgment is due to the Royal Air Force and to the Postgraduate Medical School of London for research facilities and supply of equipment

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Hormonal Control of the Development of the Thymus of the Fœtal Rabbit

THAT the fœtal adrenal is physiologically active has been firmly established, for example, Jost and Jacquot have shown its importance in the development of fœtal liver glycogen[1-3] That the adrenal itself is influenced in its development by a structure or structures within the skull—generally assumed to be the hypophysis—has been demonstrated in the rabbit[4,5] and rat[6,7] by fœtal decapitation, following which there is a considerable reduction in the adrenal by full term Since it is well recognized that in the adult animal adrenal hormones have a thymolytic action, it was considered possible that the development of the fœtal thymus might be influenced by the fœtal adrenals To test this hypothesis rabbit fœtuses were decapitated at 20–21 days of development and the thymus glands were weighed when the fœtuses had reached almost to term The results were clear cut, for in the operated fœtuses the thymus on average was 72 per cent heavier than in their litter mate controls[5]

The operation of decapitation of the fœtus, performed to remove the pituitary, does not affect the development of the fœtus, as judged by gross weight[8,9] The operated fœtuses, together with their litter mates, continue to develop within the uterine cavity for a further eight days It was thought that the resulting increase in thymus weight might be the result of the reduction in the development of the adrenals secondary to the decapitation of the fœtus which abolishes fœtal pituitary secretions, in particular, fœtal adrenocorticotrophic hormone (ACTH)[6]

However, it could be argued that the increase in size of thymus in the decapitated fœtuses might be due to the absence of other fœtal pituitary hormones apart from ACTH, or even to the removal of some other structure in the head, such as the brain or the pineal To investigate this point further, it was decided to inject ACTH into the fœtus, at the same time as the decapitation was performed, to replace the fœtal ACTH, and to investigate the effect of this combined procedure on the development of the adrenals and the thymus A long-acting preparation of ACTH in gelatin was used to avoid having to repeat the injection into the fœtus

Eight fœtuses were decapitated by the method described by Jost[10] between the 21st and 22nd day of development, and 0 2 ml of 'Acthar' gel was injected subcutaneously in the interscapular region of each fœtus just before it was returned to the uterus The fœtuses were recovered after a further seven days of intra-uterine development, by Cæsarian section, and the adrenals and thymus removed and weighed to the nearest 0 1 mgm The weights were expressed per 100 gm of body-weight, and were compared with 15 litter-mate controls

The results are shown in Table 1 There is no significant difference between the weights of the decapitated fœtuses injected with ACTH and the litter-mate controls The adrenals were found to be slightly larger in the decapitated fœtuses injected with ACTH compared with the control fœtuses, but this difference is not significant The thymus of the experimental fœtuses showed no significant increase in weight over the thymus of the controls

These results provide confirmation of the view that the increase in weight of thymus found in decapitated fœtuses[5] is due to the retarded adrenal development, caused by the absence of fœtal ACTH following the decapitation

Table 1 THYMUS AND ADRENAL WEIGHTS OF THE RABBIT FŒTUS FOLLOWING DECAPITATION AND INJECTION OF ACTH

Fœtuses	No	Age at investigation (days)	Body-weight (gm) headless	Adrenal weights (mgm / 100 gm)	Thymus weight (mgm / 100 gm)
Decapitated + ACTH (at 21–22 days)	8	28–29	23 4±1 8	25 4±4*	275 3±13*
Controls	15	28–29	23 0±1 5	19 6±1*	261 6±13*

* These differences are not significant

These findings also provide an explanation for some of the endocrine abnormalities present in the human anencephalic fœtus It has long been known that the pituitary of these fœtuses is very small or absent[11], and the small adrenals and large thymus reported in this human abnormality[12] are likely to be secondary to the deficiency of fœtal ACTH

I am indebted to Prof A Jost for his help with the technique involved in intra-uterine surgery of the fœtus, to the Medical Research Council for a grant for apparatus, to Armour Laboratories for a supply of ACTH, and to Prof E W Walls for his help and encouragement in this work

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Luteinizing Hormone in Persistent Vaginal Œstrus

THE placement of experimental lesions in the anterior part of the hypothalamus may be followed by persistent vaginal œstrus in the guinea pig and rat Such animals possess relatively small ovaries which have no fresh corpora lutea Various attempts have been made to explain these effects in terms of an altered output of gonadotrophins by the pituitary gland The original view that the secretion of luteinizing hormone was blocked[1] was supported by Van Dyke *et al* [2], who found that in animals with persistent œstrus induced by lesions, the ovarian interstitial tissue showed a high lipid content indicative of deficiency in luteinizing hormone. Recently, Taleisnik and McCann[3] reported a pituitary luteinizing hormone content in animals with persistent œstrus which was lower than in intact animals However, after gonadectomy the amount of luteinizing hormone of persistently œstrous rats showed an increase, but the level of hormone in plasma remained low, indicating that the pituitary release of the hormone is blocked On the other hand, Hillarp[4] concluded, also on the basis of the condition of the ovarian interstitial tissue, that during persistent œstrus luteinizing hormone was still being secreted in considerable amounts In other work, the possibility was stressed that in persistent œstrus induced by lesions, secretion of follicle stimulating hormone may be enhanced, because in animals which had been operated during the first two weeks after birth, puberty occurred at an earlier age than in control animals After puberty, a number of these animals showed persistent œstrus[5] A third possibility has been advanced by D'Angelo and Kravatz[6], who assumed that in persistently œstrous rats secretion of follicle-stimulating hormone may be decreased This assumption was based on their finding that the serum of unilaterally gonadectomized

Table 1

Donor rats	Persistent œstrus	Regular cycles Early di-œstrus	Late di-œstrus	Pro-œstrus	Œstrus
	A	*B*	*C*	*D*	*E*
Recipients:					
No. of animals	21	16	16	5	11
Body-weight (gm.)	50·4 ± 0·9	50·5 ± 0·8	50·6 ± 1·5	50·8 ± 1·2	51·4 ± 1·5
Ventral prostate (mgm.)	20·0 ± 1·52	14·2 ± 0·53	19·0 ± 1·80	15·8 ± 1·03	14·4 ± 0·93
Both testes (mgm.) ± *S.E.*	189·2 ± 9·2	157·2 ± 7·3	164·9 ± 10·0	137·8 ± 21·8	147·2 ± 11·9

Student's *t*:	Prostate weight	Testes weight
A—*B*	3·61	2·60
A—*E*	3·14	2·72
A—*C*		1·76
B—*C*	2·56	0·92
C—*E*	2·27	1·12

rats with persistent œstrus contained less follicle-stimulating hormone than that of cycling animals after removal of one ovary.

There is no conclusive evidence for any of these hypotheses. Cook[7] was unable to detect differences between separate pituitary contents of follicle-stimulating and luteinizing hormone when the pooled glands of rats with persistent œstrus were compared with those of intact or operated rats with regular cycles.

We are investigating gonadotrophin contents in pituitary gland and serum of rats with persistent œstrus following a basal lesion in the anterior part of the hypothalamus; the lesions involved the dorsal and caudal end of the optic chiasm. This preliminary report concerns results on pituitary luteinizing hormone. Assays were carried out in the following manner. Individual pituitary glands were homogenized in saline solution with a Potter–Elvehjem apparatus, the final volume of each homogenate being 12 ml. Twice daily 0·5 ml. of each homogenate was injected beneath the skin into one hypophysectomized immature male rat (body-weight 30–40 gm.) for six days, resulting in a total dose of 0·5 equivalent of one single hypophysis. The weight of the ventral prostate served as an index of the luteinizing hormone potency of the pituitary homogenate. The control group consisted of twenty-five rats with a lesion which showed normal cycles, and twenty-three intact rats. Since no differences were apparent between the results obtained in these two categories they were combined in this work. Gans and De Jongh[8], using a slightly different method for assay of luteinizing hormone, have found that pituitary luteinizing hormone fluctuates during the œstrous cycle with a peak during late di-œstrus followed by a decline in pro-œstrus. In the present work, therefore, the data on control rats were related to the phase of the œstrous cycle prevailing at the time of death. The finding of Gans and De Jongh was confirmed as may be seen from Table 1. Animals with persistent œstrus possessed a high pituitary luteinizing hormone potency, comparable with that noted in the late di-œstrus phase of the normal cycle. Both the weights of the ventral prostate and of the testes of recipients of pituitary homogenates derived from these two groups of individuals were significantly higher than those of recipients of homogenates obtained from rats in œstrus or early di-œstrus.

These findings show that a hypothalamic lesion causing persistent œstrus and inhibition of ovulation does not necessarily block the production of luteinizing hormone within the pituitary gland. The high pituitary content may be the result of an enhanced production or an inhibited release of luteinizing hormone. It is of great interest that preliminary results on serum luteinizing hormone have brought to light a marked contrast between the conditions of persistent œstrus and late di-œstrus; while the high pituitary luteinizing hormone content of late di-œstrus has a parallel in the high serum content, the serum of rats with persistent œstrus possesses luteinizing hormone in only barely detectable amounts. If the latter finding can be confirmed, it would seem that a hypothalamic lesion which does not necessarily affect production of luteinizing hormone may inhibit its release from the gland.

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Morphological Changes following Transplantation and Chronic Obstruction of the Rabbit Appendix

EXPERIMENTS reported by Sholpo[1] from the Moscow Medical Institute are claimed to be evidence in support of the theory that morphology and function depend on environment. In a series of 46 animal experiments Sholpo transplanted the rabbit cæcal appendix to various sites in the small bowel, almost all in the ileum. After three months the transplanted appendices showed marked reduction in the size of the lymphoid tissue. The results were summarized as follows:

"The vermiform appendix appears to be a convenient object for experimental researches on re-activity and plasticity of tissues of the digestive system. When surgically inserted into the digestive tube at different levels of the latter, the appendix finds itself in (an) unusual functional condition. The operation results in a substantial reconstruction of the wall of this organ: the glandular layer develops intensively, the lymphoid layer reduces both in thickness and in numbers of rows of lymph follicles. The appendix of (the) rabbit begins to display considerable muscular activity which is wanting under normal conditions".

It seemed to us unlikely that the changes which Sholpo described could occur as a result of an altera-

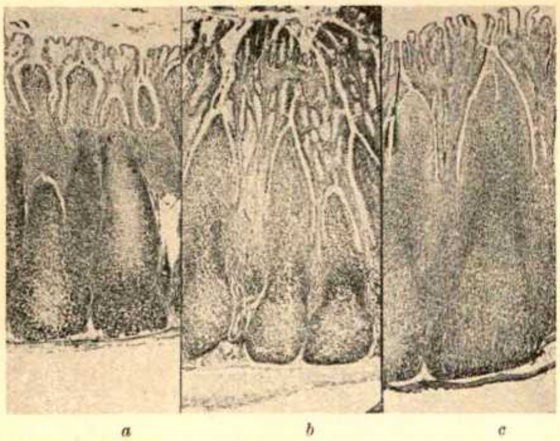

Fig. 1. The microscopical appearance of the wall of (*a*) the appendix; (*b*) the saccus rotundus and (*c*) an agminate patch. The villi of the glandular layer are on the top, the main thickness is formed by the lymphoid tissue and a thin muscular layer lies beneath

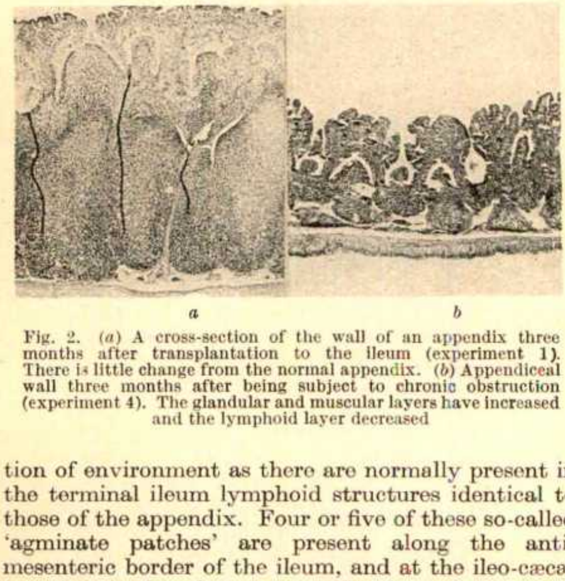

Fig. 2. (*a*) A cross-section of the wall of an appendix three months after transplantation to the ileum (experiment 1). There is little change from the normal appendix. (*b*) Appendiceal wall three months after being subject to chronic obstruction (experiment 4). The glandular and muscular layers have increased and the lymphoid layer decreased

tion of environment as there are normally present in the terminal ileum lymphoid structures identical to those of the appendix. Four or five of these so-called 'agminate patches' are present along the anti-mesenteric border of the ileum, and at the ileo-cæcal junction is found a large collection of lymphoid tissue, the saccus rotundus. The similarity of the microscopical structure of these lymphoid areas to that of the appendix is shown in Fig. 1. Their function also is similar[2]. We therefore felt that an environment compatible with the development of an agminate patch was unlikely to result in the structural alteration of the lymphoid tissue in the appendix, yet we too had observed changes similar to those found in Moscow in appendices transplanted into the ileum. However, as we became more practised at performing the anastomosis we found that these changes occurred less frequently, and stenosis at the suture line became less common.

Table 1

Operation	No. of rabbits	Died	Histologically normal after 3 months	Showing marked histological change after 3 months	With obvious anastomotic stenosis
1. Appendico-ileostomy	6	0	5	1	1
2. Appendiceal inter-position	6	0	3	3	2
3. Ileal diversion	6	3	3	0	0
4. Cæcal obstruction	6	0	0	6	—

Four experiments were therefore devised, the first three exactly like those of Sholpo and the fourth providing obstruction without transplantation: (1) The base of the appendix was separated from the cæcum and the tip anastomosed to the ileum. (2) The appendix was used as a replacement for an excised piece of ileum of equal length. (3) The ileal stream was diverted through the appendix into the cæcum by an end-to-end anastomosis. (In each operation the blood supply to the appendix was preserved and the anastomosis performed with 5/0 arterial silk sutures.) (4) In a further six rabbits the appendix was not disturbed but subjected to a chronic obstruction by producing a proximal cæcal stenosis with carbolized tape.

The normal histological appearance following three months transplantation is shown in Fig. 2*a*. After three months of chronic cæcal obstruction the glandular layer had developed, the lymphoid thickness reduced and the muscle thickness increased (Fig. 2*b*). In only one of the four transplanted appendices which showed a structural change was there no demonstrable suture line stenosis and here there appeared to be a functional obstruction as there was hypertrophy of the proximal ileum.

Sholpo's thesis is therefore rejected. The changes he described following appendiceal transplantation are, we suggest, the result of chronic obstruction as a result of the anastomosis and not the result of altered environment.

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Increased Iron Absorption without Increase in the Rate of Erythropoiesis

IT is generally agreed that under normal conditions in man the total body iron is maintained at a relatively constant level by some mechanism which regulates the amount of iron absorbed from the gastro-intestinal tract. Apart from manipulating intra-luminal conditions within the intestine, numerous experimental conditions are known which increase iron absorption and which have in common an accelerated erythropoietic rate. These include anoxic hypoxia[1], cobalt administration[2], erythropoietin administration (Mendel, G. A., unpublished observations), pyridoxine deficiency[3], acetyl phenylhydrazine induced hæmolytic anæmia[4] and anæmia induced by loss of blood[5]. The rate of erythropoiesis has been considered of major importance under 'physiological' conditions in regulating iron absorption[6,7]. In certain disease states, such as hæmochromatosis, however, iron absorption may be increased without measurable increase in erythropoietic rate[8]. It is the purpose of this preliminary communication to report an experimental condition in which gastro-intestinal iron absorption is increased above normal in the virtual absence of erythropoiesis.

Erythropoiesis in the bone marrow of young adult *CF* No. 1 female mice was greatly reduced by the

Table 1 EFFECT OF HYPOXIA ON IRON ABSORPTION AND ERYTHROPOIESIS IN NORMAL AND SPLENECTOMIZED MICE TREATED WITH STRONTIUM-89

Treatment	Percentage of iron-59 absorbed from gastro-intestinal tract (72 hr)	Percentage of intravenous administered iron-59 incorporated into red blood cells (24 hr)	Average reticulocyte count at time of iron-59 administration (per cent)
Normal controls	15 5 ± 3 98 (8)	38 8 ± 2 57 (8)	4 4
Splenectomy strontium-89	11 6 ± 1 32 (10)	4 1 ± 0 36 (6)	0 2
Normal hypoxic	33 1 ± 1 93 (10)	56 5 ± 4 85 (6)	4 2
Splenectomy strontium-89 hypoxic	30 3 ± 4 48 (8)	4 0 ± 0 02 (6)	0 1

No of animals in each group in parentheses

* Standard error of mean

intraperitoneal administration of 4 μc /gm of strontium-89_{2+} chloride Jacobson[9] has shown that animals so treated rapidly develop compensatory ectopic erythropoiesis in the spleen For this reason splenectomy was performed on the day prior to the administration of strontium-89 Eleven days after injection of strontium-89, the rate of erythropoiesis as measured by the incorporation of 1 μc of intravenously administered ferric iron-59 citrate into red cells was reduced from the normal of 38 8 per cent to 4 1 per cent (Table 1) Reticulocyte values were correspondingly reduced The gastro-intestinal absorption of 1 5 μc of force-fed ferrous iron-59_{2+} sulphate was measured, as previously described[2], using total body counting in a Geiger–Muller well counter Iron absorption was somewhat reduced from the normal value of 15 5 per cent to 11 6 per cent in the splenectomized animal treated with strontium-89 It should be noted that in these experiments iron absorption was measured over a 3-day period to allow time for the elimination of unabsorbed radioiron from the gut The incorporation of intravenously administered iron into red cells was measured during the 24-hr period coinciding with time of maximum iron absorption[2]

The influence of hypoxic anoxia on the gastrointestinal absorption of iron-59 sulphate was studied in groups of normal and splenectomized mice injected with strontium-89 Hypoxia was produced by placing the animals in a chamber in which a gas mixture containing 10 per cent oxygen was maintained Twenty-four hours after being placed in the hypoxia chamber, intravenous or intra-gastric iron-59 was administered Twenty-four hours later, the animals were removed from the chamber Normal mice made hypoxic showed a marked increase in iron absorption (33 1 per cent) as well as an increase in erythropoietic rate (56 5 per cent) (Table 1) The splenectomized mice treated with strontium-89 that were made hypoxic likewise showed a marked increase in iron absorption (30 3 per cent) but no increase in the rate of erythropoiesis (4 0 per cent)

Mazur[10] has presented evidence that the transfer of iron from liver ferritin to the plasma under hypoxic conditions in rabbits, guinea pigs and dogs is mediated by the enzyme xanthine oxidase The present demonstration of increased iron absorption independent of increased utilization of iron by the bone marrow under hypoxic conditions suggests that a similar enzymatic mechanism may be of importance in the regulation of iron absorption The hypoxic splenectomized animal injected with strontium-89 should provide a useful model for the study of enhanced iron absorption that is independent of accelerated erythropoiesis The details of these experiments as well as related studies will be reported at length elsewhere.

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* The Argonne Cancer Hospital is operated by the University of Chicago for the U S Atomic Energy Commission

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PHARMACOLOGY

Hypertensive Effects of Bradykinin in Rats

BRADYKININ obtained from ox plasma[1] and synthetic bradykinin cause intense vasodilatation in many mammalian species Intravenous injections of bradykinin lower arterial blood pressure in anæsthetized animals. On a molar basis, bradykinin is only ten times less active than acetylcholine and as active as histamine[1] Two crude bradykinin-containing extracts from ox plasma—one prepared by us and the other kindly supplied by Prof Rocha e Silva—were shown to have the typical depressor action in normal rats, but frequently produced a striking hypertensive effect in rats nephrectomized 16–24 hr before These results are similar to those obtained with anephrotensin prepared from rat serum[2]

It seemed of interest to investigate whether pure bradykinin has the same hypertensive action in nephrectomized rats as the crude extract Forty-six male rats (180–200 gm) were nephrectomized under brief bromethol anæsthesia, 8–24 hr later they were again anæsthetized with allobarbitone and their blood pressure recorded from a carotid by a membrane manometer Twenty-four sham-operated animals were used as controls Synthetic bradykinin (Sandoz) supplied in sterilized ampoules, and a natural (89 per cent) purified product prepared by Dr Elliott were diluted with Tyrode solution and injected into a femoral vein Three ranges of dose were used (μgm / 100 gm body-weight) low (0 005–0 1), medium (0 15–0 5) and high (0 55–6 0) Angiotensin-amide (5-valyl) and anephrotensin were tested in the same animal Anephrotensin was obtained from the serum of dogs nephrectomized 48 hr before, 0 005–0 1 ml of the purified extract was given intravenously

In the intact rat with normal blood pressure (110–130 mm mercury) pure bradykinin, whether natural or synthetic, decreased arterial pressure Occasionally a small rise followed the hypotensive response In nephrectomized rats three different types of response were observed (*a*) a rise in blood pressure, (*b*) a rise in blood pressure preceded by a transient and slight drop, and (*c*) a weak depressor effect, which was usually less pronounced than the effect of the same dose in a normal rat

The type of response obtained depended both on the level of arterial pressure at which the injection was given and on the dose Out of a total of 61 injections of 'low' doses given to nephrectomized rats with an arterial blood pressure less than 100 mm (60–100 mm), 62 per cent purely hypertensive, 36 per cent diphasic and 8 per cent hypotensive responses were obtained. The incidence of hypertensive effects decreases when higher doses are used But when low and medium doses were injected into nephrectomized rats with blood pressures of 60 mm or less, a hypertensive effect was observed in all instances In nephrectomized rats with high arterial pressures (100–140 mm), a hypertensive effect of low doses of bradykinin was observed in 31 per cent and after medium doses in 17 per cent

When intact rats (in which bradykinin is uniformly hypotensive) were treated with a ganglion-blocking drug such as pentolinium, bradykinin produced a hypertensive effect in 76 per cent and a diphasic response in 24 per cent In rats with low blood pressure due to pentolinium, bradykinin had a hypertensive effect in all instances

These results in anæsthetized rats show that the hypotensive action of bradykinin is converted into a hypertensive one when basal pressure is lower than usual This was seen in nephrectomized animals investigated 8–24 hr after removal of the kidneys and in intact rats treated with pentolinium; a purely hypertensive action of bradykinin is more frequently observed with small doses The hypertensive action of bradykinin seems to be more dependent on the initial level of the blood pressure than on the absence of the kidneys In contrast, dog anephrotensin produces stronger hypertensive effects in nephrectomized rats than in normal animals, irrespective of the initial blood pressure

The fact that low and medium doses of bradykinin in pentolinium-treated or nephrectomized rats cause hypertensive effects will have to be taken into account when such animals are used in tests for angiotensin in extracts of blood which contain bradykinin

We wish to thank the Gildemeister Foundation for financial aid for the work reported We are indebted to Dr Elliott, Dr Boissonnas (Sandoz, S A) and Dr Gaunt (Ciba, Ltd) for their generosity in supplying natural bradykinin, synthetic bradykinin and angiotensinamide (5-valyl)

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Release of Noradrenaline from the Spleen of the Cat by Acetylcholine

Acetylcholine contracts the spleen of the dog[1] and cat[2]. In those species the effect of acetylcholine in contracting the spleen is not blocked by atropine, but is blocked by dibenzyline and reduced by prior treatment of the animal with reserpine, thus it appears that the action of acetylcholine may be mediated by releasing noradrenaline from its stores in the spleen, which afterwards causes a contraction The work reported here provides further evidence that acetylcholine liberates noradrenaline from the cat spleen

The cat spleen was prepared for the collection of samples of its venous blood by a method similar to that in ref 3, in which a large vein branching from the main splenic vein was cannulated In addition a branch of the splenic artery was cannulated to permit intra-arterial injections into the spleen Heparin was given intravenously The splenic nerves were divided to exclude the reflex liberation of noradrenaline Acetylcholine bromide (1 5 mgm) was diluted with the animal's own plasma immediately before use, and injected into the splenic artery Splenic venous blood was collected during the next 40-sec period by temporarily occluding the main splenic vein, so diverting the flow through the cannulated branch, the blood was centrifuged and the plasma stored at – 10° C until required for assay Control samples of plasma were collected before acetylcholine injections Plasma noradrenaline was assayed on the blood pressure of the pithed rat recorded with a strain-gauge transducer as described in ref 4 The acetylcholine in the plasma was assayed on the blood pressure of the cat, to see whether there was sufficient to interfere with the assay for noradrenaline It was found that the volume of plasma injected into the rat contained less than 2 μgm of acetylcholine As a further precaution against acetylcholine interfering with the noradrenaline assay the pithed rat was injected with 1 mgm of atropine and 1 mgm of hexamethonium This treatment blocked both the muscarinic and nicotinic effects of 1 mgm of acetylcholine

Table 1 Effect of Acetylcholine (1 5 mgm) injected into the Splenic Artery on the Noradrenaline Content (mgm /ml) of Venous Plasma from the Cat Spleen before and after Phenoxybenzamine (10 mgm /kgm)

	Before phenoxybenzamine			After phenoxybenzamine		
Cat No	Control	After acetylcholine	Increase	Control	After acetylcholine	Increase
1	30	30	0	30	50	20
2	60	100	40	80	170	90
3	50	50	0	50	75	25
4	7 5	10	2 5	10	20	10
5	15	15	0	15	25	10
6	30	30	0	35	70	35
7 (reserpine-treated)	10	8	−2	7 5	7 5	0
8 (reserpine-treated)	20	20	0	12	12	0

The results are summarized in Table 1 The injection of acetylcholine into the splenic artery produced contraction of the cat spleen, but only in two out of six experiments was this accompanied by a detectable increase in the noradrenaline content of the splenic venous plasma

Brown and Gillespie[3] found that phenoxybenzamine (dibenzyline) increased the amount of noradrenaline which appeared in the venous plasma after stimulating the splenic nerve, therefore phenoxybenzamine (10 mgm /kgm) was injected intravenously into the cats After phenoxybenzamine the injection of acetylcholine did not contract the spleen, but the amount of noradrenaline appearing in the venous plasma was increased in every experiment. This observation provides a parallel with the experiments of Brown and Gillespie[3], who found an increased output of noradrenaline into the splenic vein after splenic nerve stimulation in the presence of phenoxybenzamine They suggested that phenoxybenzamine prevented the access of noradrenaline to receptor sites so that more was detected in the venous blood However, phenoxybenzamine also has anticholinesterase activity[5] An anticholinesterase would increase the effect of injected acetylcholine in releasing noradrenaline, and the effect of nerve stimulation if acetylcholine were an intermediary in the release of noradrenaline produced by sympathetic nerve stimulation. Evidence for such an intermediary action of acetylcholine in the sympathetic nerves to the spleen has recently been obtained[2].

Two cats were pre-treated with reserpine as described in ref. 6 in order to deplete the spleen of noradrenaline. In these cats acetylcholine did not produce a contraction of the spleen, nor did it cause an increase in plasma noradrenaline either before or after phenoxybenzamine.

These experiments show that the intra-arterial injection of acetylcholine releases noradrenaline from the spleen of the cat. When the noradrenaline stores were depleted by reserpine the intra-arterial injection of acetylcholine failed to increase the noradrenaline content of the splenic vein blood. These observations provide evidence that the sympathomimetic effect of acetylcholine on the spleen is due to release of noradrenaline from stores within the spleen.

We thank Smith Kline and French (Australia) for a gift of phenoxybenzamine (dibenzyline).

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Protection by Antioxidants against Lethal Doses of Carbon Tetrachloride

Rees, Spector and Sinha[1] have shown that 'Phenergan', 10-(2-dimethylamino-isopropyl) phenothiazine hydrochloride, protects against necrosis of liver cells in rats dosed with carbon tetrachloride. Afterwards, Gallagher and Rees[2] found that 'Phenergan' maintained normal concentrations of oxidized pyridine nucleotides in the liver despite the administration of carbon tetrachloride. These findings, together with those of McLean[3,4] and Gallagher, Gupta, Judah and Rees[5] that 'Phenergan' protects rats against liver necrosis due either to vitamin E deficiency or to administration of thioacetamide, suggested that 'Phenergan' operates on a general metabolic mechanism involved in the maintenance of cellular and mitochondrial integrity. The value of 'Phenergan' as an alternative to vitamin E indicated that its action might be associated with antioxidant properties. Therefore, the antioxidants[6,7] α-tocopherol, sodium selenite and *NN*¹-diphenyl-*p*-phenylenediamine were investigated as possible protective agents in carbon tetrachloride poisoning.

Female Wistar rats weighing 150–180 gm. were used. They were observed for 14 days after carbon tetrachloride had been administered.

(*a*) *Vitamin E*. Table 1 shows that 95 I.U. of α-tocopherol acetate administered intraperitoneally 40 hr. before 4 ml. of undiluted carbon tetrachloride/kgm. was given by stomach tube protected all of 15 rats against death. The death-rate was also reduced by administering 2 doses of 95 I.U. vitamin E at 24 and 6 hr. before carbon tetrachloride. Doses of 95 I.U. given with, and 5 hr. after, carbon tetrachloride were of negligible protective value. A smaller dose (19 I.U.) of α-tocopherol given daily for 3 days prior to administering carbon tetrachloride reduced the death-rate to 72 per cent of 18 rats as compared with 98 per cent of 43 rats given carbon tetrachloride only.

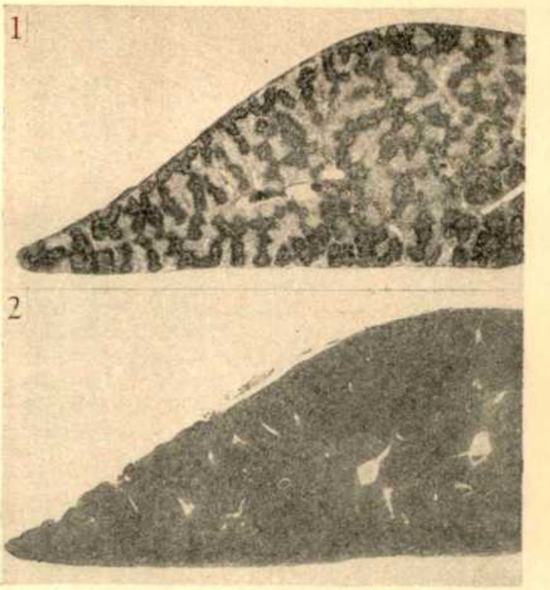

Fig. 1. Sections of liver stained with hæmatoxylin and eosin. (× 5). (1) Rat killed 24 hr. after receiving 4 ml. carbon tetrachloride/kgm. by stomach tube; the section shows widespread centrilobular necrosis and fatty change. (2) Rat killed 24 hr. after receiving 4 ml. carbon tetrachloride/kgm. by stomach tube. The animal had been given 100 mgm. DPPD in 0·3 ml. arachis oil intraperitoneally at 48, 24 and 0 hr. before carbon tetrachloride; the histology is almost normal

(*b*) *Sodium selenite*. Intraperitoneal injections of 0·5, 0·25, 0·25 and 0·25 mgm. sodium selenite at 72, 48, 24 and 6 hr. before the administration of 2·7 ml. carbon tetrachloride/kgm. reduced the death-rate to 51 per cent of 18 rats as compared with 89 per cent of 18 rats given this dose of carbon tetrachloride only (Table 2).

(*c*) *NN*¹-*diphenyl-p-phenylenediamine* (DPPD). Three intraperitoneal injections of 100 mgm. DPPD

Table 1. Effect of α-Tocopherol in the Prevention of Carbon Tetrachloride Poisoning

No. of rats	Treatment with α-tocopherol: Time (hr. before or after carbon tetrachloride)	Treatment with α-tocopherol: Dose (I.U.)	Carbon tetrachloride (ml./kgm.)	Deaths: No.	Deaths: Per cent
43	—	—	4·0	42	98
15	−40	95	4·0	0	0
10	−24, −6	95	4·0	6	60
15	0	95	4·0	12	80
15	0, +5	95	4·0	13	87
18	−72, −48, −24, −6	19	4·0	13	72

Table 2. Effect of Sodium Selenite in the Prevention of Carbon Tetrachloride Poisoning

Group	No. of rats	Treatment with sodium selenite: Time (hr. before carbon tetrachloride)	Treatment with sodium selenite: Dose (mgm.)	Carbon tetrachloride (ml./kgm.)	Deaths: No.	Deaths: Per cent
1	18	—	—	2·7	16	89
2	18	72	0·5			
		48	0·25			
		24	0·25			
		6	0·25	2·7	10	51
3	18	72	0·5			
		48	0·25			
		24	0·25			
		6	0·25	—	0	0

Table 3. Effect of NN^1-Diphenyl-p-phenylenediamine in the Prevention of Carbon Tetrachloride Poisoning

No. of rats	Treatment with DPPD: Time (hr. before carbon tetrachloride)	Treatment with DPPD: Dose (mgm.)	Carbon tetrachloride (ml./kgm.)	Deaths: No.	Deaths: Per cent
32	—	—	4·0	32	100
19	48, 24, 0	100	4·0	0	0
11	48	100	4·0	6	55
17	0	100	4·0	10	59
12	48, 24, 0	50	4·0	8	67
11	48, 24, 0	10	4·0	7	64

in 0·4 ml. arachis oil at 48, 24 and 0 hr. before giving 4 ml. carbon tetrachloride/kgm. protected all of 19 rats against death (Table 3). One dose of 100 mgm. DPPD either 48 hr. before or with carbon tetrachloride reduced the death-rate to 55 and 59 per cent respectively of 11 and 17 rats, as compared with 100 per cent of 32 rats given carbon tetrachloride only. Courses of 3 injections of 50 or 10 mgm. DPPD during the 48 hr. prior to administering carbon tetrachloride also reduced the death-rate.

The photomicrographs illustrate the protective effect of DPPD against histologically observable damage to the liver by carbon tetrachloride. Fig. 1 shows a section of the liver of a rat killed 24 hr. after having received 4 ml. carbon tetrachloride/kgm. The liver is typical for rats with severe acute carbon tetrachloride poisoning, showing centrilobular necrosis and fatty change. This contrasts with the virtually normal appearance of the liver section in Fig. 2, which is typical for rats killed 24 hr. after the same dose of carbon tetrachloride but which have been given 3 intraperitoneal injections of 100 mgm. DPPD during the 48 hr. before administration of carbon tetrachloride.

It seems likely that the antioxidants protect rats against the lethal effects of large doses of carbon tetrachloride by acting on a general permeability mechanism, and thus prevent the loss of mitochondrial and cellular pyridine nucleotides which would otherwise result from the physical damage to the cell produced by carbon tetrachloride.

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HÆMATOLOGY

Thromboplastic Activity of Red Cells

The problem concerning the relationship of the thromboplastic activity of red cells to that of platelets which Inglis and Halliday[1] have recently studied has, in part, been answered by the work of my associates and me.

It was repeatedly noted that in very mild hæmophilia the prothrombin consumption carried out by my original method was often normal when whole blood was clotted, but not when the platelet-rich plasma was clotted under otherwise identical conditions[2]. This observation led to an investigation of the clotting factor in red cells and its probable role in the generation of thromboplastin. It was found that the activity of hæmolysed erythrocytes (hæmolysate) is similar to that of platelets but quantitatively much greater[3,4]. It is significant that, when a concentrated extract of platelets is added to normal native platelet-poor plasma that has been in contact only with silicone-coated surfaces, the consumption of prothrombin is too small to be determined; whereas, when the plasma is first brought in contact with a glass surface or treated with a minute amount of thrombin, the addition of a platelet extract brings about good consumption of prothrombin[5]. In marked contrast, the addition of an extract of red cells results in a high consumption of prothrombin even when added to native plasma that has been exposed only to silicone-coated surfaces[6].

These findings suggest that platelets can enter the coagulation reaction only after a plasma factor has been activated. Because this activation can occur by exposure to glass, the agent has been designated 'contact factor'. It appears that a platelet constituent interacts with the activated contact factor and that the resultant product is either very similar to, or identical with, the clotting agent found in the red cell. The latter factor, which appears to be a phospholipid[7], does not require contact factor to enter into the clotting reaction. From recent work it appears that some of the clotting agent formed from the platelets during clotting is removed by the red cells[8].

Whether the clotting activity of the intact red cell, which is feeble, has physiological significance remains unanswered. The alternative view that the red cell may be constantly removing this potent agent, which perhaps is being produced continuously, also deserves recognition and consideration.

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We freely acknowledge the priority of Prof. Quick in discovering and reporting the platelet-like thromboplastic activity of red-cell hæmolysate. No reference was made to his work, as it was our opinion that it would be well known to all workers in this field.

The activity reported by us is, however, manifested by non-hæmolysed cells. According to previous reports, non-hæmolysed red cells are capable of exerting only feeble platelet-like activity. Prof. Quick concurs with this. Our work, however, has shown that this is true only in regard to the initial stages of coagulation, but that after the formation of fibrin becomes evident red cells which are still intact develop much greater activity. We have not, as yet, investigated whether this is due to the envelope of the red cell being attacked by thrombin. The activity may be due to the same element in the red cell as is responsible for the platelet-like activity of red cell hæmolysate; but if so, this element can act while still attached to an intact cell, provided that its activity is released by coagulation initiated in some other way This activity is not manifested in the prothrombin consumption test described by Prof Quick because the clot is separated by centrifugation, which also effectively removes the red cells That centrifugation has this effect also demonstrates that the activity observed is not due to red cells which have been hæmolysed Some of our recent observations tend to show that, prior to the red cell developing this activity, it has a contrary inhibitory effect This is in agreement with findings reported by Prof Quick

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P_1 Antigen in the Human Fœtus

It is well established that in the human fœtus and new-born the P_1 antigen is incompletely expressed[1,2] In the course of testing blood from 403 fœtuses between the ages of 12 and 28 weeks we have confirmed that the incidence of P_1 is below the adult-level, however, it was found to be considerably higher in young than in old fœtuses Moreover, in the early fœtuses the strength of the agglutination also tended to be greater

Table 1

Crown-rump length	P_1^+	P_1^-	Total	Percentage P_1^+
Less than 10 cm	50	36	86	58 14
More than 10 cm.	67	250	317	21 14
Adults (ref 3)	1,984	641	2,625	75 58

Taking a dividing line at 10 cm it can be seen from Table 1 that the distinction between these two groups of fœtuses is highly significant

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IMMUNOLOGY

Selective Secretion of Circulating Antibodies in the Milk of the Rat

PASSIVE immunity is transmitted from mother to young in the rat, as in the mouse[1], partly before birth, but chiefly after birth by way of the milk and the gut of the suckling. Postnatal transmission continues until the twentieth day of age[2], when the capacity of the gut of the young rat to absorb antibody terminates although antibody continues to be secreted in the milk The lactating female rat was chosen for investigating the transmission of antibody from the circulation to the milk.

Table 1 CORRELATION TABLES OF MILK WHEY AND SERUM TITRES OF RATS AFTER PRIMARY (*A*) AND SECONDARY (*B*) IMMUNIZATION

A

Milk whey titre

1/1	10	20	40	80	160	320	640	1 280	2,560
1/1,280				1	4				
640					1	3			
320				6	7	5			
160				9	10	3	3		
80		2	9	2	6	5	3		
40		4	5	6	2	1	1		
20			1	2	1	1			
10			1		1				

Serum titre

B

Milk whey titre

1/1	10	20	40	80	160	320	640	1,280	2,560
1/1,280									
640									
320						1	2		
160					1	10	6	2	2
80					4	13	2		
40			1	3	9	5			
20		1	4	10	1	1			
10		6	2	1					

Serum titre

Female rats were actively immunized within a day or two of the first littering with *Brucella abortus* Two injections, one of 0 5 and another of 0 75 ml of the World Health Organization standardized *Brucella abortus* agglutination concentrate, washed and resuspended in a five-fold dilution in saline, were given two days apart subcutaneously in the neck region Paired samples of blood and milk were collected daily, or on alternate days, and the serum and milk whey titrated in parallel. The same procedure was repeated at the termination of subsequent pregnancies of these same animals

Antibody appeared in the serum five days after the primary immunization and by the ninth day had attained titres of 1/160–1/640 Thereafter the titres declined gradually to 1/40–1/80 by the end of the first lactation and were at or near this level at the time of each subsequent immunization The secondary response was more variable the majority of animals had attained comparable serum titres of 1/160–1/320, while a few animals showed titres of 1/640–1/2.560, by the seventh day

The appearance of antibody in the milk was strikingly different after primary, as compared with subsequent, immunizations After primary immunization antibody appeared in the milk one or two days later than in the serum, and by the tenth day reached maximum titres equalling or exceeding those in the serum Thereafter these declined more rapidly than in the serum After secondary immunization the milk titres did not rise appreciably, as did the serum titres, and remained nearly constant throughout lactation, seldom equalling and never exceeding the serum titres This difference in the ratio of milk to serum titres is shown clearly by comparing the data in *A* with *B* in Table 1 Evidently the antibody formed in response to a primary immunization appears in the milk much more readily than the antibody formed afterwards

It has been known since the pioneer work of Ehrlich[1] on mice that circulating antibodies, whether actively or passively acquired, do appear in the milk If it were assumed that the antibody was derived exclusively from that in the circulation, as seems probable, then that which was produced in response to primary immunization was transmitted more readily than that synthesized later This interpretation involves a selective transmission to the milk of certain molecular types of antibody in preference to others The only alternative interpretation would require the assumption of local production and direct secretion into the milk of antibody in response to the primary subcutaneous injection of antigen in the neck region, for which there is no evidence.

Selective transmission of antibodies and of labelled globulin has been demonstrated repeatedly in recent years across the fœtal membranes and is known to occur in the guinea pig[3], rabbit[4], monkey[5] and man[6] It occurs across the neonatal gut-wall in the mouse[7], rat[8] and hedgehog[9], though it has not been observed in the ruminants, horse or pig It occurs also across the yolk-sac wall of the chick[10] Hitherto, so far as we are aware, it has been recognized only in transmission across the fœtal membranes and the neonatal gut, so that this is the first instance recognized in an adult and in a glandular secretion

It will be a matter of considerable interest to determine whether the relative rates of transmission of different antibodies from the circulation to the milk of the mother rat correspond to, or are independent of, those across the gut wall of the suckling rat, or whether these two membranes, across which the antibodies must pass in transit from the maternal to the neonatal circulation, exercise selection on a similar or dissimilar basis

I am much indebted to Prof F W Rogers Brambell for helpful advice and encouragement in this work and Mr W A Hemmings and Dr R. Halliday for their interest, and to Dr A W Stableforth and Mr W T Rowlands, of the Animal Health Division of the Ministry of Agriculture, Fisheries and Food for supplies of antigen

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PATHOLOGY

5′-Adenylic Acid Deaminase in Dystrophic Mouse Muscle

THE discovery[1], in 1955, of a hereditary myopathy in a strain of mice has provided a useful subject for the investigation of muscular dystrophy Although the ultimate cause of the mouse disease may not be identical with the cause of any of the human muscular dystrophies, it is a reasonable assumption that many of the secondary changes will be similar A number of workers have reported quantitative enzyme changes in the dystrophic mouse muscle Some activities, for example, cathepsins[2] and glucose-6-phosphate dehydrogenase[3], show an increase, others, for example, lactic dehydrogenase[4] and phosphorylase[5], are decreased

5′-Adenylic acid deaminase, which converts adenosine-5′-phosphate into inosine-5′-phosphate (apparently irreversibly) is known to be present in much higher concentration in skeletal muscle than in any other tissue Its physiological function is, however, uncertain The experiments reported here show a sharp decrease in the activity of this enzyme in dystrophic mouse muscle

Four pairs of female mice, of the Bar Harbor strain, 3–5 months old, were used The animals of each pair were litter-mates, the 'normal' mice were heterozygous with respect to the recessive gene responsible for the disease Muscle from the hind limbs was chopped finely and homogenized for 3 min with about 5 volumes of ice-cold distilled water in a Potter–Elvehjem homogenizer rotating at high speed The homogenate was kept in ice for about 30 min , and diluted (usually 1 · 50) with distilled water Suitable volumes were shaken gently at 37° C for 10 min in a medium (total volume, 1 ml) containing citric acid–sodium citrate buffer, *p*H 6 7 (0 1 *M*) and 5′-adenylic acid (sodium salt) (5 μmoles) Perchloric acid (1 ml , 10 per cent) was then added, after centrifuging the sample, the ammonia content of the supernatant was measured with Nessler's reagent Homogenates produced no measurable ammonia from adenosine under the same conditions

The reference base was 'non-collagen N', determined by standing samples of the homogenate overnight at 30° C with 0 1 *N* sodium hydroxide; they were then centrifuged and total nitrogen of the clear supernatant determined by a micro-Kjeldahl procedure (Such 'non-collagen N' represented on the average about 90 per cent of the total nitrogen of the tissue)

The results are shown in Table 1 It may be seen that the mean activity of the muscle from the affected animals was only about one-third that of the normals The activities bore no relation, in either group, to the age of the mice

Mixtures of homogenates from normal and dystrophic muscle were also tested. The combined activities were equal to the sum of the activities when tested separately, thus making it unlikely that

Table 1 5′-ADENYLIC DEAMINASE ACTIVITY IN SKELETAL MUSCLE FROM DYSTROPHIC AND NORMAL MICE

Ammonia produced (μmoles/mgm of non-collagen nitrogen)

	Normal	Dystrophic
	192	51 6
	127	60 8
	217	64 8
	206	54 6
mean	186	57 9

Muscle homogenates were incubated with 5′-adenylic acid as described the low activity of the dystrophic muscle is due to an inhibitory factor

The pH-activity curves in citrate buffer for normal and dystrophic muscle were similar, with a peak at 6 7 The relative activity of the two was approximately the same in succinate buffer (pH 6 4) as in citrate buffer, although the measured activities were about 25 per cent lower

This work was aided by research grants from the Muscular Dystrophy Group of Great Britain and from the Muscular Dystrophy Associations of America, Inc

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Identification of Antibody in β_2-Globulins of Mouse Serum and Ascitic Fluid

MICE given intraperitoneal injections of antigen–adjuvant mixture[1-3] produce large amounts of ascitic fluid which contains specific antibodies Inbred *AKR* mice injected intraperitoneally with isologous tumour cell fragments in modified Freund's adjuvant developed large amounts of peritoneal fluid which contained specific cytotoxic and neutralizing antibodies[2,4] These observations had immediate practical importance since immunological studies have been limited by the small amount of antiserum obtainable from each mouse Since little or no information on the biophysical properties of mouse ascitic fluid is available, an investigation was made of its electrophoretic and immunological characteristics

Female *AKR* mice were injected intraperitoneally with complete Freund's adjuvant, incomplete Freund's adjuvant from which *Mycobacterium butyricum* was omitted, or complete Freund's adjuvant containing isologous lymphoma cell fragments Female *CF*1 mice were injected with crystalline bovine serum albumin in complete Freund's adjuvant Complete Freund's adjuvant (85 parts Drakeol mineral oil, 15 parts 'Arlacel A', 1 mgm of *Mycobacterium butyricum*/ml) was modified so that the aqueous phase was due only to the water contained in the packed lymphoma cell fragments or crystalline bovine serum albumin Antigen–adjuvant mixtures with little or no aqueous phase were more effective in causing ascites formation than conventional water-in-oil emulsions Animals received three 0 25 ml injections one week apart Approximately 2 weeks after the last injection ascitic fluid was obtained by paracentesis and blood by cardiac puncture Serum and ascitic fluid were clarified by centrifugation Moving-boundary electrophoretic analyses were made in a Spinco model *H* instrument (barbital buffer pH 8 6, $\mu = 0{\cdot}1$) The component containing antibody was identified by the boundary disturbance test[5] In this procedure antigen is added to the buffer in the electrophoretic cell Combination of antigen with antibody in a specific component forms a precipitate which interrupts the light and produces a clear area in the electrophoretic pattern Ascitic fluid was fractionated by starch block electrophoresis (barbital buffer pH 8 6, μ = 0 075), and the fractions tested for precipitins by the interfacial ring test

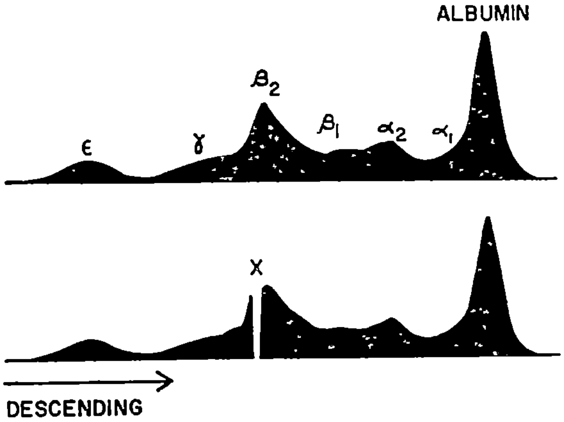

Fig 2 Electrophoretic boundary disturbance patterns of ascitic fluid from *CF*1 mice immunized with bovine serum albumin Top, control, bottom, 1 μgm bovine serum albumin/ml buffer

Normal *AKR* serum (Fig 1*A*) consisted of seven discernible components conventionally designated as γ-, β_2-, β_1-, α_2-, α_1-globulin, albumin, and a prealbumin component, ρ The concentration boundary is designated as ε Comparison of the electrophoretic patterns and analysis of the relative percentage of each component were made Serum pools and ascitic fluid pools from *AKR* mice injected with complete Freund's adjuvant (Figs 1*B*, 1*D*) or with complete Freund's adjuvant with lymphoma cell fragments (Figs 1*C*, 1*E*) had increased relative percentages of α_2- and β-globulins compared with normal serum The α_2-globulins were in greater concentration in the sera of immunized animals (Figs 1*B*, 1*C*) than in corresponding ascitic fluids (Figs 1*D*, 1*E*) The prealbumin component present in all serum pools was not detectable in ascitic fluid

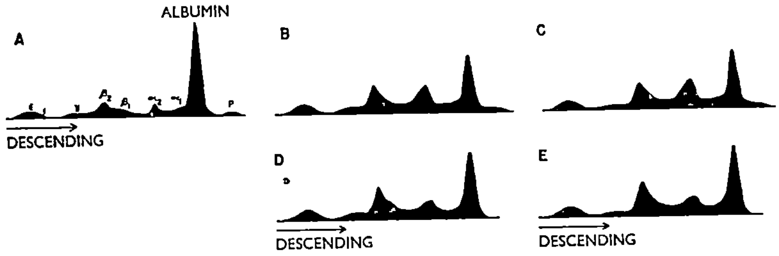

Fig 1 Electrophoretic patterns of *AKR* mouse sera and ascitic fluids *A*, Normal *AKR* mouse serum, *B*, serum from mice injected with complete Freund's adjuvant, *C*, serum from mice injected with lymphoma cell fragments in complete Freund's adjuvant *D*, ascitic fluid from mice injected with complete Freund's adjuvant, *E*, ascitic fluid from mice injected with lymphoma cell fragments in complete Freund's adjuvant

Animals injected with incomplete Freund's adjuvant did not produce ascites The *Mycobacterium butyricum* component of the adjuvant mixture was necessary for the induction of ascites by the immunization procedure described

Ascitic fluid from *CF*1 mice contained anti-bovine serum albumin precipitins A well-defined boundary disturbance (*X* in Fig 2) was produced in the β_2-globulin component when ascitic fluid (475 µgm. anti-bovine serum albumin protein/ml) was treated with 1 µgm of bovine serum albumin/ml. buffer Eluates obtained from starch-block electrophoresis of ascitic fluid contained anti-bovine serum albumin precipitins in fractions corresponding to β-globulins The *CF*1 serum was also shown to contain anti-bovine serum albumin precipitins in the β_2-globulin region by the boundary disturbance method

Serum and ascitic fluid from *AKR* mice had a similar distribution of electrophoretic components with one major difference Ascitic fluid did not contain prealbumin Relative concentrations of α_2- and β-globulins were greater in serum and ascitic fluid from immunized animals than in serum from normal mice, however, there was very little change in the relative concentration of γ-globulin This raised the question as to which component contained the antibody Boundary disturbance experiments showed that the anti-bovine serum albumin precipitins of mouse ascitic fluid and serum were contained in the β_2-globulin Prolonged immunization of the mouse might result in localization of antibody in a serum component other than β_2-globulin

Mouse serum contains a prealbumin component not detected in ascitic fluid The relative concentration of α_2- and β-globulins was greater in the serum and ascitic fluid of immunized animals than in the serum of normal mice, whereas γ-globulin remained relatively unchanged Anti-bovine serum albumin precipitins have been demonstrated in the serum and ascitic fluid of immunized mice and have the electrophoretic properties of a β_2-globulin

This work was supported by research grant *CY*-5188 from the National Institutes of Health, U S. Public Health Service

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Nature of Increased Protein and DNA in Chronic Hepatic Injury

Excess protein and deoxyribonucleic acid (DNA) are found in chronic hepatic injury, for example, after administration of ethionine[1-3], butter yellow[4,5], 2 acetyl aminofluorene[6,7], and a high fat–low choline–low protein diet[1] This suggested that the excess protein or DNA is an abnormal product of the hepatocytes[1-3] This is supported by the incorporation of ethionine into hepatic proteins[2], presumably the abnormal proteins These proteins, or DNA, have been related to cancer[1,7] This communication is to point out that the excess protein formation results from alterations in cell population, rather than from formation of abnormal proteins

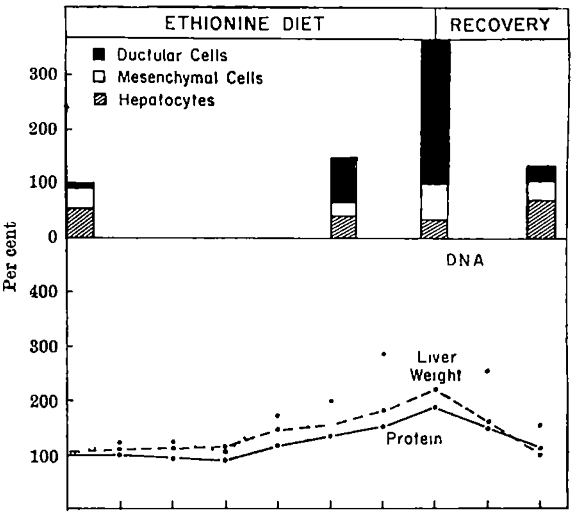

Fig 1 Changes in liver weight, hepatic protein and DNA content, and cell population during ethionine intoxication

A group of 42 rats was fed a synthetic diet supplemented by 0 5 per cent ethionine, with groups killed weekly up to seven weeks An additional 12 rats were similarly treated and then placed on the synthetic diet supplemented by 1 per cent methionine They were killed after one week or two weeks Twenty rats fed the synthetic diet alone were controls

From the third to seventh week the liver enlarged and its total protein content nearly doubled (Fig 1), with collagen (determined as hydroxyproline) accounting for only 5 per cent of the increment The total DNA content increased almost five-fold, while the RNA/DNA ratio decreased from 4 to 1 8 While in the control liver the ductular cells accounted (on the basis of differential cell counts) for only a small fraction of the population, with a ductular cell hepatocyte ratio of 1 10, after seven weeks on ethionine the ductular cells predominated, the above ratio being 7 1 Calculating the change in the total number of cells, the ductular cells increased about 50 times, while the number of hepatocytes decreased slightly (Fig 1) After two weeks of methionine supplementation, total protein and DNA reverted almost to control-levels, parallel with the disappearance of almost all ductular cells and a restoration of the cytological pattern of the liver After administration of 0 3 µc tritiated thymidine/gm body-weight at the seventh week of ethionine intoxication, incorporation of thymidine determined by scintillation counting was about 30 times the control (Table 1) Radioautographs demonstrated the label almost exclusively in ductular cells (Fig 2)

The rise in DNA is accounted for by the proliferation of bile ductules, qualitatively and quantitatively,

Table 1 Incorporation of Thymidine-^{3}H into Liver DNA, and the Distribution of Radioautographic Label in Different Cell Types in Control Rats and Rats Intoxicated with Ethionine

Diet	Total activity in DNA c /min	No of labelled cells per 100 high-power fields Hepatocytes	Mesenchymal cells	Ductules
Control	1,760	4	6	3
Ethionine (7 weeks)	47,250	12	18	200

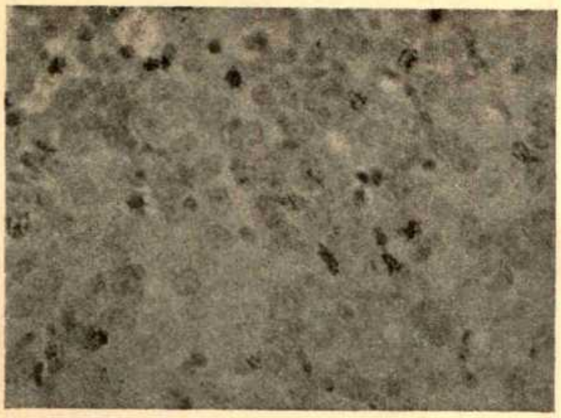

Fig. 2. Radioautograph of liver of rat fed ethionine for 7 weeks and then given tritiated thymidine. Labelled cells are all ductular cells. Hæmatoxylin. (× 120)

as shown by cell counts and autoradiography. Allometric analysis of the individual results for DNA and total protein shows a close relationship, indicating that the increase in liver weight and total protein are functions of proliferation of ductular cells. The smaller rate of increase in total protein and RNA than in DNA is explained by the much smaller cytoplasm to nucleus ratio of the ductular cell. In addition, the disappearance of excess protein and ductules during recovery run parallel. After a high-fat – low-protein diet, bile duct ligation and administration of α-naphthyl isothiocyanate and 2-acetyl aminofluorine, the same chemical and histological relationships hold true.

The excess protein and DNA are thus the structural protein of another cell type pre-existing in the normal liver rather than the accumulation of abnormal proteins as a reflexion of metabolic alterations of the hepatocytes. That the average cell size becomes smaller[5,6] is also explained by the proliferation of this new cell type. Radioautographs demonstrate that the previously observed increased uptake of thymidine-^{3}H (ref. 2) in ethionine intoxication is merely a reflexion of proliferation of ductular cells. No evidence exists that proliferation of ductular cells in the rat is related to carcinogenesis. The correlated approach applied emphasizes the value of morphological controls in the study of liver injury.

This work was supported by research grant *A*–3846 Path. from the U.S. Public Health Service and by contract *DA*–49–007–*MD*–790 from the U.S. Army Medical Research and Development Command.

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BIOLOGY

Translocated Heat Injury in Plants

TRANSLOCATED heat injury is common in burns of human beings[1] but is apparently not recorded in plants. When one of the two primary leaves of pinto bean (*Phaseolus vulgaris* L.) or cowpea (*Vigna sinensis* ((Torner) Savi) or the cotyledons of National Pickling cucumber (*Cucumis sativus* L.) was heated in water (5 sec. at 70° C. was suitable) and the opposite leaf abraded as in mechanical inoculation with viruses, the heated leaf was killed and the unheated leaf was severely injured. There was no apparent injury to the petioles and stem through which the injury stimulus passed. When the heated leaf was removed from the plant within 4 hr. after heating, the translocated injury to the opposite leaf was prevented, or recovery was favoured. Translocated injury still occurred if heating followed abrasion by as much as 12 hr., or if abrasion followed heating by as much as 16 hr., but comparable injury never occurred from abrasion or heat only. As the ratio of the area of the heated leaf to that of the unheated leaf was increased by removal of part of the leaf area, translocated injury was increased. The time of day to produce maximum translocated injury quickly was in the morning, but translocated injury could be produced at any time of day. Translocated injury was favoured by light and high temperatures in the environment. As the age of plants was increased from 12 to 23 days the degree of translocated injury was increased. The temperature coefficient for translocated heat injury was about 10. This translocated heat injury had various effects on infections which will be reported elsewhere. While no chemical responsible for translocated heat injury has been demonstrated, these results suggest a similarity between translocated heat injury in plants and animals.

This work was aided by a grant (*G* 9820) from the National Science Foundation.

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A Distinction between Bolting and Flowering Effects on Senescence

IT is well known that flowering and fruiting processes bring about the senescence of most annual species of plants. Molisch[1] interpreted this senescence as due to the mobilization of food materials into the fruits. His interpretation has been questioned by Leopold, Kamien and Janick[2], for staminate as well as unpollinated pistillate plants of spinach show senescence after flowering. Both staminate and pistillate spinach plants develop a large seed stalk with the commencement of reproductive development, and it may be that Molisch's interpretation could be made more correct by including the mobilization of food materials into the stalk as an explanation of the imposition of senescence. The application of gibberellin to spinach which has not experienced photoperiodic induction will result in the formation of a stalk without the further development of flowers, providing an opportunity for distinguishing between the effects of bolting and of flowering on senescence.

Spinach plants of the 'Spica' variety planted on November 14, 1960, were grown under natural winter day-length. On March 31, 1961, twenty plants were selected for uniformity and divided into four groups of five plants each. Two groups were placed under short days (8 hr.) and two groups under long days (17·5 hr.). Day-lengths were shortened with curtains or lengthened with about 100 ft.-candles of tungsten light. Within each photoperiodic regime, one group was sprayed with 1,000 p.p.m. of gibberellic acid with the other group acting as a control.

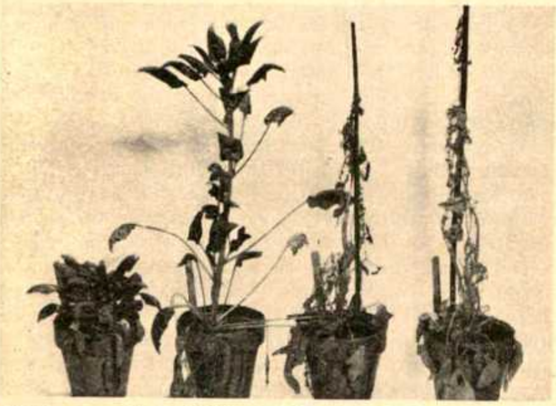

Fig. 1. Separation of bolting and flowering effects on senescence in spinach. Treatments from left to right: 1, short day; 2, short day + gibberellic acid; 3, long day; 4, long day + gibberellic acid. Photographed 49 days after treatment

Under conditions of long days, all plants bolted and flowered and afterwards showed senescence. The dates of death of plants treated with gibberellic acid and controls were essentially the same. Four of the ten plants were staminate and died in 33–51 days while the six pistillate plants died in 46–56 days. The final height of the plants treated with gibberellic acid was only slightly greater than the controls. Under the short-day treatment all plants were non-senescent at the end of 46 days, although the treated plants had elongated to an average height of 11 in. (Fig. 1). At this time two plants each from the gibberellic acid treatment and control were transferred from short- to long-day treatment. These flowered in two weeks and all showed normal senescence, the plants dying between 45 and 51 days from this date. Of the six remaining plants left under short days, four were abandoned as a result of insect infestation. The two remaining, one treated and one control, were non-senescent at the end of 74 days, a total of 120 days from the start of the original treatments, at which time the experiment was terminated.

The results demonstrate that the stimulation of an amount of stem growth in vegetative plants comparable with that produced in flowering spinach plants does not produce the stimulus for senescence which the bolting and flowering do. We suggest this as further evidence that the simple mobilization of materials is not the principal cause of senescence following reproduction, for the development of the small staminate inflorescences induces senescence whereas in contrast the growth of the massive central stem of plants treated with gibberellic acid had no detectable effect. For rapid growth of the stalk there must be a large mobilization of food substrates into the stalk and yet the death of the plant does not follow. It would appear that not all mobilizing forces within the plant induce senescence.

This work was supported by a grant from the Rockefeller Foundation.

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Biosynthesis of Gossypol by Excised Cotton Roots

GOSSYPOL, a toxic phenol, occurs primarily in the resin glands of cotton seed but also has been isolated from the tap-root bark of the cotton plant[1]. A study of the distribution of gossypol in the cotton plant revealed that the roots, especially the feeder roots, contained high levels of gossypol. This was true for strains of cotton in which the plants and seed were free of resin glands as well as for strains which contained many glands in plants and seed. The high levels of gossypol in the roots and the minute amounts in the seed of the glandless plants suggest that the gossypol was synthesized in the root tissue.

Since techniques for the growth of excised root tissue in sterile nutrient solutions have been developed[2,3] it seemed feasible to investigate the biosynthesis of gossypol by cotton root tissue. Excised root tips incubated in a sterile nutrient solution were used to determine if gossypol is synthesized by growing cotton root tissue. Dawson[4] found that excised roots of the tobacco plant synthesized nicotine.

The technique used for the culture of the excised root tissue was similar to that of Robbins with the tomato[3].

Cotton seed from both glanded and glandless strains were delinted with concentrated sulphuric acid and washed well with water saturated with calcium oxide. The seeds were sterilized by suspending them for 5 min. in a 0·5 per cent sodium hypochlorite solution ('Clorox' diluted 1 : 10). The treated seeds were washed three times with sterile distilled water and then transferred to sterile plates containing nutrient agar. The plates were incubated at about 30° C. until the roots had emerged from the seed to a length of 0·5–0·75 in.

The root tips were excised with a sterile razor blade and sampled randomly (Table 1, samples 1*a* and 2*a*) to determine the initial gossypol content. The remaining tips were transferred aseptically to 500-ml. Erlenmeyer flasks containing 150 ml. of sterile nutrient solution. The solution was identical, except for the inclusion of 0·05 p.p.m. of iodine, to that of Robbins[3], which contained the following in mμmoles per litre: thiamine 20, pyridoxine 200 and nicotinamide 200. The flasks were incubated for about 4 weeks. Some of the roots grew well, while others made little or no growth. At the end of the incubation period the roots were washed with water and, after separating the new growth (Table 1, 1*b* and 2*b*) from the original tips (Table 1, 1c and 2c) that were used to initiate the root culture, were dried. Both of these parts and the tips that were removed as control samples (Table 1, 1*a* and 2*a*) at the time of transfer into the nutrient solution were analysed separately for total gossypol content as I have already described[5].

Table 1. GOSSYPOL CONTENT OF EXCISED COTTON ROOTS GROWN IN NUTRIENT SOLUTIONS*

No.	Description	No. of cultures	Mean gossypol content (per cent)
	Glanded strain (1)		
1 *a*	Tips at time of transfer		0·080
1 *b*	New growth in nutrient solution	2	2·300
1 *c*	Incubated tips, new growth removed	2	6·320
	Glandless strains (2)		
2 *a*	Tips at time of transfer	2	0·051
2 *b*	New growth in nutrient solution	2	2·018
2 *c*	Incubated tips, new growth removed	2	1·361

* Values are expressed on an air-dried basis

The results of the analyses which are expressed on an air-dried basis are presented in Table 1. The concentration of the gossypol in the root tips (1*a* and 2*a*) at the time of transfer into the nutrient solution was low, although the level in the glanded strains was somewhat higher than that in the glandless strain. During incubation, however, the gossypol content of the original tips (1*c*, 2*c*) increased very markedly. This change was more apparent for the glanded strain, a 78-fold increase, than for the glandless strain. Moreover, the concentration of gossypol in the new root growth (1*b*, 2*b*) was many times higher than that observed in the original root tips at the time of transfer into the nutrient solution.

Thus these results clearly indicate that gossypol was synthesized by the excised root tissue. Furthermore a sample of root tips from the glandless strain that made no growth when incubated in the nutrient solution contained 2·315 per cent gossypol. This observation indicates that the root tips of the cotton plant have the enzyme systems for the production of gossypol even when they make no growth.

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Juvenile Stage in Cultivated Forms of *Brassica oleracea*

Stokes and Verkerk[1] showed that while chilling induces flowering in Brussels sprouts, there was a juvenile stage during which chilling was ineffective. This is reflected in the horticultural practice of overwintering small seedlings derived from an August sowing of Brussels sprouts, which then grow vegetatively for a whole season without flowering. A similar procedure is successfully followed in the cultivation of spring and some autumn and cattle cabbage, suggesting the existence of a juvenile stage in these also, as if the plants, as a result of a too early sowing, are too large (that is, beyond the juvenile stage); winter chilling induces premature flowering (bolting) in the following spring[2,3]. I have, from August sowings, successfully overwintered outside, seedlings of autumn and winter cabbage (3 cultivars), kale (cottagers, hearting, marrow stem and thousand-headed), kohl rabi (4 cultivars), sprouting broccoli (2 white and 2 purple cultivars) and heading winter cauliflower or broccoli (9 cultivars), as well as those of spring cabbage. With all these cultivated forms a high proportion have, when transplanted to permanent quarters either in the October or April following sowing, continued to grow vegetatively, whereas plants from a July sowing all flowered in the spring following. This experience suggests that in all these cultivated types a juvenile stage exists during which chilling in ineffective in inducing flowering.

This feature was even more marked in plants raised from seed of a wild, although perhaps not indigenous, cabbage collected from the Yorkshire cliffs. Plants of this, from a sowing made on July 7, did not flower in the following spring, whereas all the plants from a corresponding sowing of the cultivated forms enumerated above did flower.

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Kinetin-induced Meiotic-Prophase Acceleration and Stasis in *Tradescantia* Anthers cultured on Media Deficient in Sugar

THE ability of kinetin to accelerate mitotic division has been demonstrated for *Paramecium*[1], and an apparent effect of accelerating prophase has been inferred from onion root-tip work[2]. That the acceleration may be a direct consequence of a hormonal acceleration of discrete phases of cellular metabolism has been suggested[3,4]. The former workers found that synthesis of deoxyribonucleic acid (DNA) could be induced in tobacco pith tissue by kinetin; the latter found that it increased the activity of deoxyribonuclease and ribonuclease from bean hypocotyls. It is known that indole-3-acetic acid is also required for stimulation of division by kinetin and it has been shown that it is also required for the effect on nucleic acid metabolism[1-4]. In contrast with the stimulation of nuclear and cellular division, most workers have found that in higher concentrations kinetin causes abnormalities of division and eventually mitotic activity ceases.

We planned to determine whether acceleration could be detected in the prophase of meiosis in anthers of *Tradescantia paludosa*, in which the prophase stages investigated (from pachytene and diplotene onwards) are of sufficient duration to do so. The anthers were cultured on a modification of the basic medium used by Taylor[5] in which 4 per cent lactose was substituted for the equivalent of sucrose, to which kinetin at a concentration of 0·25 p.p.m. and/or different sugars were added. This medium provides semi-starvation conditions, in spite of which approximately two-thirds of the meiocytes in an anther proceed through both stages of the meiotic division with regular nuclear products when cultured at 27° C.

It was found that, among a group of fifteen anthers, excised at pachytene or very early diplotene, seven anthers treated with kinetin had advanced to diakinesis or beyond in 24 hr., and one was at late diplotene; six of the untreated anthers from the same buds were at early or mid-diplotene and one at diakinesis. In addition, in a test involving comparisons between the two lobes of an anther, one treated with kinetin, the other not, five of the treated lobes markedly outstripped the control, and only one showed apparent retardation. We conclude that kinetin accelerates prophase stages beyond pachytene. Since synthesis of DNA in the meiocytes of *Lilium longiflorum* is already completed before this stage[6] but synthesis of ribonucleic acid (RNA) reaches a peak during it, it is suggested that an enhancement of RNA synthesis, as indicated by observation of enhanced ribonuclease activity (ref. 4), may be responsible for this acceleration.

A large number of tests indicated that in anthers cultured for 48 hr. at mid-diplotene, kinetin caused a cessation of kinetic activity during the meiotic process that we have termed 'meiotic stasis' (Fig. 1). The observed pattern ('random stasis'), in which all the stages from diplotene to anaphase 2 have been detected, suggests that the phenomenon is caused by depletion of the cellular energy or substrate reserves, as a result of the accelerated development of prophase. Citrate, when added at a concentration of 83 p.p.m., markedly alleviated this nuclear stasis, and hence it appears probable that energy through the tricarboxylic acid cycle may be more critical than substrate.

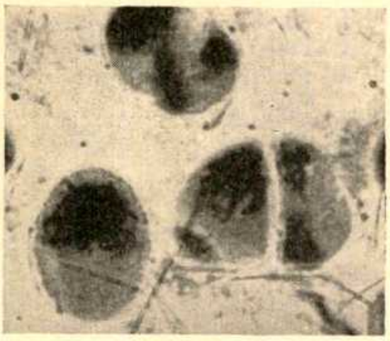

Fig. 1. Microsporocytes in stasis (see text) from a mid-diplotene anther cultured for 48 hr. sucrose-deficient medium containing kinetin (× 665)

In describing the inhibition of mitosis in onion root-tips induced by high concentrations of kinetin (1–5 p.p.m.) Guttman[2] mentions binucleate cells, massed prophases and small, dense cells with heavily stained nuclei. These observations coincide quite closely with our own observations of microsporocytes in kinetin-induced stasis. It appears probable that these abnormalities in mitosis also were a result of energy starvation brought about in the root-tips by nuclear acceleration induced by kinetin.

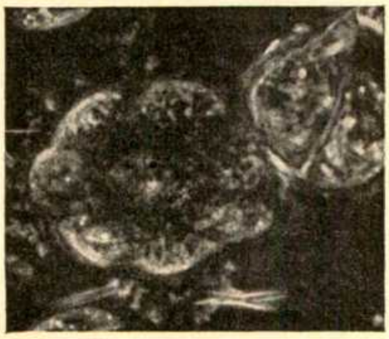

Fig. 2. 8-microspore 'tetrad' produced by an immediate division of the microspore nuclei, from a mid-diplotene anther cultured for 48 hr. on a sucrose-sufficient medium containing kinetin (× 665)

When citrate or sucrose was supplied, mid-diplotene meiocytes in kinetin-treated anthers frequently underwent three successive nuclear divisions within 48 hr. (Fig. 2). In contrast, under natural conditions on the plant, a period of 7 days elapses between the completion of the second meiotic division and the first mitotic division of the microspore[7]. Hence the action of kinetin in this instance apparently involves an elimination of almost the whole of the long intervening metabolic period in the microspores. This would suggest that it controls at least the conditions for the replication of DNA, since in *Tradescantia* microspores the replication occurs at the end of the interphase[8]. This evidence therefore supports that of Patan *et al.*[3] from mitotic work with tobacco pith tissue that the induction of DNA synthesis in the interphase nucleus is a significant feature of kinetin activity.

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Plankton on the North Kenya Banks

During the investigations of the East African Marine Fisheries Research Organization into the resources of part of the coast of East Africa a hitherto uncharted shallow area was located off the north Kenya coast in the vicinity of Lamu[1]. Subsequent investigations have indicated that this area, designated the North Kenya Banks, has a good fish population and is of importance as a potential fishery[2,3].

Due to lack of specialists it has been difficult to make an extended and continuous investigation of the various facets of this marine environment which, together, result in the production of a good fishery. However, during plankton investigations along the East African coast, facts were discovered which substantiate the claim that the North Kenya Banks is a fertile area and potentially a good fishery.

Part of the plankton investigations constituted taking a series of lines of samples from the coast (c. 100 fm., or 50 fm. when the fringing reef permitted) outwards for some 40 or 50 miles. These lines of stations were worked from Cape Delgado, near the southern Tanganyika border, to Dick's Head, near the northern Kenya border; the outward lines from the coast were at approximately 30–50-mile intervals. The plankton net used was a Currie and Foxton 70-cm. net[4] without the meter. A filtration coefficient of 1 : 1 has been assumed (reasonable in the light of former experience), in view of the short duration of the haul, the speed of hauling (c. 1 m./sec.) and the evident lack of clogging. All were vertical samples from, usually, both 200 m. and 50 m. to the surface at each station. In shallower water the samples were from the bottom to the surface. All samples were duplicated, the first being for numerical and specific analysis, the second for dry weights. These latter samples were dried to a constant weight at 50° C. in a controlled oven. The resulting weights were applied to the height of the water column from which they were taken, the final result being expressed as mgm./m.^{3} of sea-water.

Full analysis of the results is not yet complete, but the preliminary results are of much interest. Towards Cape Delgado the plankton dry weight is about 8–12 mgm./m.^{3}, with no great variation in off-shore samples. Farther north in the Mafia area the coastal water is rather richer with *c.* 20–30 mgm./m.^{3}, decreasing to *c.* 8–12 mgm./m.^{3} in off-shore waters. This trend towards a relatively enriched coastal water increases to the north, and in the Dick's Head area in-shore figures are *c.* 30–40 mgm./m.^{3}, the off-shore figures being *c.* 10–15 mgm./m.^{3}.

A preliminary Survey Report of the 1960 cruise of the Russian research vessel *Vitiaz*, for the International Indian Ocean Expedition, shows the contouring of the plankton dry weight in the top-most 100 m. of the Indian Ocean. It agrees in showing that water with a low plankton dry weight (7·5 mgm./m.^{3}) is much nearer the coast-line in the Cape Delgado area than farther north in the Dick's Head area.

The stations worked over the North Kenya Banks showed the highest figures. Thus, among others, plankton dry weights of 65·1, 55·0 and 62·8 mgm./m.^{3} were recorded at the bottom end of the range, with the largest reading being 161·6 mgm./m.^{3}. This sample was taken at a depth of 108 m., the position being 2° 25·5′ S. 40° 54′ E.

These readings show that the water over the North Kenya Banks is demonstrably richer than the remainder of the Tanganyika–Kenya coast. Further readings will have to be taken before it can be stated confidently that this applies throughout the year, although there is reason to believe that this is, in fact, the case.

It is worth noting that the colour of the sea over these Banks was the 'bottle-green' associated with such temperate water as the North Sea, and neither the clear blue of the off-shore East African waters nor the dirtier blue or pale greens of the in-shore waters was in evidence.

Of interest is the consideration of the plankton dry weight underneath one acre of water over the North Kenya Banks. Assuming a mean depth of 80 m. and a plankton dry weight of 100 mgm./m.^{3} the resulting figure is 32·27 kgm. plankton dry weight beneath 1 acre of water.

Analysis of the preserved samples has not yet been completed, but random examinations give every indication of a richer population of fish eggs and larvæ compared with the rest of the coast. This poses the problem, to be investigated later, of whether the North Kenya Banks are a breeding ground, with the fish converging from outlying waters to breed, or whether the North Kenya Banks are mainly a feeding ground, the breeding being resultant and dependent on this.

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CYTOLOGY

Increase in the Dense Cytoplasmic Bodies in Radiation-induced Giant Cells of the Cultured Fibroblast

The formation of giant cells after the irradiation of cell cultures was described by Puck and Marcus[1] in HeLa cells. The phenomenon also occurs in cultured fibroblasts. After a dose of 1,000 r., a high percentage of the cells in the cultures cease to undergo mitosis but continue to grow in bulk, forming characteristic giant cells which survive for about two weeks under normal culture conditions. Dickson, Paul and Davidson[2] have shown that the giant cells contain twice the deoxyribonucleic acid, ribonucleic acid, protein and lipid of the normal cell and that the synthesis of these compounds appears to be normal. The primary radiation effect, therefore, would seem to be on the mechanics of mitosis.

The non-irradiated, mouse subcutaneous fibroblast (strain *L* (ref. 3)) is roughly circular in thin sections under the electron microscope, with a single nucleus and cytoplasm containing typical mitochondria, sparse granular endoplasmic reticulum, a diffuse Golgi apparatus, numerous vesicles and 'dense cytoplasmic bodies'. The last-named are a characteristic feature of these and other cultured cells[4]. They are circular bodies, approximately 0·5 mμ in diameter, with a single outer membrane and opaque ground substance, and occur three or four to a cell in the thin sections. In a few cases the outer membrane is duplicated for part of its length, or even laminated. Rarely, a myeloid body is seen. Other structures are present, the ground substance of which is intermediate in density between that of the dense bodies and that of the cytoplasmic vesicles.

Pinocytosis can be demonstrated easily in these cells by growing them in a medium containing 2 per cent 'Thorotrast' ('Testagar'). After an hour, granules of thorium dioxide are present throughout the cytoplasm on the inner surface of membrane-bound vesicles. A few of the dense bodies also contain one or two granules at this stage. If cells treated with the 'Thorotrast' are returned to a normal medium for a further 24 hr., all the intracellular granules are segregated within the dense bodies which, therefore, correspond to the residual bodies of Palade[5].

In the giant cells developed from the fibroblasts after irradiation, the nucleus is large and convoluted so that thin sections often give an impression, seldom confirmed on light microscopy, of multi-nucleation. Individually the cytoplasmic structures are identical with those of the non-irradiated cells, though the Golgi apparatus may appear more diffuse. The striking feature of these cells, however, is the increase in the number of the dense bodies, which occur in groups of 12–15 in each cell in the thin sections. As in the normal cell, they are approximately 0·5 mμ in diameter, with a smooth outer membrane and opaque ground substance. Their internal structure is more variable, most have some lamellations and myeloid bodies are fairly common. After 'Thorotrast' treatment for 1 hr., the dense bodies of the giant cells, in contrast to those of the normal cells, show a considerable concentration of granules (Fig. 1). 'Thorotrast'-treated giant cells, returned to a normal medium for a further 24 hr. show the substance to be entirely localized in the dense bodies.

It is concluded that, as a result of continuous pinocytosis, when cell division in cultured fibroblasts

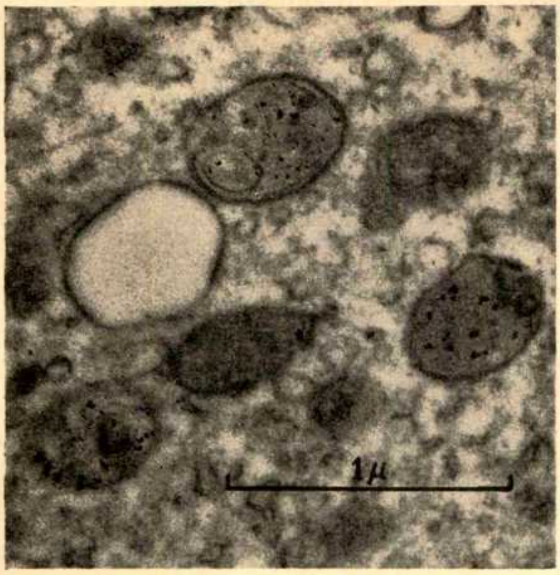

Fig. 1. Dense cytoplasmic bodies in an irradiation giant cell after 1 hr. in a medium containing 'Thorotrast'. The structures have internal membranes and contain granules of thorium dioxide

is prevented by radiation, the dense cytoplasmic bodies increase in number. The build-up of these structures, possibly with a high enzyme content, may be a factor in the death of these cells. The greater concentration of 'Thorotrast' in the dense bodies of the giant cells suggests that the rate of pinocytosis is greater than in the normal cells. Such a change could be correlated with the increased volume of the cell and the relatively decreased surface area available for the diffusion of nutrients.

I am grateful to Dr. J. Paul, of the HERT Tissue Culture Laboratory in the Department of Biochemistry, University of Glasgow, and to Miss M. Struthers, for technical assistance with the cultures.

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MICROBIOLOGY

Incorporation of Molecular Oxygen during Microbial Utilization of Olefins

THE utilization of various alkanes by certain bacteria involves the incorporation of molecular oxygen into the conversion products[1] and into the bacterial cells[2]. The attack of the olefin 1-hexadecene at the saturated end of the molecule[3] probably is not different from that of the alkanes. Instances where the oxidative attack occurs at the double bond in olefins[3,4] have now been examined with the use of oxygen-18. Also, the cells of an ethylene-utilizing bacterium were analysed similarly.

The yeast *Candida lipolytica* A.T.C.C. 8661 was incubated at 30° C. for 4 days with continuous shaking in a closed 4-litre flask containing 800 ml. of the mineral salts solution of Bruyn[4], supplemented with 30 ml. of 1-hexadecene or 1-octadecene. The gas phase contained 80 per cent of labelled oxygen. The culture was extracted with diethyl ether. Chloroform was added to dissolve the diol crystals in the extracted unutilized olefin and the diols were purified by column chromatography[5]. The diols were recrystallized twice from *n*-hexane and dried *in vacuo* over phosphorus pentoxide (Table 1).

Table 1

	Hexadecane-1,2-diol	Octadecane-1,2-diol
Substrate	1-hexadecene	1-octadecene
Yield of recrystallized product	32·5 mgm.	70·6 mgm.
Melting point	85° C.	86·5° C.
Bromine test for double bond (ref. 6)	Negative	Negative
Periodate test for diol (ref. 7)	Positive	Positive
Purity, by periodate titration (ref. 8)	97·2 per cent	96·0 per cent
Analysis, found	C, 73·91 per cent; H, 12·75 per cent	75·12 per cent; H, 12·91 per cent
theory	C, 74·4 per cent; H, 13·2 per cent	75·4 per cent; H, 13·2 per cent
Formaldehyde produced by periodate oxidation (ref. 9)	Positive	Positive

The *Candida* cells from the octadecene culture were also analysed for oxygen-18. Cells of the 'ethylene bacterium' were recovered from mineral-salts medium in an atmosphere containing 10 per cent ethylene and 30 per cent of labelled oxygen. Cells of this bacterium were also obtained under comparable conditions with 1 per cent sodium acetate instead of the ethylene.

Isotopic composition of the products was determined by established procedures[10,11] (Table 2).

Table 2

Product	Substrate	Atom percentage excess ^{18}O		Percentage of oxygen in product derived from O
		O_2	Product	
Hexadecane-1,2-diol	1-Hexadecene	2·66	1·50	56·4
Octadecane-1,2-diol	1-Octadecene	5·47	1·69	31·0
Octadecane-1,2-diol	1-Octadecene	5·50	1·62	29·6*
Octadecane-1,2-diol	1-Octadecene	5·50	1·99	36·2*
Candida lipolytica cells	1-Octadecene	5·50	0·057	1·04
'Ethylene bacterium' cells	Ethylene	5·02	0·194	3·87*
'Ethylene bacterium' cells	Ethylene	5·02	0·164	3·27*
'Ethylene bacterium' cells	Ethylene	4·62	0·075	1·62
'Ethylene bacterium' cells	Sodium acetate	5·36	0·006	0·11

* Duplicate flasks in the same experiment. The orange-yellow pigmented ethylene bacterium was isolated from soil. It is a Gram-positive, aerobic, motile, non-sporulating rod. The minimal purity of the ethylene used in these experiments was 99 mole per cent.

Although a large fraction of the diol oxygen was derived from molecular oxygen, the results do not show whether one or both oxygen atoms of the diols came from the atmosphere. Assuming that the oxygen in any one position in the diol molecule was introduced by only one mechanism, the minimal theoretical value for incorporation is 50 per cent. The decidedly lower values for octadecane-1,2-diol in all probability indicate that an exchange had taken place whereby the oxygen-18 originally incorporated was partially replaced by oxygen-16 derived from water. The value of 56·4 per cent in the case of hexadecane-1,2-diol suggests that both oxygen atoms were derived initially from the atmosphere, but better evidence is required.

The cells of *Candida lipolytica* were also enriched in oxygen-18, as expected[2] from the assimilation of labelled intermediate diol. The 15- to 35-fold enrichment in oxygen-18 of the bacterial cells grown on ethylene over those grown on sodium acetate is indicative of an incorporation of molecular oxygen in the primary attack on the ethylene. By analogy, these results may be taken to imply, but not prove, that oxidation of ethylene by this bacterium takes place via ethylene glycol formed by the incorporation of molecular oxygen. Ethylene glycol was shown to serve as a sole source of carbon and energy for the 'ethylene bacterium'.

This work was supported in part by the U.S. National Science Foundation, the U.S. National Institutes of Health and the U.S. Office of Naval Research.

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Antigenic Structure of *Entamœba histolytica*

IMMUNOLOGICAL investigations on the specific antigens of *Entamœba histolytica* are complicated by the presence of concomitant bacterial flora in the culture. The precipitin reaction in cases of clinical amœbiasis has been investigated[1,2]. Menendez[3] reported the presence of precipitin antibodies in the serum of rabbit immunized with cultures of *Entamœba histolytica*. In the present work the precipitating antigens of these parasites were analysed by agar-gel diffusion and intragel absorption techniques.

Entamœba histolytica, strain *E.H.* 1, was used in this work. The associated bacterial flora in the culture were *Escherichia coli* and *Pseudomonas pyocyaneus*. *Ent. histolytica* grown in Row's hæmoglobin media at 37° C. for 48 hr. was treated with dihydrostreptomycin sulphate to kill the bacteria. In each test, materials from thirty culture tubes were pooled and centrifuged at 2,000 r.p.m. for 5 min. The supernatant fluid was discarded and the deposit was re-suspended in Ringer's solution so as to contain about 100–150 parasites/low-power field. Sera of rabbits hyperimmunized with cultures of *Ent. histolytica* were used and are referred to as 'antiamœba' sera in this communication. Sera were also cultivated against *E. coli* and *Ps. pyocyaneus*.

Diffusion of antigen and antibody in agar gel was carried out according to the method of Feinberg[4] as adapted by Ghosh and Mukerjee[5]. In order to eliminate the reactions due to the antigens of associate bacteria, controls were set up with *E. coli* and *Ps. pyocyaneus* antigens in each test. It can be seen in Fig. 1*a* that 'antiamœba' serum gave rise to three precipitin bands against *E. coli* and two bands against *Ps. pyocyaneus* antigens. But when the amœba culture was used, a number of bands appeared between the antigen and antiserum wells and it was not possible to delineate the specific bands of amœbæ. This was due to the presence of antibodies against *E. coli* and *Ps. pyocyaneus* in the 'antiamœba' serum and the corresponding antigens in the antigenic preparations of amœba. By incorporation of 50 per cent (v/v) anti*coli* and anti*pyocyaneus* sera in the media, it was possible to absorb the bacterial antigens and identify the three specific precipitin bands of amœbæ.

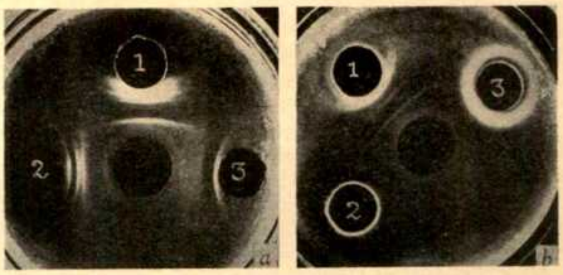

Fig. 1. *a*, Central well; antiamœba serum; 1, amœba antigen; 2, *E. coli* antigen; 3, *Ps. pyocyaneus* antigen. *b*, Intragel specific absorption 50 per cent anti*coli* and anti*pyocyaneus* sera (v/v) in gel. Arrangements of the wells are the same as in Fig. 1*a*. Note unmasking of three specific bands of amœba

On adding 'antiamœba' serum to the media all the bands, including those due to *Ent. histolytica*, were eliminated.

Since the rabbit's blood was used both for the preparation of Row's hæmoglobin media and as the source of antisera, no precipitin band appeared against media controls.

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GENETICS

Skewed Recombination in a Rare Interspecific Jute Hybrid

Islam and Rashid[1] and Swaminathan *et al.*[2] have recently described the morphological and cytological characteristics of the F_1 hybrids between the two cultivated jute species, *Corchorus olitorius* L. and *C. capsularis* L. The F_1 plants studied were intermediate in phenotype, but an interesting genetic situation was found when we examined the F_2 and F_3 generations of two hybrids of *C. olitorius* (♀) × *C.*

capsularis (♂). Chromosome associations during microsporogenesis were analysed in the two F_1 hybrids, and five bivalents and one quadrivalent, or one trivalent and a univalent, occurred in several cells at diakinesis and metaphase I (ref. 2). One of the bivalents was heteromorphic. The F_2 progenies of these two hybrids contained more than 40 per cent of non-viable seedlings. Also, about 11 per cent of the F_2 population had various types of chlorophyll deficiency, abnormalities in leaf shape and poor vigour.

The varieties of *C. olitorius* and *C. capsularis* used in this work differed prominently from each other in branching habit, leaf index, flower size, number of stamens, size and shape of capsules, and seed colour. The F_2 population of one cross, comprising 138 individuals, was analysed for these characters according to the hybrid index procedure of Anderson[3]. The results (Fig. 1) show that there is a preponderance in the population of plants with an index value approaching that for *C. olitorius*. While a wide range of segregation was observed for the shape and size of capsule, no plant had the *capsularis* type of globose fruit.

Chromosome associations were studied in seven F_2 plants, and differential segments and inversion loops at pachytene, a heteromorphic bivalent at metaphase I, and a dicentric bridge and an acentric fragment at anaphase I were recorded. Two plants proved to be trisomics with $2n = 15$ and in both of them the capsules occurred in clusters of 5–7 as in the *capsularis* parent (Fig. 2), thereby indicating that the extra chromosome may be a *capsularis* one carrying the gene for this trait. In the F_3 population raised from one of the hybrids, phenotypic segregation was even more prominently skewed in the direction of *olitorius* than in the F_2.

The cytological results, the occurrence of a high proportion of unthrifty and non-viable seedlings and the skewed segregation behaviour observed in F_2 and F_3 progenies indicate that the genomes of *C. olitorius* and *C. capsularis* have differentiated in the direction of increased coherence[4], which automatically decreases the possibility for effective recombination. It has not been possible, so far, to make this cross with *C. capsularis* as the pistillate parent. Whether or not cytoplasmic factors have any role in determining coherence and the direction of skewness in segregation can be ascertained only when the reciprocal hybrid becomes available. From the point of view of breeding, the occurrence of trisomics in the hybrid progenies is a useful feature, since a way is thus open for substituting whole pairs of chromosomes that cohere but do not recombine.

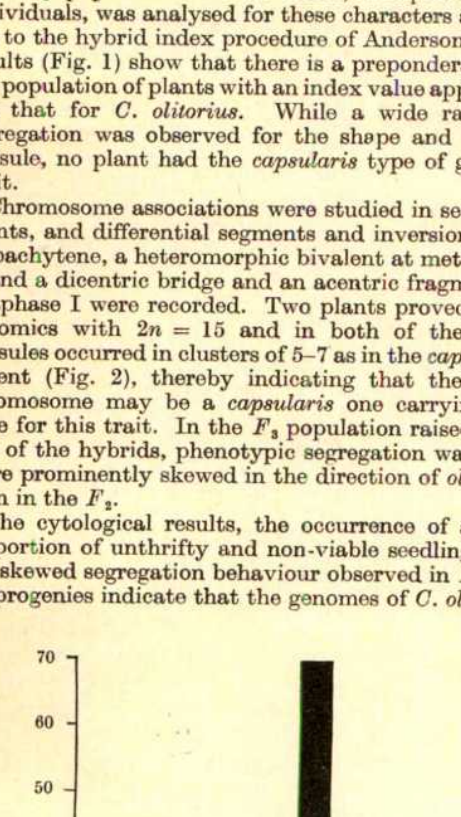

Fig. 1. Histogram showing the number of F_2 plants under each index value in the cross *C. olitorius* × *C. capsularis*. The population was scored for six different morphological characters and to each character typical of *C. olitorius* an index number of 1 was assigned. Thus plants totally like *C. capsularis* will have an index value of 0 and those like *C. olitorius* will have a value of 6. A value of 0·5 was given when the phenotype for a character was intermediate between that of the parents

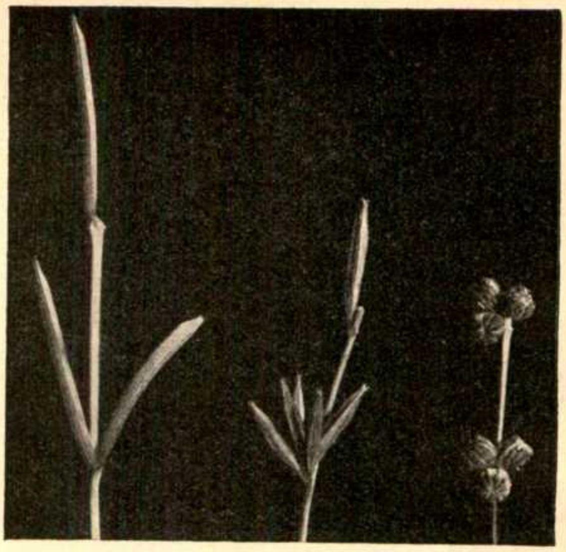

Fig. 2. Fruiting branches of (left to right), *C. olitorius* (♀), the trisomic F_2 with bunched capsules and *C. capsularis* (♂). 3/8 nat. size

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Hæmoglobin Polymorphism in Fishes

MERISTIC and morphometric characters have been the most commonly used traits in the investigation of races or sub-populations of fishes. The differences in such characters among various populations may be due to variation in genetic as well as environmental factors. However, the relative influence of these two factors is usually unknown[1]. Consequently, it would be advantageous to use gene-frequency data based on Mendelian characters, the expression of which may be considered not to be influenced by environmental variation.

In mammals, especially man, this has been successfully done with blood types and hæmoglobin types[2,3]. Variation of blood-groups within fish species has been described by several authors[4–8]. Although zone electrophoresis of fish hæmoglobins has been reported by Buhler and Shanks[9] and by Chandrasekhar[10],

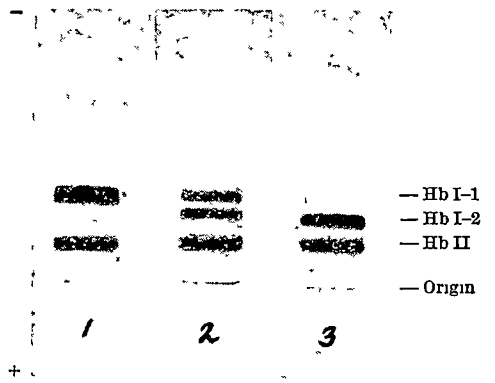

Fig. 1. Hæmoglobin patterns of whiting. 1, Type Hb I-1; 2, type Hb I-1-2; 3, type Hb I-2

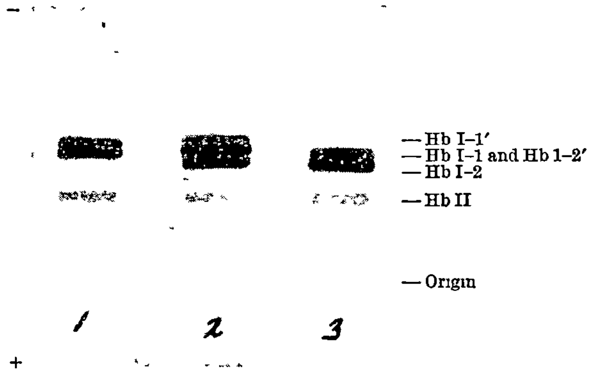

Fig. 2. Hæmoglobin patterns of cod. 1, Type Hb I-1; 2, type Hb I-1-2; 3, type Hb I-2

no intraspecific variation in electrophoretic mobilities was detected.

This communication describes hæmoglobin polymorphism in two species of fish, namely, whiting and cod. Polymorphism was detected by a modification of the agar electrophoresis technique described by Giri[11]. The present experiments were carried out with phosphate buffer (pH 7.3, ionic strength 0.02). Only freshly prepared oxyhæmoglobin solutions have been used.

In whiting (*Gadus merlangus*) three different electrophoretic patterns were found (Fig. 1). The whiting hæmoglobins were run at room temperature for 30 min. at 200 V. between electrodes. The patterns all have a slow-moving component (Hb II) in common. In addition, they may have either one or both of two faster-moving components (HbI-1 and HbI-2). The three types of individuals are called type Hb I-1, type Hb I-1-2, and type Hb I-2. Both in type Hb I-1 and in type Hb I-1-2 the Hb I-1 component is accompanied closely at the cathodic side by a weak minor component. No correlation of hæmoglobin type with sex or size of the specimen could be detected.

Apart from the common Hb II component the situation observed here is similar to the hæmoglobin polymorphism described in man, cattle and sheep. In these species, the underlying genetics have been elucidated[3]. By analogy a genetic hypothesis involving two alleles may be tentatively adopted to explain the genetics of the hæmoglobin variation observed in whiting. According to this hypothesis two co-dominant allelic genes Hb I^1 and Hb I^2 determine the hæmoglobin components Hb I-1 and Hb I-2 respectively. Type Hb I-1 and type Hb I-2 are considered as homozygous for the corresponding allele, and type Hb I-1-2 would represent the heterozygote. This hypothesis receives some support from the fact that the distribution of the supposed genotypes is consistent with the distribution calculated according to the Hardy–Weinberg law (Table 1). There is no significant heterogeneity between the samples represented in Table 1.

In cod (*Gadus morrhua*) three distinct patterns were again observed (Fig. 2). The hæmoglobin components of cod require longer time and cooling of the apparatus for their resolution. They were run for 60 min. at 200 V. between electrodes. It should be noted that although the same designations (Hb I-1, Hb I-2, and Hb II) have been used for cod and whiting hæmoglobin components, it is not known whether the two species have any component in common.

Both the Hb I-1 and the Hb I-2 component of cod hæmoglobin are accompanied at the cathodic side, by a minor component, Hb I-1′ and Hb I-2′ respectively. The Hb I-2′ component was inseparable from the Hb I-1 component in the supposed heterozygote as well as in an *in vitro* mixture of hæmoglobin from individuals of type Hb I-1 and Hb I-2. This situation is similar to that described for serum transferrin types in mice[12]. Since storing the cod hæmoglobin solutions tends to increase the relative amount of minor components, they may represent denaturation products.

A two-allele genetic hypothesis may also be adopted to explain the findings with cod. The results obtained are presented in Table 2. A highly significant difference in gene frequencies can be seen between the Bornholm sample and the three other samples. Apart from the Bornholm material the samples are consistent with the Hardy–Weinberg distribution and show similar gene frequencies. The poor fit ($P = 0.0004$) of the Bornholm sample is most easily explained by assuming that it represents a population homozygous or nearly homozygous for the Hb I^2 allele with some admixture of migrants from a population having gene frequencies similar to those

Table 1. DISTRIBUTION OF HÆMOGLOBIN TYPES IN SAMPLES OF WHITING FROM DIFFERENT AREAS

Type Supposed genotype	HbI-1 HbI^1/HbI^1	HbI-1-2 HbI^1/HbI^2	HbI-2 HbI^2/HbI^2	Total	Frequency of HbI^1 allele
Inner Danish Waters (Kattegat, The Sound, and Fehmarn Belt)	16	78	72	166	0.33
Southern North Sea (near Holland)	10	40	46	96	0.31
Northern North Sea (near Aberdeen)	10	21	21	52	0.39
Irish Sea	4	18	16	38	0.34
Totals observed	40	157	155	352	0.34
Totals expected according to the Hardy–Weinberg law	39.9	157.2	154.9		

Table 2. DISTRIBUTION OF HÆMOGLOBIN TYPES OBSERVED IN SAMPLES OF COD FROM DIFFERENT AREAS COMPARED WITH THE DISTRIBUTION EXPECTED ACCORDING TO THE HARDY–WEINBERG LAW OF GENOTYPE FREQUENCIES

Type Supposed genotype		HbI-1 HbI^1/HbI^1	HbI-1-2 HbI^1/HbI^2	HbI-2 HbI/^{2}HbI^2	Total	Frequency of HbI^1 allele
The Sound	observed	93	112	37	242	0.62
	expected	91.7	114.6	35.7		
Fehmarn Belt	observed	27	30	12	69	0.61
	expected	25.6	32.8	10.6		
Great Belt	observed	37	50	15	102	0.61
	expected	37.7	48.6	15.7		
Baltic Sea, near Rønne, Bornholm	observed	2	2	74	78	0.04
	expected	0.1	5.8	72.1		

observed in the Sound and Fehmarn Belt This interpretation, although supported by results from Danish tagging experiments[13], should be tested by further investigation

In addition to cod and whiting, distinct intraspecific variation in hæmoglobin patterns has been observed in sole (*Solea solea*) and eel-pout (*Zoarces viviparus*) These preliminary results suggest that hæmoglobin polymorphism may be of rather common occurrence in fishes and that zone electrophoresis of hæmoglobins may become a valuable tool in taxonomic investigations of fishes at the sub-specific level

This work was supported by a grant from the Carlsberg Foundation Acknowledgment is due to Dr B. B Parrish, Marine Laboratory, Aberdeen, Scotland, to Dr M Roessingh, Rijksinstituut voor Visserij Onderzoek, Ijmuiden, Holland, and to Dr L Birkett, Fisheries Laboratory, Lowestoft, Suffolk, England, for providing samples of whiting blood

I am indebted to Dr O Frydenberg for suggesting this type of work to me and for helpful discussion and suggestions

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VIROLOGY

Inactivation of Rous Virus by Phospholipase *A*

Rous virus is known to consist of protein, ribonucleic acid (RNA) and lipid, about 40 per cent of which is in the form of phospholipids[1,2] Partially purified preparations of the virus are inactivated by a wide range of lipid solvents—organic solvents, bile salts and synthetic detergents—and the loss of infectivity is associated with the shedding from the virus particles of protein-like material[3]

Anderson and Ada[4] have shown that Murray encephalitis virus—an arthropod-borne virus containing RNA, protein and lipid—is inactivated by phospholipase *A* This communication reports a similar loss of infectivity in Rous virus preparations incubated with phospholipase *A*

Partially purified Rous virus suspensions were made from fresh fowl-grown tumours according to the method of Carr and Harris[5] as modified by Bather[6]. Phospholipase *A*, supplied by Miss H Doery of the Commonwealth Serum Laboratories, had been purified from the venom of the black snake (*Pseudechis porphyriacus*) The concentration of enzyme used in these experiments was 100 μgm per ml of 0 9 per cent sodium chloride , each sample was boiled for 10 min to ensure that phospholipase *A* was the only functional enzyme present

Equal volumes of virus suspension (in phosphate-citric buffer *p*H 7 2) and phospholipase *A* were mixed and incubated at 37° C for 30 min As a control, the virus suspension was mixed in 0 9 per cent sodium chloride and kept at the same temperature and for the same time as the experimental tubes The infectivity of the control and treated samples was estimated by titration in young chicks[5]

Table 1 shows the loss of infectivity which resulted from the incubation of Rous virus with phospholipase *A* in two experiments Experiments with MH_2 tumour virus preparations have yielded similar results (Table 2)

Table 1 EFFECT ON INFECTIVITY OF ROUS VIRUS INCUBATED WITH PHOSPHOLIPASE *A* (100 μGM /ML) AT 37° C FOR 30 MIN

		Dilution of sample and No of tumours/No of chicks inoculated at each dilution							Infectivity
		10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
1	Control	2/2	2/2	4/4	4/4	4/4	4/4	0/4	$10^{5\,5}$
	Treated	2/2	2/2	0/2	0/4	0/4	0/4	0/4	10^{1}
2	Control	2/2	2/2	2/2	4/4	4/4	4/4	4/4	$>10^{6}$
	Treated	2/2	1/2	0/2	0/4	0/4	0/4	0/4	10^{1}

Table 2 EFFECT ON INFECTIVITY OF MH_2 VIRUS INCUBATED WITH PHOSPHOLIPASE *A* (100 μGM /ML) AT 37° C FOR 30 MIN

		Dilution of sample and No of tumours/No of chicks inoculated at each dilution							Infectivity
		10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
1	Control	1/2	2/2	2/2	4/4	4/4	1/4	0/4	$10^{4\,7}$
	Treated	0/2	0/2	0/2	0/4	0/4	0/4	0/4	0
2	Control	2/2	2/2	4/4	4/4	2/4	0/4	0/4	$10^{4\,0}$
	Treated	0/2	0/2	0/4	0/4	0/4	—	—	0

Since phospholipase *A* splits specifically only fatty acids bound in the – position, it was concluded from these results that Rous virus (and probably the other fowl tumour viruses as well) are inactivated by the cleavage of fatty acids from phospholipids contained in their structure

This result would support a previous suggestion[3] that the integrity of at least some of the protein bound to the phospholipid moiety in the extranucleoid region of Rous virus particles is essential for infectivity It may be that disruption of this phospholipid-protein wrapping is so radical as to cause irreversible injury to the virus RNA, and that it is this secondary effect which destroys the reproductive capacity of the virus On the other hand, the lipoprotein shell of Rous virus may allow the virus particles to react with some intracellular system and this 'acceptance' may be a necessary prerequisite for virus replication

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, December 4

Institute of Metal Finishing (in the Gower Room, Hotel Russell, London, W C 1), at 3 30 p m —Dr G L J Bailey "The Changing Face" (Presidential Address)

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W 12), at 4 p m —Dr H E Huxley "Mechanism of Muscular Contraction" *

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place London, W C 2, at 5 30 p m Discussion on "Backward Waves in Waveguides", opened by Dr P J. B Clarricoats, Mr R A Waldron and Mr G H B Thompson

Institution of Mechanical Engineers, Applied Mechanics, Theory of Materials of Construction Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "Can Plastic Components be Designed Rationally ?"

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 6 p m —Prof W K Estes (Indiana) "Current Foci of Research in Mathematical Behaviour Theory" * (Further lectures on December 7 and 11)

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S W 1), at 6 30 p m —Scientific Film Evening

Tuesday, December 5

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1 15 p m —Dr H Kalmus "Biological Time-keeping" *

Institution of Electrical Engineers, Measurement and Control Section (at Savoy Place, London, W C 2), at 5 30 p m — Prof L F Bates, F R S "Domains and Domain Patterns"

Parliamentary and Scientific Committee, General Committee (in Committee Room 12, House of Commons, London, S W 1), at 5 30 p m —Dr D W Hill and Sir Charles Goodeve, F R S "The Developing and Financing of Research Associations"

Royal Society of Medicine, Pathology Section (at 1 Wimpole Street, London, W 1), at 5 30 p m —Symposium "On Histochemistry and Cytochemistry" Speakers Dr N. M Hancox, Mr S J Holt and Mr A J Hale

University of London (at Imperial College of Science and Technology, London, S W 7), at 5 30 p m —Prof M G Fleming Inaugural Lecture

Institution of Polish Engineers in Great Britain, Group for Economic, Social and Management Studies (at 55 Princes Gate, Exhibition Road, London, S W 7), at 7 p m —Mr E A Cooper-Key "Management Training in the Armed Forces and in Industry"

Royal Photographic Society of Great Britain (at 16 Princes Gate, London, S W 7), at 7 p m —Dr R G W Ollerenshaw "Design for Projection a Study of Legibility" (Eleventh Renwick Memorial Lecture)

Science Forum (at Friends House, Euston Road, London, N W.1), at 7 30 p m —Dame Kathleen Lonsdale, F R S "Statistical Ethics"

Wednesday, December 6

Institute of Physics and Physical Society, Colour Group (at the Northampton College of Advanced Technology St John Street, London, E C 1), at 3 p m —Dr B H Crawford "Colour Matching and Visual Adaptation"

Institution of Agricultural Engineers (at Cromwell Hall, Earls Court, London), at 4 p m —Dr A H Willis "Agricultural Engineering Problems in Australia"

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1) at 5 p m —Prof C. R Rao "Efficient Estimates and Optimum Inference Procedures in Large Samples"

Institute of Biology (in the Lecture Theatre of the British Museum (Natural History), Cromwell Road, London, S W 7), at 5 15 p m —Dr J M Tanner "Human Growth in its Comparative and Evolutionary Aspects" (Second Darwin Lecture)

Institute of Petroleum (at 61 New Cavendish Street, London, W 1), at 5 30 p m —Dr J A Carruthers and Mr K J Marsh "Charge Relaxation in Hydrocarbon Liquids flowing through Conducting and Non-conducting Pipes", Mr K J Wigley and Dr J A Carruthers "The Estimation of Electrostatic Potentials, Fields, and Energies in a Rectangular Metal Tank containing Charged Fuel"

British Institution of Radio Engineers, Computer and Medical Electronics Groups (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Discussion on "Possible Uses of Computers in Medical Diagnosis" opened by Dr A D Booth

Institution of Electrical Engineers, Education Discussion Circle (at Savoy Place, London W C 2), at 6 p m —Discussion on "Teaching Protective Systems" opened by Mr G. Heywood

Wednesday, December 6—Friday, December 8

Royal Photographic Society of Great Britain, Scientific and Technical Group (at the Royal Society of Arts, John Adam Street, Adelphi, London, W C 2), at 9 30 a m daily—Symposium "Document Copying by Photography"

Thursday, December 7

Royal Society (at Burlington House, Piccadilly, London, W 1), at 4 30 p m —Sir Gavin De Beer, F R S "The Origins of Darwin's Ideas on Evolution and Natural Selection" (The Wilkins Lecture)

Institute of Refrigeration (at the Institute of Marine Enginineers, The Memorial Building, 76 Mark Lane, London, E C 3), at 5 30 p m —Dr J C Fidler "Developments in Storage of Fruits"

Institution of Electrical Engineers (at Savoy Place, London W C 2), at 5 30 p m —Prof M W Thring "Magnetohydrodynamics" (Parsons Memorial Lecture)

Royal Institution, Library Circle (at 21 Albemarle Street, London, W 1), at 5 30 p m —Mr K D Vernon "Forgotten Worthies of the Royal Institution 2 William Savage and Thomas Webster"

Royal Meteorological Society (in the Conference Hall, County Hall, London, S E 1), at 5 30 p m —Mr C E Wallington "Meteorology and Gliding"

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Dr F Kingsley Sanders "Infective Nucleic Acids and Virus Synthesis" * (Sixteenth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

Oil and Colour Chemists' Association, *London Section* (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W 1), at 7 p m —Mr A Macmaster "Aircraft Paints"

Royal Institute of Chemistry, London Section (at the Northampton College of Advanced Technology, St John's Street, London, E C 1), at 7 p m —Mr T M Dunn "Ultra-violet Spectroscopy"

Thursday, December 7—Friday, December 8

Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals (at Church House, Great Smith Street, London, S W 1), at 2 30 p m on Thursday and 10 a m on Friday—Winter Meeting

Friday, December 8

Association of Applied Biologists (at the British Museum (Natural History), South Kensington, London, S W 7), at 10 50 a m — Paper reading meeting

Institution of Electrical Engineers, Medical Electronics Discussion Group (at Savoy Place, London, W C 2), at 6 p m — Discussion on "The Peltier, Hall and Seebeck Effects"

Royal Institution (at 21 Albemarle Street, London, W 1), at 9 p m —Dame Kathleen Lonsdale, F R S "Restless Atoms in Solids"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Senior Demonstrator (with a degree with biochemistry as a major subject and preferably some teaching or postgraduate experience) in Biochemistry at the University of Melbourne, Australia —The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, December 15)

Lecturer (with an honours degree with special interest in inorganic and/or physical chemistry, and preferably experience in university teaching) in Chemistry at the University College of Townsville, University of Queensland—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (December 16)

Experimental Officer or Senior Experimental Officer (with Higher National Certificate, degree or similar qualification) in the Department of Electrical Engineering—The Registrar, The University, Manchester 13 (December 18)

Organic Chemist or Biochemist (with a good honours degree or equivalent qualification), for research in structural carbohydrate chemistry and immunochemistry—The Secretary, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S W 1 (December 30)

Lecturer in Geography, a Lecturer in Physics, and a Lecturer in Psychology at the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, December 31)

Lecturer (medical graduate of at least two years standing with experience in teaching and research) in Anatomy—The Secretary, The Medical College of St Bartholomew's Hospital, West Smithfield London, E C 1 (December 31)

Official Fellows (2) in Physics, to carry responsibility for the teaching of theoretical and experimental physics—The Warden, Wadham College, Oxford (December 31)

Assistant in the Sub-Department of Pharmacology, University College of The West Indies, for duties which include research, demonstration and some teaching for M B , B S , degrees of London University—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W C 1 (January 1)

Lecturers or Assistant Lecturers (2) (with a major interest in the theory of machines, and preferably an interest in metallurgy, machine design or engineering drawing) in Mechanical Engineering at the University College of The West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 1)

Lecturer or Assistant (preferably with a special interest in solid state physics) in Physics—The Secretary, Queen's College (University of St Andrews), Dundee (January 8)

Technicians (2) in the Department of Anatomy, Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 10)

Chair of Anatomy in the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 15)

Chairs (4) in Engineering in Monash University, Melbourne—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall London, S W 1, or The Registrar, Monash University, P O Box 92, Clayton, Victoria, Australia (Australia and London, January 31)

Senior Lecturer (with an appropriate science qualification and wide experience of clinical biochemistry) in Clinical Biochemistry at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, January 31)

Official Fellow and Tutor in Physics, or a Lecturer in Physics—The Principal, St Hugh's College, Oxford (February 1)

Imperial Chemical Industries Research Fellows in Chemistry, Physics, Metallurgy, Engineering, Geology, Agricultural Science or related subjects—The Registrar, University of Wales, University Registry, Cathays Park, Cardiff (March 5)

Assistant (graduate in astronomy, or in physics or electrical engineering (electronics) with some training or interest in astronomy) to the Director of the Jeremiah Horrocks and Wilfred Hall Observatories—Chief Education Officer, Municipal Building, Preston

Assistant Professor (with a Ph D in pharmacology), and an Assistant Professor (with a Ph D in pharmacognosy or plant chemistry)—Prof J R Murray, Director of the School of Pharmacy, University of Manitoba, Winnipeg, Canada

Biochemist for fundamental research on collagen and gelatin—The Director of Research, British Gelatine and Glue Research Association, 2*a* Dalmeny Avenue, Holloway, London, N 7

Chief Laboratory Technician—Head, Applied Physics Department, Northampton College of Advanced Technology, St John Street, London E C 1

Lecturers, Assistant Professors, and Associate Professors in the Department of Mathematics (Edmonton campus or Calgary campus)—Prof M Wyman, Mathematics Department, University of Alberta, Edmonton, Alberta, Canada

Lecturers (with a first- or second-class honours degree in mathematics, physics and most branches of engineering), to teach students for the B Sc degrees of London University—The Registrar, Royal Military College of Science, Shrivenham, near Swindon, Wilts

Librarian (with a knowledge of chemistry or physics) in the Department of Chemical Engineering and Chemical Technology—Head of the Department of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, London, S W 7

National Coal Board Research Assistant (good honours graduate or equivalent) in the Department of Pure and Applied Chemistry, to continue a study on the origin of coal tar components—The Reader in Industrial Chemistry, Northampton College of Advanced Technology, St John Street, London E C 1

Research Assistant (with a good honours degree, or its equivalent, in physiology, biochemistry or zoology, and preferably experience in some field of hormone research or immunochemistry) in the Physiology Department, to study the physiology of prolactin with special reference to its assay in body fluids—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref 61/31

Research Assistant (with experience of numerical analysis and the writing of computer programmes) in the Mathematics Department—The Registrar, Brunel College of Technology, Woodlands Avenue, Acton, London, W 3

Senior Laboratory Technician in the Bacteriology Department, Connaught Hospital, Walthamstow, London, E 17—The Secretary, Forest Group H M C, Langthorne Road, Leytonstone, London, E 11

Senior Lecturer in Physics, and a Lecturer in Physics—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N 7

Senior Research Assistant (preferably with some research experience) in the Department of Physics, for a programme of work on the magnetic properties of thin films, provisionally for one year in the first instance—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey

Technician or Junior Technical Officer (female, preferably with "A" level G C E or equivalent), to assist in biological research at Pollards Wood Research Station, Chalfont St Giles, Bucks—The Secretary, Institute of Cancer Research, 33 Sumner Place, London, S W 7, quoting Ref 301/B/83

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

British Medical Bulletin, Vol 17, No 3 (September, 1961) Human Genetics Pp 177–264+xv (London British Council, 1961) 20*s* [59

Wye College (University of London) Annual Report of the Department of Hop Research from 1st April, 1960, to 31st March, 1961 Pp v+90+2 plates Hop Research at Wye College in 1960 By H S Darling Pp 4 (Wye, Ashford Wye College, University of London, 1961) [79

Ministry of Agriculture, Fisheries and Food Bulletin No 148 Incubation and Hatchery Practice By Dr W M Allcroft Pp v+65+8 plates (London H M Stationery Office, 1961) 5*s* net [79

Learning Theory and the Therapy of Neurosis By R Metzner. (*The British Journal of Psychology*, Monograph Supplements, No 33.) Pp v+29 (Cambridge At the University Press, 1961) 7*s* 6*d* net [79

Department of Scientific and Industrial Research National Engineering Laboratory Annual Report 1960 Report of the National Engineering Laboratory Steering Committee with the Report of the Director of the National Engineering Laboratory Pp iv+72+4 plates (Edinburgh and London H M Stationery Office, 1961) 5*s* net [89

The Institute of Navigation Report of the Council, 1960–61 Pp 16 (London Institute of Navigation, 1961) [259

University of London University College Calendar, 1961–62 Pp lxi+504 (London University College, 1961) [259

Other Countries

New Zealand Forest Service Forest Research Institute Research Notes No 25 The European House Borer *Hylotrupes bajulus* L , a Possible Pest for New Zealand By R H Milligan Pp 19 (Wellington Government Printer, 1961) [79

Guide Book to the Royal Botanic Gardens, Melbourne Pp 28 (Melbourne Royal Botanic Gardens, 1961) 4*s* (Australian) [89

United States Department of the Interior Geological Survey Water-Supply Paper 1591–A Urban Growth and the Water Regimen By John Savini and J C Kammerer Pp iv+43+1 plate Professional Paper 402–A Mechanism of Gravity Drainage and Its Relation to Specific Yield of Uniform Sands By W O Smith Pp iii+12 20 cents Professional Paper 411–B Transverse Diffusion in Saturated Isotropic Granular Media By Akio Ogata Pp iii+8 15 cents (Washington, D C Government Printing Office, 1961) [89

Smithsonian Miscellaneous Collections Vol 143, No 4 Comparison of Tektite Specimens from Empire, Georgia, and Martha's Vineyard, Massachusetts By Roy S Clarke, Jr , and Maxwell K Carron Pp i+18+6 plates (Publication 4465) (Washington, D C Smithsonian Institution, 1961) [89

Bulletin of the Museum of Comparative Zoology at Harvard College Vol 125, No 3 Two Jurassic Turtle Skulls—a Morphological Study. By Thomas S Parsons and Ernest E Williams Pp 41–108+6 plates (Cambridge, Mass Museum of Comparative Zoology at Harvard College, 1961) [259

United States Department of Agriculture Farmers' Bulletin No 2168 Controlling Potato Insects By W A Shands, B J Landis, and W J Reid, Jr Pp 16 (Washington, D C Government Printing Office, 1961) 10 cents [259

Smithsonian Miscellaneous Collections Vol 142, No 4 Cenozoic and Cretaceous Echinoids from Trinidad and Venezuela By C Wythe Cooke Pp 36+14 plates (Publication 4459) (Washington, D C Smithsonian Institution, 1961) [259

Work Half Done Bernice P Bishop Museum Annual Report for 1960 Pp 30 (Honolulu, Hawaii Bernice P Bishop Museum, 1961) [259

Institut Royal Météorologique de Belgique Contribution No 67 Etude des Propriétés Statistiques des plus Fortes Marées à Ostende (Belgique) Par Dr R Sneyers Pp 11 (Bruxelles Institut Royal Météorologique de Belgique, 1961) [259

Proceedings of the United States National Museum, Smithsonian Institution Vol 113, No 3453 Notes on Australian Flies of the Family Conopidae By Sidney Camras Pp 61–76 Vol 113, No 3454 New and Previously Known Millipeds of Panama By H F Loomis Pp 77–124 Vol 113, No 3455 New General and New Species of Lacebugs from the Eastern Hemisphere (Hemiptera Tingidae) By Carl J Drake and Florence A Ruhoff Pp 125–183 (Washington, D C Government Printing Office, 1961) [259

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Editorial and Publishing Offices of "NATURE"
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Telephone Number Whitehall 8831 Telegrams Phusis Lesquare London

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EXCELLENCE AND LEADERSHIP IN A DEMOCRACY

THE need to foster outstanding qualities among democratic peoples has formed the theme of a series of articles in *Daedalus* A number of draft papers were prepared and considered at a closed conference held at Middletown, Connecticut, during April 28–30 The final papers have now been brought together and published in the *Proceedings of the American Academy of Arts and Science* (90, No 4, Autumn 1961) Among the papers is one by Robert S Morrison, director of medical and natural sciences at the Rockefeller Foundation, who discusses the need for new types of excellence

Morrison began his inquiry by means of an informal survey among a representative group of friends Almost all found time to formulate thoughtful analyses of future needs This fact serves to document an awareness among intellectual leaders for something different and a good deal better than now exists All seemed well satisfied with the progress being made in expanding knowledge of the physical world and in applying that knowledge to the reduction of poverty and suffering Most of present pre-occupation is with organization, interdisciplinary co-operation, decision making, and value problems Science is increasingly confronted with large problems the parts of which are so interdependent that they cannot be dissected without spoiling them for future study A contribution by Warren Weaver shows how the scientific problems of the future merge without perceptible transition into the classical problems of the humane arts

"I have a very strong conviction", he wrote, "that the 'new forms of excellence' required by science over the next half-century will in considerable part consist of techniques for dealing with broader problems, larger problems, problems which arise when a moderate number of small problems are organically interrelated "

One can start with the very obvious and over-simplified examples "The day is past", Dr Weaver claims, "when science can properly treat food supply as an isolated problem, birth-rates as another, public health as another The whole body of servo-mechanism and feedback theory, many of the techniques of operations research, the emerging knowledge concerning information and communication theory—these are all groping toward the capacity to deal with problems of organized complexity

"We have been good at producing bits of information, fragments of knowledge We now need to devote more attention to the fitting together of these bits, to broaden interrelations, and hence to broader utilization We have to humanize science, and give more general meaning and structure—and even value—to it "

Most of the other correspondents to Dr Morrison's inquiry came directly to the need for synthesizing, interdisciplinary research, better organization, and better decision-making, without bothering to analyse how All recognize that it is the extraordinary dimensions of this pile which shape the demand for new forms of excellence

It is science which has (1) given so much information that no man can master more than a small portion of it, (2) given reality to the concept of one world, (3) given the populations of some parts of that world so many material possessions and so much power over their own destinies that for the first time the paramount question is not "How do I get enough to stay alive?" but "What do we do with a life that is more or less guaranteed?"

Each gift calls for its own sort of expertize, and, taken together, requires a form of human activity so new that there is no word to describe it The rapid accumulation of raw information and digested knowledge places unprecedented burdens on the minds and characters of those who follow the learned professions It is not cheering to know that this year's class of new medical students are confronted with four times as much to learn as their teachers encountered a short twenty years ago By the time these young men finish their courses they should return immediately to the basic sciences they studied as freshmen There will be as much that is new to them as there was when they studied the basic sciences the first time

It is not only the possession of specialized bits of knowledge that sets a specialist off from his fellows but also his different way of looking at things, and this may erect even more serious barriers Whole schemes of thought and discourse have developed to deal appropriately with the findings provided by separate skills, until ultimately the specialities are divided from one another not only by the possession of special information and manipulative skills but also by wholly different ways of thinking and talking Nowhere is this more clear than in the field of psychology Here there are psychoanalysts, learned theorists, conditioned-reflex addicts, physiological psychologists and ethologists, all looking at the same set of phenomena in such wholly different ways that they can scarcely hold sensible conversations with one another

The unsatisfactory nature of this state of affairs is widely recognized and lies behind the persistent cries for more 'interdisciplinary' research and better 'generalists' How does one set about becoming excellent in a general way ? If there is substance to the view that specialization arises from the invention of special methods of observation and is perpetuated by human incapacity to store and recall more than a small fraction of our exponentially increasing pile of data, it seems reasonable to give primary attention to simplifying these functions or transferring them to other hands Our best minds might then become freer to deal with conceptual and communication problems which worry us so much

There seems reason to hope that many of the basic scientific specialities are less separated by technique than they used to be The electron microscope was invented by physicists, but methods for its use have been worked out by chemists, cytologists, histologists, microbiologists, and an occasional physiologist It is beginning to give a view of biological work which is clearly more unified than anything before Now that instruments exist for analysing events at what is essentially molecular level, the attention of all specialities is beginning to focus at the same point As a result, the histologists, biochemists and physiologists are now holding livelier conversations with one another than at any time It is increasingly difficult to differentiate the basic science departments on the basis of the instrumentation used within them Basic biochemical apparatus, electron and light microscopes, cathode-ray oscillographs, and radiation counters may well be found in any of them Part of the increasing homogeneity is due to the recognition that all biological events can be traced to biochemical ones This means, in turn, that all biologists must learn to speak the *lingua franca* of biochemistry—and ultimately of atomic physicists, at least in so far as the nature of the chemical bond between atoms is concerned

The splitting of our scientific culture into language blocks does not doom man to the splendid isolation of the 'Tower of Babel' The separate ways, if pursued long enough, can lead to an even more splendid and imperial synthesis of crystal clarity. It is possible for a single individual to select and give a meaningful synthesis to the most significant facts and concepts of economics, sociology, history, business management, and political science—*vide* W W. Rostow, *The United States in the World Arena* As knowledge expands beyond a certain point, the burden on the individual can become less For it is much easier to grasp and to remember interrelated schemes in which a set of observations developed in one language can be interpreted in terms of another than it is to hold on to a mass of unrelated items

It also seems probable that the pursuit of excellence in the technology of gathering and processing data will help to free mankind from the burdens of specialization In the early stages of any natural science, the great advances come from devising new ways of observing Recent technical advances have reduced many of the essential manipulative skills to semi-automatic mechanical processes There is now, for example, a machine which will automatically analyse a complex protein for all its constituent amino-acids in a matter of hours Only a few years ago whole teams of skilled chemists were trying to find out how the job could be done at all

The second problem—how to handle the data after they are accumulated—is more difficult It remains for the future to devise an excellence which will make the most of the speed and accuracy with which mechanical computers handle large volumes of data, while at the same time maximizing the associative capacities and instinct for relevance contained within our own brains Enough progress has been made to suggest that computer techniques will be useful not only in storing and recalling existing information, that is, in improving the library function There are great possibilities for the development of machines which will analyse and correlate data as they occur For example, neurophysiologists and psychologists are now much interested in the possibility of correlating electrical events in the nervous system with functions such as perception, learning and memory

New forms of excellence are required for developing these new technologies for the accumulation and analysis of data More interesting, perhaps, is the challenge the new techniques offer for the development of even newer excellences It will not be easy to relinquish to the machine the arts and techniques which have taken decades to master, and to concentrate on the even more demanding tasks of generalization, synthesis and judgment which only men can carry out Perhaps the greatest demand for ingenuity will fall on the field of education, once it is decided to hand over to other means the mere memorization of information, the computing of sums, and such other routine intellectual tasks as may be found appropriate It will be an excellent teacher who shows how to programme school and college experience so as to maximize the capacity to grasp general principles, detect relevance where none was seen before, and create new conceptual schemes When that day comes, all education will be general education since the technical problems which now divide the specialities will in large part have been handed over to the computers, and "we will have taught ourselves a language we can all use in common"

Dr Morrison also examines the set of problems arising from the emergence of "one world" When radiation fall-out affects both the just and unjust of all peoples in the world, it is time to invoke Waddington's concept of 'stochastic' or 'statistical morality' which calls for a new kind of excellence that must be invented to deal with this new state of affairs The whole future of the race is at stake in such matters as the uncontrolled expansion of its numbers in the 'population explosion', or the decline in its quality brought about by the genetic effects of advances in modern medicine in combination with our present-day casual breeding patterns

There is a school of thought, particularly well exemplified by the tobacco industry, which attempts to shrug off the evidence for all such dangers as 'only statistical' At present the evidence available suggests that men who smoke a package or more of cigarettes a day are likely to die earlier than men who do not smoke at all Yet smoking habits have little changed The explanation seems to lie in the fact that men still find it very difficult to pay serious attention to contingencies that lie more than a few years in the future and are coupled to present events with a probability of less than one

After examining the need for improving standards of government, Dr Morrison shows how compulsive are the mass uses of indoctrination techniques. There is great need for responsible people to be constantly aware of the dangers inherent in specialities and in propaganda machinery. There is need for a combination of the highest sort of intellectual and moral attainments In the first place, it should be unnecessary in a democracy to point out that a speaker has no business to try to make the final decision for his hearers This would be, in fact, the simple way out and is common procedure in dictatorships, theocracies, and other controlled societies Democracy rests on the assumption that people in general will reach the right decision if they are given the truth But the truth is impossible to define in abstract terms, and even in concrete matters like the effect of radiation on genetic anomalies, is not much more than a fluctuating area of agreement

The telling of this kind of truth cannot be guaranteed by constitutional articles or perjury laws As more and more features of our life fall under the control of those who wield a specialized body of knowledge, control measures must be sought These can be found only in the individual moral and intellectual excellence of those who develop, hold, and disseminate knowledge

THE HISTORY OF SCIENCE

The Search for Order

The Development of the Major Ideas in the Physical Sciences from the Earliest Times to the Present. By Cecil J Schneer Pp xvii+398 (London English University Press, Ltd, 1960) 21*s*. net

THIS book belongs to the class, of which there have been several examples of late, of bird's-eye views of the history of physical science from early times to the present The chief defect of this type of work is that it inevitably omits everything but the direct ancestry of the science of our time, and makes what is in fact an almost indescribably devious course, constantly doubling back on itself, appear like a straight line Of its kind, however, it is among the best The story is told in a clear, straightforward and interesting manner, and, apart from an excessive number of small inaccuracies that could well have been avoided, the subjects dealt with are on the whole presented faithfully Those subjects include, mainly, physics, with some chemistry and a brief glance at geology and evolution. Despite a picture of a spiral nebula on the dust cover, there is no cosmology and next to no astronomy

There are a few surprising passages For example, the conflict between physicists and geologists over the age of Earth is presented as a conflict between "historical knowledge" and ordinary physical knowledge, as though these were different in kind, instead of as an instance of the different conclusions that can be drawn from two incomplete sets of data On a smaller scale, while it is certainly true that J. J. Thomson "lived on into the electronic era", the essence of the fact scarcely appears in that expression of it, and there are numerous quotations of which the authorship is either not given or indicated ambiguously.

But the chief criticism to which the book is open concerns its underlying attitude, which, though it appears overtly only here and there, can be felt throughout—the attitude, namely, that identifies present knowledge, so far as it goes, with truth. Whatever is is right, and ever will be, and whatever else was is wrong, and will never be again We are told, for example, that "the [relativity] theory must for ever remain ", and that "the assertion of the unity of all living things through the theory of evolution, is contradicted only by the ignorant or the intellectually vain" What one should learn from the history of science is that the fact that one is living now is a mere accident, and that the science of our time will appear to a historian of the future as crude as that of the seventeenth century appears to us, while some of the latter crudities (the transmutability of elements has already done so) may then have returned to displace our present superstitions The present time is an utterly undistinguished point in an indefinitely long sequence, it is not the culminating moment "for which the floating forceless ages waited" Unfortunately, few historians of science make this their central theme HERBERT DINGLE

PSYCHICAL RESEARCH

William James on Psychical Research

Compiled and edited by Gardner Murphy and Robert O. Ballou. Pp viii+339 (London Chatto and Windus, Ltd, 1961) 30*s* net

CONDITIONS in New England around the mid-nineteenth century were such as to throw up a number of people of exceptional talents Among them were the brothers James—William, investigator of the paranormal, and Henry, the novelist We are concerned here with the former, the elder of the two by one year They had much in common, especially a marked streak of pragmatism Its manifestations were different, practical and empirical for William, introspective and self-observational for Henry. So we find William taking Medical School at Harvard, and receiving an invitation to teach physiology to students, which soon led, as one would expect, to the borders of psychology and the associated problems of the mind

William James on Psychical Research deals with the last forty years (1870–1910) of William's life by presenting his papers, combined with an illuminating introduction and retrospect. The collection is valuable, more perhaps on account of the personalities involved than for the content of the contributions themselves. As a commentary on the state of psychical research, and indeed of much advanced philosophical thought of the period, it is admirable But if the reader is to enjoy these pages as he should—and as they deserve to be enjoyed—he might try the experiment of overlooking a good deal of the descriptions of séances, media, occult happenings and so on—which naturally aroused a fair amount of suspicion and controversy—and concentrate on the courage and sincerity of the people in question. We need pass no verdict on what they did, or what they thought it all meant what stands out is a monument of intellectual valour.

James was always asking 'Is it really so?" Herein lay his strength, devoted as he was to his friends and their common purpose. Not always could he go the whole way with them.

Seen against the background of the *fin de siècle*, one is struck by the power for good of Anglo-American co-operation: the foundation in 1882 of the Society for Psychical Research in England with a 'cell'—later a full society—in the United States.

In Great Britain the movement got away to a flying start with the active support of such figures as Henry Sidgwick, Edmund Gurney and Frederic Myers, not to mention Lord Rayleigh. Sidgwick's critical powers and Gurney's passion for hard work were essential, but the genius of the group was undoubtedly Myers, his life (largely) a tragedy and his death heroic. To his contemporaries he was a literary and historical scholar of exquisite sensibility, a Fellow of Trinity College, Cambridge, who, having no love for teaching, became an inspector of education. But as some will recollect his name as the author of the great poem *St. Paul*, the question can scarcely be avoided: What is the relation between these verses and his intense search for the nature of the survival of personality after death? We may never know, except to be humbled by the magnitude of the endeavour, for he was not one to count the cost or heed the wounds. Indeed, as Myers himself wrote: "Desperate patience of a brave desire."

An event of consequence, not mentioned by the compilers, was the short but brilliant life of the Metaphysical Society (1869–80). Among its members were Shadworth Hodgson, who founded the Aristotelian Society, and G. C. Robertson, who initiated *Mind*. Both those were closely connected with the Society for Psychical Research, having no doubt sharpened their wits and deepened their perception in discussions aforetime with such giants as Cardinal Manning, Father Dalgairns and Dean Stanley.

Meanwhile, James continued to press for experimental evidence. We gain little information as to how this was answered. There was much checking of mediumistic phenomena, trances and so on, but surely something more directly physical could have been attempted, even if the results were negative. It is worth remembering that the Einthoven string galvanometer appeared in 1903, and made possible an enormous increase in sensitivity for the measurement of minute electrical effects. Seemingly, comparatively little was done by direct instrumental means, or deliberate laboratory controls.

Prof. Gardner Murphy in the retrospect already mentioned, and writing about 1958, refers to four accomplishments of William James which make him a leading character in the history of psychical research. These are (i) he gave the subject the necessary prestige, (ii) he set things well and truly on their feet in the United States, (iii) he kept facts well to the fore, and (iv) he discovered the famous Mrs. Piper as a wholly exceptional medium. Now, long after the event, these do not appear to be very striking achievements, but in the intellectual climate of the time they counted for much.

But they were symptomatic of a fundamental quality. Prof. R. B. Perry wrote that James always "Knew there was more", and that is the outlook of the born explorer. Somebody once said to James, "the great field for new discoveries is always the unclassified residuum". (How forcibly indeed this was illustrated, for example, in the discovery of argon by Rayleigh and Ramsay.) Evidently it must be a basic tenet for students of the paranormal, from whatever direction they may approach it.

Nevertheless, it was left to Myers to pose the elemental question "Is the Universe friendly?". We cannot tell. What matters is that this timely volume shows William James and his circle following the light as they saw it, and leaving behind them a cherished example of dedicated research.

F. I. G. RAWLINS

CHEMISTRY IN INDUSTRY

Ammonia

Manufacture and Uses. By Dr. A. J. Harding. Pp. xiii+41+7 plates. (London: Oxford University Press, 1959. Published under the auspices of Imperial Chemical Industries, Ltd.) 6*s*. 6*d*. net.

Electrolytic Manufacture of Chemicals from Salt

By Dr. D. W. F. Hardie. Pp. xii+74+7 plates. (London: Oxford University Press, 1959. Published under the auspices of Imperial Chemical Industries, Ltd.) 7*s*. 6*d*. net.

THE books under review are the second and third in a series which is being published by Imperial Chemical Industries, Ltd., with the assistance of the Science Masters' Association and the Association of Women Science Teachers. The aim of the series is to help correlate academic chemistry as taught in schools, colleges and universities with chemistry as it is applied industrially.

Dr. Harding's book on ammonia has chapters on its manufacture, its properties and technical data, and its uses. There is also a bibliography and a short appendix on the application of physical chemical principles. The first chapter is by far the longest: it is concerned with the sources of the raw materials, the purification of hydrogen and nitrogen, and the actual synthesis process itself. Apart from the underlying chemistry, the mechanics and economics of the processes are briefly described.

Dr. Hardie, in his discussion of the salt industry, has chapters on the fundamentals of salt electrolysis, raw materials and energy, the manufacturing processes, packing, storage and transport of the products, production statistics and trends, physical and chemical properties of the products, principal uses of the products, and an outline of historical development. In addition, there are a bibliography, a brief list of important dates in the development of the industry, and a glossary.

Both volumes deal with their respective subjects clearly and in a way that should appeal to sixth-form student, university undergraduate and graduate alike. The authors bring home to the non-industrial chemist the interplay of chemistry, engineering and economics which is such an important part of industrial chemistry. Perhaps the most valuable service to the academic chemist is the way in which the authors show how the production of one particular chemical is co-ordinated with that of so many others in modern chemical industry. An example of this, quoted from Dr. Hardie's book, is that of chlorine production. Electrolytic chlorine production must go hand in hand with caustic soda production (sodium production is of relatively minor importance). However, recently the demand for chlorine for the production of chlorinated organic compounds has outstripped the demand for caustic soda. Hence investigations have been made into the aerial oxidation of the hydrochloric acid which is produced as a by-product of organic chlorination processes The academic chemist finds to his surprise that the obsolete Deacon and Weldon processes are likely to be resuscitated on economic grounds

These two books are well illustrated with line diagrams and plates The authors and publishers are to be congratulated on these books which keep up the high standard set by the first volume in this series *Sulphuric Acid Manufacture and Uses*, published by the Kynoch Press for Imperial Chemical Industries, Ltd in 1955 M J NEWLANDS

ISOTOPES IN HÆMATOLOGY

The Use of Isotopes in Hæmatology

By L G Lajtha Pp x+83 (Oxford: Blackwell Scientific Publications, Springfield, Ill Charles C Thomas, 1961) 21*s* net

THE application of isotopic techniques to hæmatological problems is standard procedure in modern medicine All too often their use has assumed the significance of a status symbol. Estimation of blood and plasma volume, the mean life-span of the erythrocyte and the absorption of radioactive cobalamin are procedures of established value There is therefore a place for an authoritative account covering these and related topics

The first two chapters of Dr Lajtha's book describe the estimation of plasma and red cell mass and the estimation of the mean cell life of the erythrocyte The next two are concerned with radioactive vitamin B_{12} and iron, and there are two short chapters on white cell and platelet tagging and autoradiography

The section on autoradiography is undoubtedly the best and reflects the author's vast experience in this field The technique of preparing autoradiographs is set out in precise detail and the description is warmly recommended to anyone proposing to work in this field The other sections are less happy The description and comment may well meet the need of the general hæmatologist who looks for guidance in setting up and interpreting tests involving the use of isotopes, but is unlikely to please the expert in the various fields

Surface counting techniques are mentioned in somewhat general terms, but a detailed account of how one should set about doing them and how to interpret the results is absent The estimation of mean cell life using chromium-51 is always of interest in a hæmolytic anæmia, but occasionally information about the site of destruction of the red cells may provide decisive information as to whether splenectomy is indicated or not Simultaneous iron-59 and chromium-51 studies, the former providing the additional information about the site of red cell formation, are often performed together and perhaps should have found a place in this book

Equally important is the emphasis the author places (or fails to place) on various procedures Both an *in vitro* and *in vivo* method for labelling red cells with chromium are described and little guidance is given as to which is the method of choice

Similarly, four methods of estimating absorption of vitamin B_{12} labelled with cobalt-58 are listed and discussed, namely, the fæcal excretion test, the urinary excretion test, the hepatic uptake test and the plasma test The change in plasma radioactivity after an oral dose of vitamin B_{12} does not merit consideration as a routine test of absorption of vitamin B_{12} even if material of sufficiently high specific activity were available It certainly has no place as a screening procedure On the other hand, many would regard the hepatic uptake of vitamin B_{12} labelled with cobalt-58 after an oral dose in a far more favourable light than does Dr Lajtha It is independent of the ability and willingness of the patient to collect his fæces (? for 15 days) or urine

Finally, a section devoted to the behaviour of the limited range of isotopes of use in the hæmatological laboratory and to the equipment that the hæmatologist could most profitably invest in in order to count these isotopes would have greatly enhanced the value of the book I CHANARIN

ZOOLOGICAL LITERATURE FOR 1958

The Zoological Record

Vol 95 Records of Zoological Literature relating chiefly to the year 1958 Edited by G Burder Stratton, assisted by Marcia A Edwards Pp v+2234 (London Zoological Society of London, 1961) 160*s*

THE present volume of *The Zoological Record*, relating chiefly to the literature of 1958, is bigger than ever, and provides good evidence, if this were necessary, of the great expansion of the subject in recent years The *Record*, apart from being a necessity to anyone engaged in taxonomic studies, also furnishes a good indication of the amount of work being carried out in the different groups of the animal kingdom In addition, the valuable system of classifying publications according to subject enables one to see the relative attention being devoted to the various aspects in each of the eighteen groups of animals listed A study of the Insecta, by far the largest section, comprising 660 pages, shows an increasing number of publications dealing with physiology and ecology, and in the subject index the number of pages under each heading is as follows: insect morphology 9, development 12, physiology 21, bionomics 10, ecology 19, evolution 8, geographical and faunistic 27 The main divisions show little difference from those in recent years Mollusca, with 187 pages, is followed by Protozoa 147, Pisces 141, Vermes 131, Mammalia 125, Arachnida 123, Aves 112, Crustacea 110, Amphibia 100, Reptilia 93, Trilobita 86 and in gradually diminishing size to Porifera 7 The number of new genera and subgenera is nil in Protochordata, one in Amphibia, and reaches a maximum of 949 in the Insecta It is noteworthy that the total number of new genera and subgenera has shown no diminution in recent years and as evidence of the amount of taxonomic work in progress does not support the pessimistic views of certain authorities concerning the dearth of taxonomists

This is the last volume to be issued in bound form Starting with Volume 96 (literature for 1959) the separate sections will be sent to subscribers as soon as they are published, a title-page and binding case will be forwarded when the volume is complete This will bring information of current research to users of the *Record* more quickly In addition, the format, beginning with Volume 96, will be changed to crown octavo, to give the larger type area necessary for recording the expanding volume of literature each year EDWARD HINDLE

Elsevier's Dictionary of Automation, Computers, Control and Measuring in Six Languages

English/American, French, Spanish, Italian, Dutch and German Compiled and arranged on an English Alphabetical base by W. E Clason. Pp. vi+848. (Amsterdam . Elsevier Publishing Company , London . D. Van Nostrand Company, Ltd , 1961) 140*s*

THE increase in technical and scientific knowledge has occasioned a crying need for multilingual technical dictionaries Bilingual technical dictionaries (many of which are of indifferent standard) are common enough, multilingual dictionaries are less common The present *Dictionary* is one more attempt to make good the deficit

There are 3 390 entries with an excellent index in each of the five non-English languages giving quick access to any of the entries in any of the languages used. Clearly, because of the extent of the scientific field covered, there must be a priority among the entries selected since it is not be to expected that 3,390 entries could cover such a number of highly specialized fields As with all choices, the present one cannot be expected to meet with wholesale approval or to be everyone's taste and must inevitably be a function of the compiler's bias Nevertheless I question why, for example, there are no entries for 'electronic counter', 'core', 'obey', whereas there are entries for "Westphal balance" and "mobilometer". On the other hand, it is only fair to point out that one welcomes the (unexpected) entries "tape travel" and "pigeon hole pocket". One might comment at random in this way but it does not detract from the fact that this appears to be an excellent addition to the long line of Elsevier multilingual dictionaries covering other scientific fields Its price may place it outside the average scientific pocket, but it will certainly be of help in the libraries of scientific and industrial research laboratories

The printing, binding and lay-out of the work are of the high standard and quality one has long come to expect from Elsevier. S. WHELAN

Practical Microscopy

By Dr C L Duddington Pp ix+237. (London Sir Isaac Pitman and Sons, Ltd , 1960) 30*s* net.

Histological and Histochemical Technics

By Prof Harold A Davenport Pp xxii+401 (Philadelphia and London W B Saunders Company, 1960) 49*s*

Section Cutting in Microscopy

By Dr H F Steedman Pp. vi+172. (Oxford Blackwell Scientific Publications, Springfield, Ill . Charles C Thomas, 1960) 21*s* net

SO many books on microscopy and microscopical technique have appeared in the past few years that new publications on these subjects may seem unwarranted All three books reviewed here, however, seem to have much to justify their publication Dr Duddington has addressed his work to the serious amateur, and to the elementary student of biology He assumes no previous scientific knowledge, and introduces the reader in turn to the component parts of the microscope and to some of the basic techniques of microscope manipulation The second part of the book consists of an introduction to the methods of preparing botanical, zoological and bacteriological specimens for investigation There is a short bibliography, listing most of the standard reference works on the subject, some of which may, however. prove too technical for those who have only just taken up this type of study. Perhaps a future edition might grade the references and indicate those likely to prove most useful for elementary students. The book is well produced and clearly illustrated.

Histological and Histochemical Technics, by Prof Davenport, is designed for the advanced worker, and is concerned entirely with methods of preparing specimens It provides a survey in some detail of the processes of fixation, embedding and staining, including an extensive discussion of their more theoretical aspects as well as the actual formulæ One useful chapter describes techniques of staining in the block, a method of preparation which seems to attract comparatively little interest at the present. The final chapters are devoted to a brief survey of histochemical methods. and would serve as a good introduction and source-book for those who use such methods only occasionally

Section Cutting in Microscopy, by H F Steedman, is a book largely for the expert It presents details of all the usual methods of microtomy, in addition to describing several new techniques developed by the author The approach is logical throughout and the author has made a most commendable attempt to rationalize some of the more common microscopical techniques It is pleasing to see the inclusion of a section on possible faults which may present themselves during the cutting of paraffin sections Other chapters deal very fully with the use of ester waxes, the water-soluble waxes and with the use of urea formaldehyde resin There is also a chapter on the preparation of ultra-thin sections for electron microscopy This book is a welcome addition to the reference works which one expects to find in every histological laboratory S BRADBURY

The Theory of Crystal Structure Analysis

By A. I Kitaigorodsku Translated from the Russian by David and Katherine Harker Pp. xi+275 (New York : Consultants Bureau, Inc , 1961) 12 50 dollars

IN the preface of this book, which appeared in the original Russian version in 1957, the author makes it clear that he is describing his subject-matter up to the year 1955 It is perhaps a tribute to the rate of development in this field that many of the author's analyses and opinions seem either outmoded or bizarre at the present time In particular, the analyses concerned with distribution functions have been repeated more formally and elegantly in the past few years ; the author also dismisses anomalous dispersion as of 'no importance' and the difference synthesis as 'devoid of interest' (this even drew a translators' comment).

However, much of the book is devoted to the basic theories of the Patterson method, intensity statistics and direct methods and, for these, lack of topicality is of less consequence The treatment of these topics is such as will commend itself either to the crystallographer of mathematical inclinations or to the mathematician with some knowledge of crystal structure analysis The section dealing with inequality relationships is of particular interest, being concerned. to a large extent, with the author's own excellent work. The book reads well, which is symptomatic of the quality of the translation.

To summarize. a work of considerable interest to those able to appreciate it and one the main faults of which are due to the lapse of time since it first appeared. M M. WOOLFSON

THE ROYAL SOCIETY

ANNIVERSARY ADDRESS*

By Sir HOWARD FLOREY, F.R.S.

AWARD OF MEDALS, 1961

Copley Medal: Sir Hans Krebs, F.R.S.

SIR HANS KREBS has made outstanding contributions to knowledge of the chemical pathways of metabolism. In 1932, with Henseleit, he used tissue slices to demonstrate the synthesis of urea and found that additions of ornithine stimulated the process. Ornithine was regarded as uniting with carbon dioxide and ammonia to give arginine, with citrulline as an intermediate. The enzyme arginase liberated urea and a molecule of ornithine, so that the process could begin again. Subsequent research has shown that the postulated cycle is essentially valid, although more detail has been introduced, carbamyl phosphate, argininosuccinate and adenosine triphosphate have been brought in and aspartic acid has replaced ammonia in the reaction with citrulline. The more complicated version owes much to the fundamental work initiated by Krebs in 1937 on the tricarboxylic acid cycle. This process is now seen as the main route for oxidizing the two-carbon fragment produced in the biological degradation of carbohydrates, fatty acids and amino-acids.

The initial acceptor of acetyl coenzyme *A* is oxalacetate and the completion of the cycle is marked by regeneration of a molecule of oxalacetate, and by the oxidation of one C_2 fragment to carbon dioxide and water. The product of the interaction of oxalacetate and acetyl coenzyme *A* is citric acid, and the later steps in the cycle result in the liberation of carbon dioxide and pairs of hydrogen atoms. These latter finally unite with oxygen to form water but the intermediate stages are coupled with the formation of adenosine triphosphate from adenosine diphosphate. The Krebs cycle does not allow for the net synthesis of the intermediates, which are themselves important in providing the carbon framework for many biosyntheses. The main cycle therefore needed to be reinforced by postulating ancillary mechanisms leading to the synthesis of available four-carbon intermediates. Krebs's work on the glyoxylate cycle showed that iso-citrate, by yielding succinate and glyoxylate, permitted the formation of malate by the interaction of acetyl coenzyme *A* and glyoxylate. The malate led directly to the oxalacetate needed by the main cycle, and the succinate could be used as a precursor or could permit the replacement of intermediates drawn out of the cycle by synthesis of cell substances.

The very complex overall picture is both useful and elegant. Nobody deserves more credit for this than Krebs.

A Royal Medal: Prof. C. F. Powell, F.R.S.

Prof. Powell is distinguished by his outstanding contributions to our knowledge of cosmic rays and of the fundamental particles.

He started research under Prof. C. T. R. Wilson and made important contributions to the problem of condensation of water vapour to form drops at elevated temperatures. He then worked with Prof. A. M. Tyndall at Bristol on the mobility of positive ions in gases. This was a difficult subject, bedevilled by the effects of impurities, and Tyndall and Powell raised it to new levels of definiteness and reliability.

About twenty years ago Powell turned his attention to the development of the use of photographic techniques for studying the interactions and other properties of fast particles. Up to that time such techniques were rarely used but, through collaboration with those concerned with the production of photographic emulsion, Powell established a new and powerful technique which ever since has played a most important part in the study of energetic particles.

The first applications were to the study of nuclear interactions, but quite soon the technique was applied to investigate cosmic radiation. This soon resulted in the discovery with Occhialini of the π-meson by analysis of the tracks of cosmic ray particles in emulsions exposed at high altitude in the Bolivian Andes. Further major discoveries followed from the work of the laboratory at Bristol which was so enthusiastically led by Powell. These included the τ-meson and evidence for the decay of neutral mesons into γ-radiation. Improvements in emulsion technique to increase the rate of detection of fast electrons resulted in the recent observation of multiple meson production in nuclear encounters.

Apart from these outstanding individual discoveries, Powell and his collaborators have made a great number of significant contributions to many aspects of cosmic ray and fundamental particle studies. They have introduced new and improved techniques whenever the scope of existing techniques has become inadequate. Thus they developed the use of balloons to carry emulsions to high altitudes for cosmic ray studies and they introduced the emulsion stack which made it possible to follow the tracks of very fast particles, right through to their end-points.

With the introduction of artificial accelerators capable of providing beams of particles of energy comparable with the main bulk of cosmic ray particles, Powell and his group have turned part of their attention to fruitful use of emulsion stacks to study, in more detail than hitherto, the properties of mesons and hyperons. They have, however, taken up as well the difficult problem of studying the ultra-high-energy cosmic radiation and already have obtained information of interest.

Powell has not only made many outstanding personal contributions to this work but has been, through his enthusiasm and organizing ability (which has involved large-scale international co-operation), a continual source of inspiration to everyone concerned. The pioneering work at Bristol has been, and will continue to be, of the greatest importance in the physics of high-energy phenomena and of the fundamental particles

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* Delivered at the Royal Society on November 30.

A Royal Medal. Sir Wilfrid Le Gros Clark, F R S

Sir Wilfrid Le Gros Clark is internationally known for his work in two apparently unrelated fields, namely, neuro-anatomy and primate evolution However, an important feature of his neurological work has been the way in which he has used the evidence of comparative anatomy to shed light on the evolution of the human brain His work on the connexions of the thalamic nuclei with the cortex and the hypothalamus is especially noteworthy in this respect He and his collaborators have studied the inter-relationships of various parts of the brain using in particular methods depending on retrograde and trans-neuronal degeneration He was able to show that following a small retinal lesion, localized patches of atrophy appeared in the lateral geniculate body and by these means he mapped the projection of the retina on the lateral geniculate body and on the cortex Other investigations of this type have added considerably to our knowledge of the olfactory system and have removed misconceptions concerning the relationship between the hippocampus and the rhinencephalon

Le Gros Clark is generally recognized as a leading authority on primate evolution His early work on the anatomy and behaviour of *Tarsius*, based on painstaking personal observations in Borneo, is a model of its kind During the past fifteen years he has played a leading part in the examination of many important fossil discoveries, especially in Africa His critical assessment of the Australopithecinae and the tentative conclusion that they may be closely related to the ancestral stock from which the Hominidae are derived, is one of the landmarks in this branch of anthropology He was also closely associated with the unmasking of the Piltdown forgery, thus helping to remove a puzzling discrepancy from discussions on human evolution

Apart from these major fields of work Le Gros Clark has contributed much to general anatomical and biological problems His work on muscle regeneration, for example, showed elegance and clarity, and as honorary director of the Medical Research Council Ergonomics Research Unit he has supervised research in a new and expanding field and has given a new impetus to applied anatomy

Davy Medal Prof D H R Barton, F R S.

Prof Barton's researches have for the most part been concerned with the chemistry of natural products, a field which has provided him with numerous opportunities to demonstrate his brilliance in the elucidation of complex structures, the unravelling of stereochemical complications, and the elaboration of syntheses He has always taken every opportunity to make good use of physical methods in solving problems of organic chemistry, and much of his earlier work on the applications of differences of molecular rotation, particularly in the terpene and steroid fields, has had far-reaching effects on our knowledge of stereochemical relationships. His pioneering work on conformational analysis in alicyclic systems has revolutionized concepts of the relationship between stereochemistry and reactivity More recently he has been studying long-range effects, by which, for example, a remote ethylenic linkage can exercise a marked influence on the condensation reactions of a carbonyl group in a triterpene system

His notable contributions to the establishment of the structure and intricate chemistry of caryophyllene have been followed by fruitful investigations on many other sesquiterpenes, such as tenulin, pyrethrosin, lactucin, and geigerin, while in the di- and tri-terpenoid series partial or complete structures have been established for a substantial number of natural products, these include the citrus bitter principle, limonin, the structure of which had defied elucidation for a considerable time, and clerodin, also a bitter principle His investigations on steroids and their derivatives have been prolific, and include some particularly elegant work on cevine and related steroid alkaloids The biogenetic mechanisms by which natural products are built up from simple precursors in Nature have interested him for some time, and studies on these processes led to a simple two-stage synthesis of usnic acid from methylphloracetophenone

The most remarkable results of his researches have probably been those arising from his investigations on the photochemical transformations which sometimes occur when compounds are irradiated with ultra-violet light The classical work carried out more than sixty years ago on the irradiation of santonin gave some indication of the problems involved in studying such reactions, but Barton has succeeded in establishing the structures of the three products, isophotosantonic acid lactone, photosantonic acid, and lumisantonin, and has explained mechanistically the remarkable ways in which these unusual compounds are derived, he has shown, furthermore, that other *cyclo*hexadienones, such as prednisone, behave similarly Quite recently he has used photochemical methods to bring about an attack, by a group in a favourable stereochemical position, on a non-activated carbon atom, and by most brilliant applications of this method he has achieved spectacular syntheses of aldosterone acetate (in three stages from corticosterone acetate) and of 19-noraldosterone acetate Perhaps even more remarkable is his beautiful synthesis of conessine, in which the heterocyclic ring involving C_{18} is formed by photolysis of a steroidal C_{20} azide

The brilliance and originality of Prof Barton's work has gained him an international reputation and his laboratories at the Imperial College of Science and Technology have become a rendezvous for research workers from many countries

Sylvester Medal Prof P Hall, F.R.S

In his first two papers on finite group theory, Prof Hall broke new ground in the fields of soluble groups and groups of prime-power order In 1928 he established the validity of soluble groups of some striking extensions of the classical theorems of Sylow, thereby opening a new epoch in the study of finite soluble groups Five years later he laid the foundation for all subsequent work on groups of prime-power order with a long and systematic paper, the many original and fruitful ideas of which included that of the regular *p*-group In the years between the appearance of these landmarks in the history of group-theory and the War he published many other papers on groups of finite order

The culmination of this extremely productive period occurred at the group-theoretical colloquium at Gottingen in June 1939, the central feature of which was a series of lectures by Hall, afterwards published in *Crelle's Journal* in 1940. His expositions of a classification theory for prime-power groups and of a structure theory for soluble groups, and his papers on automorphism groups and on verbal and marginal subgroups, have taken their place in the canon of essential material for any student of finite groups, and together embrace many of the leading themes of his work up to that date.

Hall's election into the Saldeirian chair of pure mathematics at Cambridge in 1953 marked the beginning of another sequence of publications of the greatest importance. In the intervening years much work had been done—notably by Russian mathematicians—on extensions and generalizations of Hall's theorems on soluble groups, and in 1956 Hall gave a systematic account of these and earlier results on the existence, conjugacy and inclusion properties of subgroups with coprime orders and indices, adding many new results of his own. Another remarkable work on finite groups, carried out in collaboration with Graham Higman, dealt with p-soluble groups and had as its most striking application certain reduction theorems for a celebrated conjecture of Burnside's.

Hall's researches have thrown much light on the interrelation of various finiteness conditions which may be imposed on groups of infinite order, and they have been illustrated by many examples constructed to delimit the range of applicability of the results. Among other types of infinite group considered by Hall may be mentioned Abelian-by-nilpotent groups, locally finite groups and periodic FC-groups.

Hall's influence on British algebraists—of whom he is the acknowledged doyen—has not been confined to specialists in the branch of algebra with which Hall's name, like that of his forerunner Burnside, will be enduringly linked.

Hughes Medal: Prof. A. H. Cottrell, F.R.S.

Prof. Cottrell's contributions to the field of structural metallurgy have been outstanding whether they be regarded from the point of view of the physicist, the metallurgist, or the engineer. Utilizing to the full the current methods of the physics of metals, and in particular the pioneer work of Taylor, Mott and Frank on dislocations, Cottrell has been able to explain, in a remarkably clear physical manner, many important mechanical properties of metals. Typical of this work is his early explanation of the yield-point phenomena in steels, which he showed to be associated with the anchoring of dislocations by solute atoms. He has become a leader in the understanding of the many and decisive ways in which crystal defects, particularly dislocations and vacant atomic sites, control the behaviour of metals in practice.

Engineers and physicists alike owe much to Cottrell's books on *Dislocations in Crystals* and on *Theoretical Structural Metallurgy*. Remarkable for their clarity of thought and composition, they have been a major cause of the current trend towards a more fundamental understanding of their materials by both engineers and metallurgists. In the past decade, British metallurgy has found much inspiration in the work of Cottrell and his associates.

In recent years Cottrell has given attention to irradiation problems arising in nuclear power developments. He has applied his ideas to the effects of irradiation on the properties of both metals and non-metals, and his name is particularly associated with irradiation-induced creep in anisotropic materials such as uranium; but much more than this has been done. It is fair to say that the effects of irradiation on the growth, swelling and creep of uranium, on precipitation in alloys, brittle fracture of steel, and energy storage in graphite have now a solid theoretical background which is largely due to Cottrell.

Leverhulme Tercentenary Medal: Sir Cyril Hinshelwood, F.R.S.

This is the first occasion on which the Leverhulme Medal has been awarded. It will therefore be appropriate to remind Fellows of the circumstances in which it was instituted. In 1960 the Council of the Society accepted an offer from the Trustees of the Leverhulme Trust Fund of a gold medal to mark the tercentenary of the Society. The medal has been named the Leverhulme Tercentenary Medal and is to be awarded every three years to the individual who, in the opinion of the Council, has made the most significant contribution in the field of pure or applied chemistry or engineering. The first Leverhulme Tercentenary Medal has been awarded to our immediate past president, Sir Cyril Hinshelwood.

Few working scientists to-day have not profited from a study of one or more of the profound and far-ranging contributions which Sir Cyril Hinshelwood has made to the advancement of natural knowledge. The range of his interests and attainments is enormous and he has touched nothing which he has not adorned. His publications illustrate not only the diversity of his learning and culture but also its basic unity.

The Leverhulme Tercentenary Medal is being awarded to him for his contributions to pure and applied chemistry. For several decades he has been recognized as one of the leading physical chemists of the world. His work on chemical kinetics is classical; it has combined simplicity and elegance of experimental approach with far-ranging theoretical implications.

He first clarified the mechanism of reactions in the gaseous phase in terms of molecular collisonal processes and molecular activation, and directed attention to the essential characteristics which arise from chain propagation in thermal systems. This work has since stimulated the application of many other methods to the determination of intermediate reactive species. He has made similar contributions to our understanding of reaction mechanisms in both the liquid and solid phases. By applying the same principles to systems involving bacterial growth he has been able to separate phases in the growth process and to examine the effect of inhibitors and the mechanism of drug resistance, and the effect of varying growth media on the growth process. He has investigated the cellular content of nucleic acids during the growth cycle and also the specific effect of elements such as potassium, sodium, phosphorus and sulphur. These researches make common ground between the chemical and biological sciences. Nor has his work been confined to basic science; he has done memorable things in the application of chemistry to useful ends.

He has not only worked as a scientist but, tirelessly, for scientists; for the University of Oxford, for international science, for scientific societies in general and for the Royal Society in particular.

We rejoice that the Leverhulme Medal is being given to a most eminent chemist, and we regard it as particularly happy that a medal which commemorates the tercentenary is going to the man who presided over that great event with such eloquence, dignity and grace.

ACTIVITIES OF THE ROYAL SOCIETY

It is a pleasure at the end of a year of office to record that I can heartily endorse all that has been said by my predecessors of the admirable way in which the interests of the Society are fostered by Dr. Martin and his staff. The smooth running of its many activities depends to a very large extent on their efforts and I personally am greatly indebted to them for all the help I have received during the past year.

The tasks of my fellow Officers are many and various. We are all very grateful to the Treasurer for the care and attention which he has devoted to the Society's affairs. The Secretaries not only have had to work with their accustomed vigour at their increasingly heavy tasks, but they have had to exercise more than their usual forbearance in dealing with my many naïve questions. I am sure the Society would wish me to thank all these Officers for their work on its behalf.

The Officers and Council always hope that the report of Council has been read by the Fellows, and in any case you will not want me to give you now a catalogue of the Society's activities. There are, however, one or two points which call for comment.

The Society has been so fortunate as to receive a great benefaction from the Wellcome Trust for the establishment of a professorship. We are, indeed, very grateful for this gift, and we are delighted that the name of Sir Henry Dale is to be permanently associated with it, for he has ably served not only the Wellcome Trust but also the Society and, indeed, science generally.

During the past year a number of Fellows have devoted considerable time to surveying the needs of biological research in Britain. Their survey leaves little room for doubt that much more could be done to encourage advances in fundamental knowledge and to foster their application. This may well apply to many branches of physical science as well.

The earning of the national living becomes increasingly difficult in a fiercely competitive world, and it would seem to be self-evident that the country should be straining every nerve to foster science and technology. We must therefore deplore the evident signs that some scientific activities, which to a large extent depend on Government aid, are to be curtailed, or at least are not to be allowed to expand at the present time. Even if this is a 'temporary measure', as it is claimed to be, damage is being done to the confidence of scientists and technologists. They observe that those who control the situation have apparently not yet recognized that the brains of the citizens are one of our greatest remaining national resources, and that the country's future prosperity will be, in a large measure, dictated by the use that is made of this asset.

There are good grounds for believing, for example, that the creation of new senior scientific positions, such as professorships in universities, is not even keeping pace with the expansion in the number of undergraduates. The opinion has been expressed by many Fellows that Britain particularly needs more professorships equipped with adequate research facilities if it is to give reasonable and proper encouragement to all its best men and women. Perhaps we can all do something towards letting it be widely known that we are by no means satisfied with the present overall position.

The maintenance of relations with foreign academies and scientists is one of the important activities of the Royal Society. During the past year this phase of our work has been fully maintained. There have, for example, been visits from Russian and Chinese colleagues, and in September very successful meetings of the Bureau, Executive Committee and General Assembly of the International Council of Scientific Unions were held in London under the sponsorship of the Royal Society. It is very much in the mind of Council to increase as much as possible all activities which impinge even remotely on maintaining contact with colleagues both from the Commonwealth and from other countries. We can only hope that we shall be able to secure means adequate for the task. Not the least of our problems is lack of accommodation for this and other responsibilities that we seek to discharge. Although I cannot give any satisfactory assurances on this point now, strenuous efforts are being made to try to overcome this very serious handicap.

EXCHANGE OF SUBSTANCES BETWEEN THE BLOOD AND TISSUES

On these annual occasions the President has commonly taken the opportunity to make some remarks about matters of scientific interest. This, I suspect, is done mainly to demonstrate that he is not entirely out of touch with scientific experiment. More than once a President has pointed out the impossibility of having a sufficiently wide knowledge to comment adequately on the many outstanding scientific discoveries that are being made under modern conditions. In following in the presidential footsteps, I have been considerably influenced by the remark made by one of my more candid friends, that I was not so highbrow as my predecessors, and by a

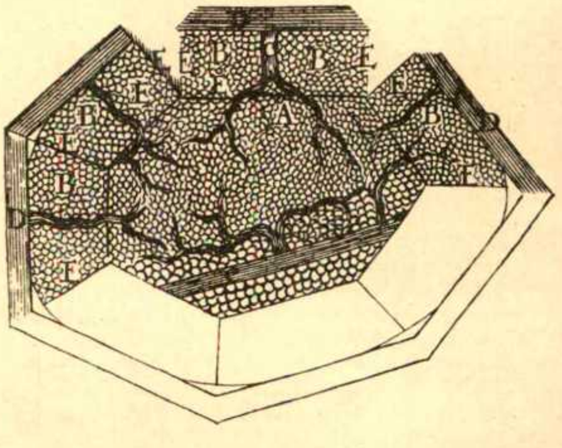

Fig. II. *Continens Cellulam ſimpliciſſimam abſque intermediis parietibus aucta in magnitudine.*

A *Cellulæ area interior.*

B *Parietes divulſi & inclinati.*

C *Arteriæ pulmonariæ truncus cum appenſis ramis, quaſi reticulato opere deſinentibus.*

D *Venæ pulmonariæ truncus parietum faſtigia pererrans ſuis decurrentibus ramis.*

E *Vas in fundo & angulis parietum commune lateralibus, & continuatis retis ramificationibus*

Fig. 1. The first illustration of the capillary circulation with its legend. Malpighi described the capillary circulation in a frog's lung in a letter to Borelli which was published in 1661

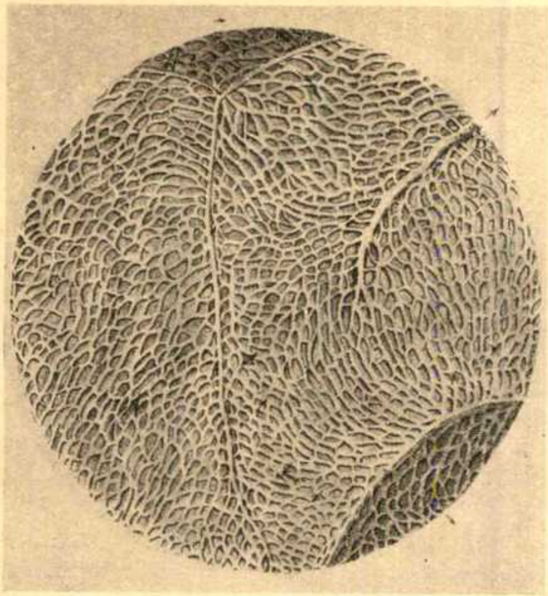

Fig. 2. The capillary circulation in a frog's lung, from a work published by Marshall Hall in 1831 entitled *A critical and experimental Essay on the Circulation of the Blood, especially observed in the minute and capillary vessels of the Batrachia and of Fishes*

comment of another colleague after I had had the honour of delivering a lecture to the Society—he said that it had been a good idea to turn the lights on and off frequently as it kept everyone awake.

Following these hints, therefore, my aim is very modest in that I propose to review and to illustrate briefly some researches in biology the history of which is almost exactly coeval with that of the Royal Society, and which have been greatly influenced by the development of special physical techniques during the past ten years.

In 1628, Harvey published his great work demonstrating the circulation of the blood with irrefutable clarity. The blood left the heart by the arteries and returned to it by the veins. It was not, however, until 1661 that an Italian, who a few years later became a Fellow of the Royal Society and whose portrait now hangs in the Society's house, discovered the vessels which connect the arteries to the veins. I refer, of course, to Marcello Malpighi. By means of his very early compound microscope he observed in the living animal what we now know as blood capillaries.

He saw and illustrated the branching of the pulmonary artery to form a network in the walls of a vesicle in the lung of a frog. The network united to join a pulmonary vein (Fig. 1).

Another Fellow of the Royal Society, Antony van Leeuwenhoek, amply confirmed and greatly extended Malpighi's observations in a large number of species, so that by 1688, in which year Leeuwenhoek sent a letter to the Royal Society describing his observations, there was sufficient evidence to justify the conclusion that blood capillaries were present in all the tissues of animals with a blood circulation.

No great progress was made in the next century and a half in the understanding of the structure of the smallest blood vessels. In 1831, Marshall Hall, a Fellow of the Society who has some claim to have discovered reflex nervous action, wrote: "It is singular that in the midst of the dispute concerning the irritability of the capillaries, there should be a doubt whether the vessels be real tubes or mere canals. There are many reasons which incline me to the latter opinion". That is to say, he thought that they were probably mere spaces in the tissues rather than tubes with their own distinctive wall (Fig. 2).

The knowledge of the structure of the capillary wall was strikingly expanded about the middle of the nineteenth century by the discovery that treatment of the blood vessels, including the capillaries, with dilute silver nitrate resulted in the appearance of a network, which was rightly considered to represent the boundaries between contiguous lining cells. This layer of lining cells, which has proved to be present in all the vessels of the body, was given the name of endothelium (Fig. 3).

As a result of this and other histological observations, the walls of the capillaries were conceived of as being composed of a mosaic of flat cells which were joined together by a cement substance, represented by the material stained black by silver nitrate. In these vessels there are few supporting cells, and the wall is, in effect, composed of endothelial cells with such fibres and tissue matrix as may be closely apposed to them.

It is through the walls of the fine blood vessels that most of the important exchanges of material between the blood and the tissues take place. The walls of the fine blood vessels appear to form a membrane which is freely permeable to gases, such as oxygen and carbon dioxide, which pass to and fro by diffusion. It is thought that substances like sodium chloride and glucose also pass freely, but that the normal capillary wall is almost, though not quite, impermeable to proteins. Ernest Starling, at the end of last century, adumbrated the theory that the capillary wall was of the nature of a semi-permeable membrane, across which the plasma proteins were able to exert an osmotic pressure, and that normally there was a near balance between the hydrostatic pressure of the blood tending to filter fluid out of the vessels and the osmotic pressure of the plasma proteins tending to draw fluid in from the surrounding tissues. This theory of Starling's was given strong support by the experiments of Landis, who measured directly the forces involved, but although Starling's theory is still almost universally accepted it has not been immune from criticism.

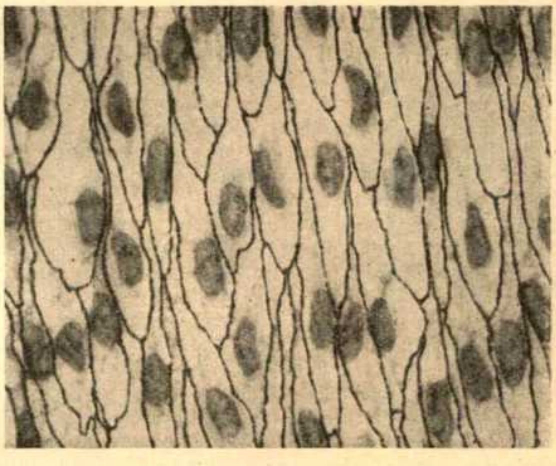

Fig. 3. Endothelium lining the aorta of a rabbit, viewed *en face*. The flat nucleated cells have been outlined by treatment with silver nitrate. (× 390)

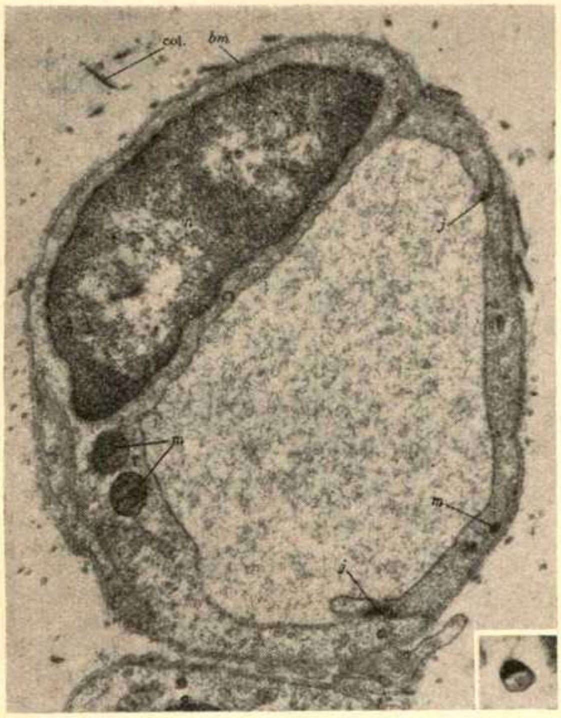

Fig. 4. Electron micrograph of a capillary, seen in cross-section, from the subcutaneous tissue of a guinea pig. At this site the wall is composed of parts of two cells; and a nucleus (*n*), mitochondria (*m*), two cell junctions showing adhesion belts (*j*), and many cytoplasmic vesicles can be seen. A basement membrane (*bm*) surrounds the vessel, and external to it collagen fibres (col.) have been cut longitudinally and transversely. (× 12,600.) Inset is a cross-section of a similar capillary viewed with a light microscope with a 2-mm. oil-immersion objective. (× 780)

Much attention has been directed to the detailed structure of the living membrane which was thought to have these semi-permeable qualities. When viewed in cross-section by the light microscope with a 2-mm. oil-immersion lens the capillary wall seems to be composed of a very thin membrane with little recognizable structure except for the nuclei of the endothelial cells, which make a bulge where they occur (inset to Fig. 4.). A basement membrane composed of polysaccharide, and not clearly differentiated from what is known as the pericapillary sheath, is also recognized as a regular constituent of the fine blood vessels.

It was conceived by some that the exchange of water and dissolved solids between the blood and the tissues took place through hypothetical pores in the intercellular cement which, on the basis of the appearances produced by silver impregnation, was thought to be present in substantial amounts. Others believed that minute pores existed in the cytoplasm of the endothelial cells themselves through which substances escaped from the blood. If either of these views were correct, it would not be difficult to visualize conditions in which Starling's hypothesis would be easily credible.

Work during the past ten years has added considerably to our knowledge of the structure of the capillary wall, knowledge which necessitates examining old theories further. This work is a good example of the influence of progress in one science on that in another. Our increased knowledge is due essentially to the invention of the electron microscope, for this remarkable instrument has opened up entirely new possibilities to the biologist who is attempting to correlate structure and function.

Another branch of the physical sciences is also involved in making this possible, for biologists are indebted to polymer chemists for embedding materials in which tissues can be cut sufficiently thin to be examined in the electron microscope.

The electron miscoscope reveals that the membrane forming the wall of a capillary, which is apparently almost structureless and might be likened to a piece of 'Cellophane' when observed in the light microscope, is composed of cells with all the usual cellular organelles (Fig. 4). As well as the nucleus there are small mitochondria, a Golgi complex and a centrosome. There are granules of ribonucleoprotein which are sometimes free and sometimes attached to vesicular structures. The junctions between the cells are regular narrow gaps about 200 Å. wide, and there is no sign of the apparently substantial band of intercellular cement substance which was thought to exist. At the cell junctions there is an electron-dense area which is known as an attachment belt or desmosome. It is thought to be the region at which the cells are most firmly attached to one another. Surrounding the vessel is a basement membrane which apparently contains very fine fibrils.

Palade, the first observer of capillary structure by the electron microscope, described intracellular vesicles which, though found in other cells, are particularly prominent in endothelium. On the luminal surface of the endothelial cell there are indentations of the bounding membrane known as caveolæ intracellulares. Similar caveolæ exist on the external surface of the cell. Between these, two surface profiles of vesicles, which are usually some 500–600 Å. in diameter, can be recognized in the cytoplasm of the cell (Fig. 5).

Palade proposed the theory that fluid is transported from the lumen of the capillary to the outside by means of these structures. According to this theory, a minute amount of plasma is enclosed in the caveola, which is then pinched off by a membrane to form an intracytoplasmic vesicle. The vesicle is ferried across the cytoplasm to be discharged on the external surface beneath the basement membrane. It is difficult to see how the cells of the capillary wall can exercise

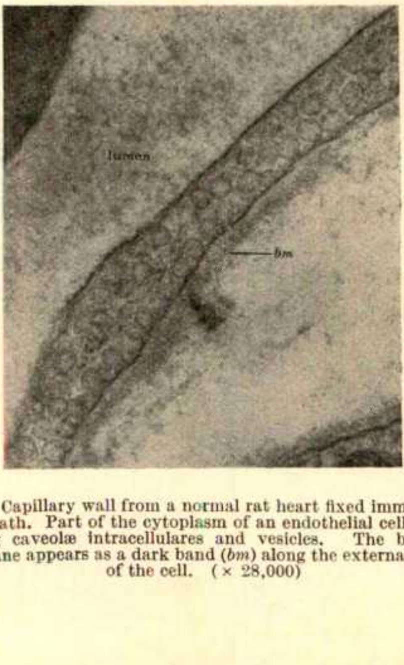

Fig. 5. Capillary wall from a normal rat heart fixed immediately after death. Part of the cytoplasm of an endothelial cell is seen, showing caveolæ intracellulares and vesicles. The basement membrane appears as a dark band (*bm*) along the external surface of the cell. (× 28,000)

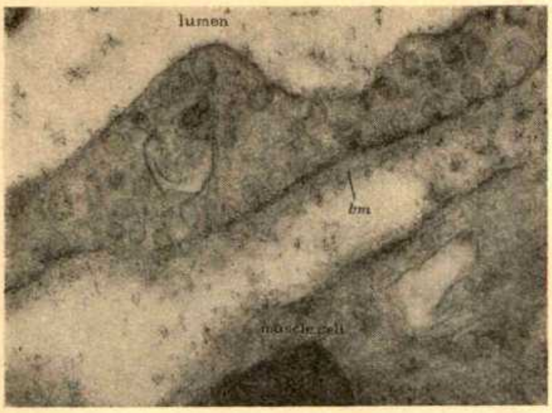

Fig. 6. Capillary from a rat heart perfused for 15 min. with saline solution containing particles of saccharated iron oxide. Particles are present in the lumen of the vessel and in the pericapillary space. They can be seen in the caveolae intracellulares on both the luminal and external surfaces of the cell, and in vesicles in the cell cytoplasm. *bm*, basement membrane. (× 33,600)

selective permeability if this is the mechanism for fluid transport, since the vesicles are very much bigger than protein molecules. To meet this difficulty Palade proposed that the selective permeability of capillaries resided in the basement membrane, which is usually a well-marked feature in the capillary wall.

Palade and Wissig attempted to show that the vesicles did, in fact, constitute a transport mechanism by injecting into the blood stream the protein ferritin, the large molecule of which contains sufficient iron to make it opaque to electrons. They found that ferritin molecules could be seen in some of the vesicles but that they also lay free in the cytoplasm. Later Palade used colloidal gold in the same way, and stated that the particles of colloidal gold were always found within the vesicles and not free in the cytoplasm, and he inferred that fluid must be transported through the endothelial cells in the vesicular system.

One of the difficulties attending such observations is to procure a sufficient concentration of the electron-opaque particles to act as efficient markers, but this can be overcome to a certain extent by the use of perfused organs. It is a standard pharmacological technique to make an isolated rat or rabbit heart beat for hours by perfusing it with a suitable oxygenated saline solution. It is possible, without stopping the heart beat, to add to the perfusion fluid a considerable amount of colloidal thorium dioxide or colloidal ferric hydroxide, the particles of which are, of course, opaque to electrons. Using such a preparation, Jennings and Marchesi have shown that the particles of ferric hydroxide pass into the open mouths of the caveolæ intracellulares on the luminal surface of the endothelium, and that they appear in the vesicles, in the cell cytoplasm, and in the caveolæ which open at the external surface. It seems fairly certain that these particles are in passage across the cell in the vesicular system in the manner proposed by Palade. There is not much evidence that the particles pass between the cells at the intercellular junctions. After they have passed through the cell there is no evidence from electron micrographs that the basement membrane forms much of a barrier to these particles, so that it is difficult to conceive that it would do so to molecules of protein (Figs. 6 and 7).

One may, therefore, picture the formation of caveolæ and vesicles as a process which is continuously altering the capillary wall and passing fluid from the lumen through into the tissue spaces. It is probable that the caveolæ are too large to account for the semi-permeable qualities of the endothelial membrane. The electron microscope, although its resolving power is sufficient to do so, has not revealed any smaller channels passing from the lumen to the outside of the capillary. Such channels would be necessary in a membrane which was semi-permeable by reason of the size of the pores in its structure. It still remains, therefore, to be explained in what structure or mechanism the semi-permeability of the normal capillary wall resides.

A morphological discovery entirely due to the electron microscope is that the capillaries do not have the same structure in all parts of the body. Those in the glomerulus of the kidney have small fenestrations through the cell cytoplasm, and those in the endocrine organs such as the pituitary body, the adrenals and the thyroid gland have gaps in the cytoplasm which are filled by a very tenuous membrane. Similar capillaries are found in the small and large intestines. There is no evidence, except in the case of the kidney, that these gaps are particularly permeable to proteins and their significance is not yet understood.

During the past forty years it has been demonstrated that certain substances which occur in the tissues of animals, such as histamine, 5-hydroxytryptamine, and bradykinin, have the property, when they are applied at great dilution, of making blood vessels more permeable than normal to proteins. Swelling or œdema is an important feature in many natural inflammations, such as those produced by bacterial invasion, and it is possible that substances of this nature which seem to be liberated in injured tissues are responsible for the increased permeability of the capillaries to proteins, leading to the accumulation of extravascular fluid. Majno and Palade have recently examined the small blood vessels in the cremaster muscle of the rat after it had been subjected to the influence of dilute solutions of the vasoactive substances that I have mentioned. They could find no

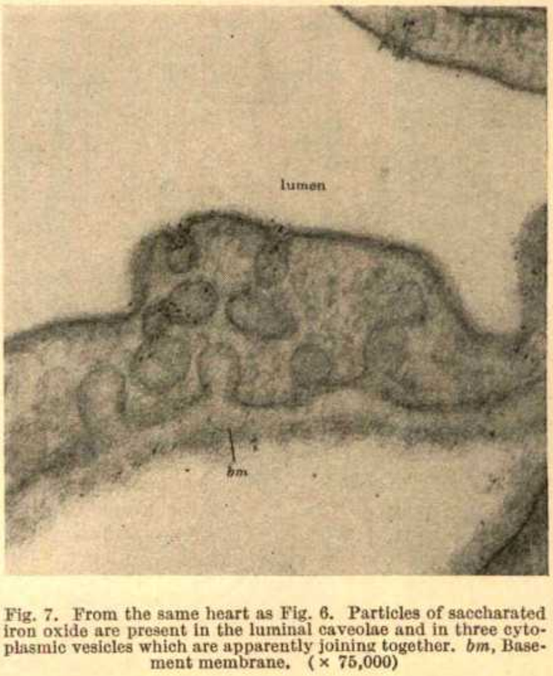

Fig. 7. From the same heart as Fig. 6. Particles of saccharated iron oxide are present in the luminal caveolae and in three cytoplasmic vesicles which are apparently joining together. *bm*, Basement membrane. (× 75,000)

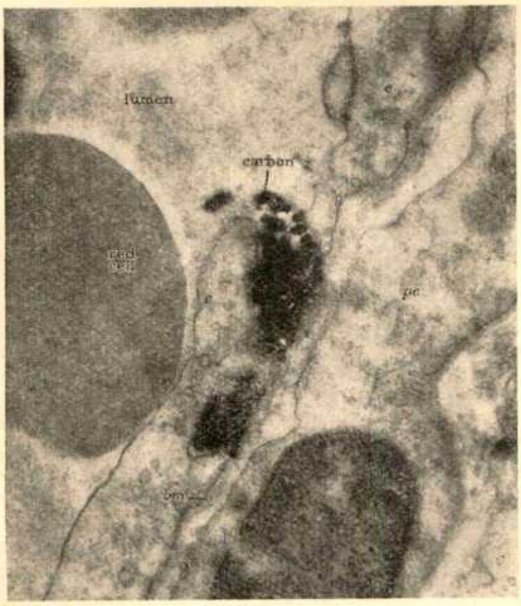

Fig. 8. Venule in the mesentery of a rat. A suspension of carbon was injected intravenously 1 min. after the mesentery had been inflamed by mechanical injury, and the tissue was fixed 3 min. later. Carbon particles have passed into a space in the vessel wall which may well be at a junction between two endothelial cells. *bm*, Basement membrane; *e*, endothelium; *pe*, periendothelial cell. (× 24,600)

alteration in the vesicular structures of the endothelium, and no holes through the endothelial cytoplasm itself, but there were discontinuities in the walls of some of the minute vessels which were probably at the junctions between the endothelial cells. These discontinuities let through not only plasma and fine particles of mercuric sulphide but also the much larger blood platelets. The plasma clotted outside the endothelial cells with the formation of fibrin, and the particles and the blood platelets were held up by the basement membrane.

In a similar way it appears that the increased permeability of small blood vessels during acute inflammation caused by mechanical trauma is due to the opening up of channels which are quite distinct from the vesicle system, and are probably between the cells. My colleague Marchesi has shown that colloidal carbon introduced into the blood stream of rats the mesenteries of which have been inflamed by a light stroke, escapes between the endothelial cells of the minute vessels in the injured area, and becomes for a time entrapped between the cells and the periendothelial tissues (Fig. 8).

Thus there is evidence to suggest that in normal vessels transport of fluid may be principally through the substance of the endothelial cells, but that in pathological conditions there may be an actual solution of continuity of the vessel wall, probably at the cell junctions, enabling much more fluid, as well as solid bodies, to pass through. This solution of continuity may be of a very labile nature, that is to say, the endothelial cells may part and join together again rapidly, but the overall effect is to allow a considerable leakage of plasma from the inside to the outside of the vessels.

The researches of the past nine or ten years have considerably increased our knowledge of the membrane through which exchanges of essential substances take place in those animals with a circulatory system. We are still far from understanding the precise behaviour of this complicated structure even under the simplest physiological conditions. What is clear is that mathematical concepts for explaining the transfer of water and large molecules through the walls of small blood vessels on mechanical principles will have to take cognisance of what is now known of their structure. The conceptions arrived at from the morphological and mathematical approaches have not yet been reconciled. Indeed, one may wonder whether the long-discarded view of Heidenhain, that lymph is secreted by the walls of the capillaries, may not have a grain of truth in it, for if the vesicle transport theory should be supported by further evidence, it may become necessary to postulate that work is done during the transport of material across the vessel walls, and this could be construed as secretion.

It is perhaps astonishing that we are still so unsure of the mechanism of the exchange of substances between the blood and tissues for, as Marshall Hall pointed out, the whole elaborate apparatus of heart and large blood vessels exists merely to subserve this exchange which takes place in the finest vessels.

Science is rarely advanced by what is known in current jargon as a 'break-through'. Rather does our increasing knowledge depend on the activity of thousands of our colleagues throughout the world who add small points to what will eventually become a splendid picture, much in the way that the Pointillistes built up their extremely beautiful canvases. What I have mentioned here are a few such points which it has taken three hundred years to place in the picture.

UNIVERSITY COLLEGE OF NORTH STAFFORDSHIRE

EXTENSION TO DEPARTMENT OF PHYSICS

THE new extension of the Physics Department of the University College of North Staffordshire, Keele, was officially opened on October 5, by Dr. F. A. Vick, director of the Atomic Energy Research Establishment, Harwell. This new extension represents a very considerable increase in the teaching facilities at Keele and the extra floor area that has been added to the Department amounts to some 18,000 sq. ft. Most of the space in this new extension is actually devoted to undergraduate and post-graduate teaching in the form of either lecture theatres, tutorial and colloquium rooms, or large teaching laboratories. Since the space that had been used in the old building for teaching has now been liberated for expansion of the Department's research activities, an increase for the latter has also been made possible, by the completion of this building.

The new building has a somewhat striking appearance (Fig. 1), and the main frontage is formed from vertical buttresses which are finished in large grey

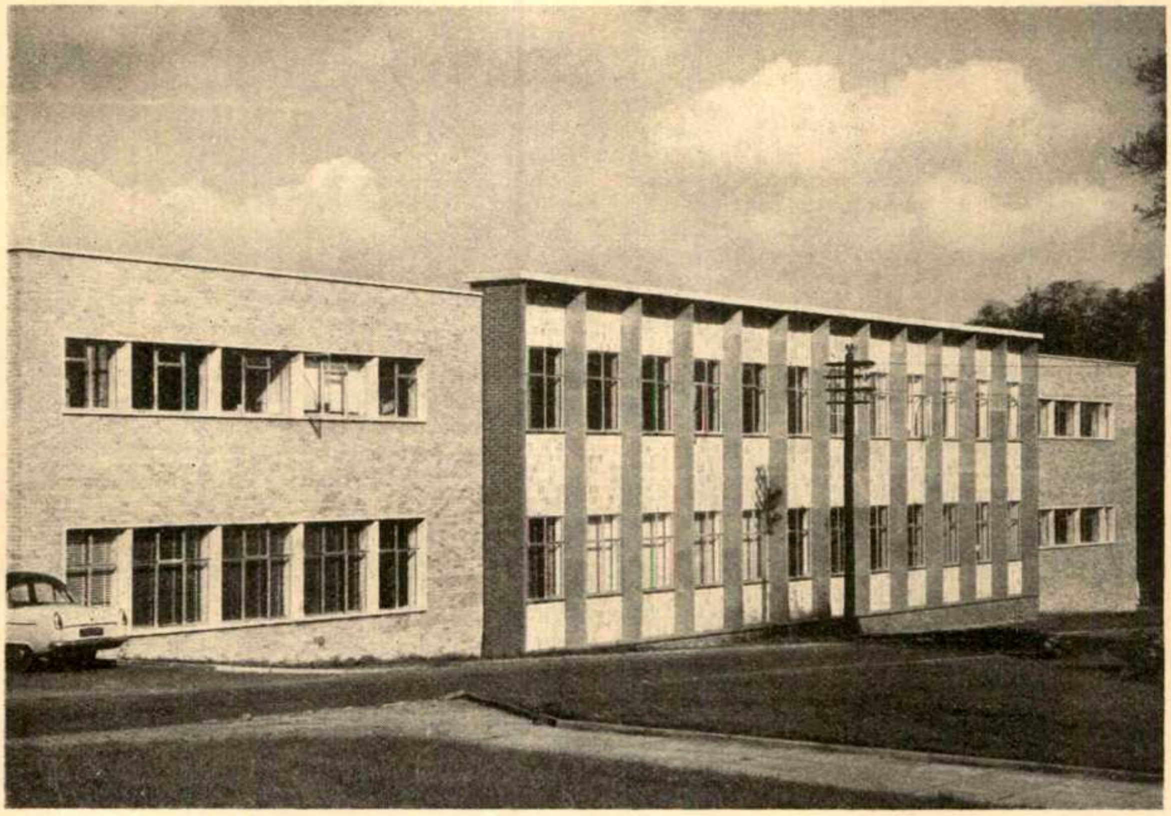

Fig. 1. New extension of the Physics Department, University College of North Staffordshire

slate slabs with inset walls of sparkling quartzite. The contrasting contours and colours provided by this southern frontage give a very pleasing impression when viewed in autumn sunlight. The building has two main floors, the ground floor being on the same level as the single-storey building which has previously housed the whole of the Department, and connects with it through a wide corridor with swing doors intervening. One somewhat unusual feature of the actual construction was the necessity of building the whole structure in five separate portions so as to meet the anti-subsidence conditions which affect the site. It is, in fact, extremely difficult to recognize these separate sections now that the building has been completed and an air of integrated cohesion exists throughout the Department, new and old buildings included. The central feature of the new wing is the large lecture theatre which is situated in the middle of the structure and continues through both floors. It has been designed not only to meet the needs of the expanding numbers in the physics courses themselves but also for the time being to house the whole of Keele's foundation year consisting of about 230 students. Corridor space has therefore been provided around the whole of this lecture theatre together with a large open entrance hall stretching up to the roof of the building itself. This has already proved of very great value as an assembly and discussion area for the large number of students that are now using the building. One feature of this hall which has produced considerable comment has been the overhead lighting, which consists of fluorescent tubes mounted within a covered glass structure which is opened to the sky through the roof. The resulting effect is almost identical during the day or night, since in daytime the sunlight itself is diffused through the whole structure over the entrance hall, while at night the fluorescent light is diffused in a very similar way and a very similar effect is obtained.

There are also two smaller lecture theatres as well as the main theatre, one which will hold up to 60 students and the other about 40. Together with these is a series of other teaching rooms, including a colloquium room for postgraduate seminars, a reading room for use by both undergraduate and research students, and a tutorial room for the tutorials which Keele still hopes to maintain as an essential part of its teaching system. The teaching laboratory accommodation consists of three large laboratories and one rather small optics laboratory serving the first honours year, since specialized teaching is still being carried out in the old building, including the teaching of optics in the second and third honours years. Two of the main teaching laboratories are arranged one above the other and are identical in size and design, each being 44 ft. long and 30 ft. wide, and are designed to provide sufficient accommodation for experimental work by up to 50 students. Each laboratory has a technician's service room leading from it, which contains all the rectifier and control equipment. The services in the teaching laboratories have been carefully kept around the walls and also fed to island frames in the centre of the room. The benches on which the experiments are performed are thus entirely flexible, and their location and assembly can be adjusted to the needs of the particular experiments that are being carried out. Electrical supplies are carried in continuous trunking with four standard types of outlet panels providing either 3-phase a.c., single-phase a.c., or d.c. voltages of various values. As well as these main teaching rooms the new wing also houses various staff rooms together with the secretarial offices and technicians' accommodation.

About 250 people attended the opening ceremony of the new extension, a large majority of these coming from outside the College, including not only representatives from other universities and teaching institutions but also various representatives of the education authorities, both locally and regionally, and also a large number from different industries and Government research stations throughout the country. Sir Alfred Owen, who is chairman of the University College Council, took the chair at the official opening ceremonies, and first called upon the new principal, Dr. H. M. Taylor, to introduce the guest of honour, Dr. F. A. Vick. In introducing Dr. Vick, Dr. Taylor referred to the rather incongruous position in which he found himself as introducing a speaker who was probably much better known to a large number of the audience than he had been to Dr. Taylor himself. He explained, however, that Dr. Vick's enthusiasm and interest in the more basic and liberal approach to the teaching of a scientist had already made its impact on Cambridge during his own time there, and he was thus already very much acquainted with Dr. Vick's thoughts and ideas if not so directly with Dr. Vick himself. The audience was indeed very fortunate in having as its main speaker not only the director of the Atomic Energy Research Establishment but also the ex-professor of physics at Keele himself.

In his own speech Dr. Vick outlined the history of the Physics Department during its eleven years existence, starting from the single room in Keele Hall and explaining that the first extension of the Department had consisted of the acquisition of two cupboards and half a room in the basement. The first main stages of the Physics Department building itself were built in 1951 and 1952, and Dr. Vick explained that they had been purposely designed for research uses as much as for teaching with the idea that the main teaching block should follow later. It was in fact this block itself that was now being opened, thus completing the steps in the original plan. Dr. Vick's historical summary was lightly sprinkled with amusing anecdotes which served to show that the major experiment at Keele in mixing the arts and sciences had often had its lighter moments. In discussing the expanding research activities of the Physics Department itself, he underlined the increasing interest of physicists in solid-state phenomena and gas discharges, and explained that Harwell itself was now much interested in these branches of the subject although it was true that they still did a little nuclear physics !

The official ceremony ended with a vote of thanks given by Prof. D. J. E. Ingram, the present professor of physics, who emphasized the very large part that Dr. Vick himself had played in the planning and design of the new physics building and said he felt sure that all who were present would agree that these efforts had produced both a highly efficient yet very handsome building. He also gave particular credit to the architects and contractors who had managed to design and build the structure at a cost of less than £100,000, which must be somewhere near a record in these days of rising building prices.

The new buildings, together with the old wing, which is now devoted almost exclusively to research, were then opened for general inspection for the rest of the afternoon and it was gratifying to see the large number of animated groups that sprang up discussing both research topics and laboratory design. Some of these design features arose from the particular circumstances at Keele, such as the anti-subsidence foundations and the heating which is entirely by inset panel radiators fed from the College boiler house. One other characteristic feature of the building was the large projection room behind the main theatre housing the ciné projector and other lantern equipment. This is a particularly useful feature for the foundation year lectures, when all types of teaching methods are being used by the different departments throughout the College.

One other very fortunate feature of the opening ceremony was that it had proved possible to bring the new building into operation just before the new academic session started, and all was in fact ready for the lectures and classes which were due to begin two days later. D. J. E. Ingram

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FUR SEALS BREEDING IN THE FALKLAND ISLANDS DEPENDENCIES

By F. A. O'GORMAN

Falkland Islands Dependencies Survey, 4 Millbank, London, S.W.1

BETWEEN December 1957 and March 1958 a Falkland Islands Dependencies Survey party of geologists and surveyors visited Livingston Island in the South Shetlands. On January 14 twenty-seven fur seals (*Arctocephalus tropicalis* (Gray)) on Cape Shirreff on the north coast were found, and on February 1 a further fifteen near Start Point in the north-west corner. There was no evidence that breeding had occurred[1].

The following year a party, led by me, was organized to carry out a preliminary investigation of the South Shetland fur seals, and during this visit a breeding group was found on Cape Shirreff on January 30. On February 29, 1960, H.M.S. *Protector* visited the South Sandwich Islands and found breeding fur seals on the south-east coast of Visokoi Island[2,3].

These are the first records of fur seals in any of these islands since 1902 when the Swedish Antarctic Expedition found one animal on Nelson Island in the South Shetlands[4], and the first record of breeding since the last century.

The Falkland Islands Dependencies consist of the sector lying south of South America between longitudes 20° and 80° west, and include the Graham Land Peninsula and the Islands of South Georgia, South Sandwich, South Orkneys and South Shetlands. South Georgia and South Shetlands saw the greatest part of the fur-sealing operations of the early eighteenth century. The present status of the fur seal on South Georgia has been discussed by Bonner[5].

The discovery of the South Shetlands in 1819 by Smith was closely followed by intensive sealing.

James Weddell[6] in 1825 reported that, between 1820 and 1822, 320,000 fur seals were killed and the colonies were virtually exterminated. At Cape Shirreff alone, 95,000 seal skins were taken in the season of 1821 by five British crews[7]. More than ninety ships were engaged in sealing around the South Shetlands during this period[8].

After this period of abundance, small catches were obtained up to 1871 when the re-developing colonies were again devastated. Between 1871 and 1890 more than 35,000 seal skins were taken[8,9]. The last definite report of the fur seal was on Nelson Island, though Hamilton[10] gives an unsubstantiated report of Canadian sealers taking animals up to 1904. Between 1925 and 1951 *Discovery*, *Discovery II* and *William Scoresby*, the ships of the *Discovery* Committee, made extensive voyages in the Dependencies and visited the South Shetlands, the South Orkneys and South Sandwich Islands without finding any fur seals[11-15]. A small number of fur seals were, however, found on South Georgia[16,17].

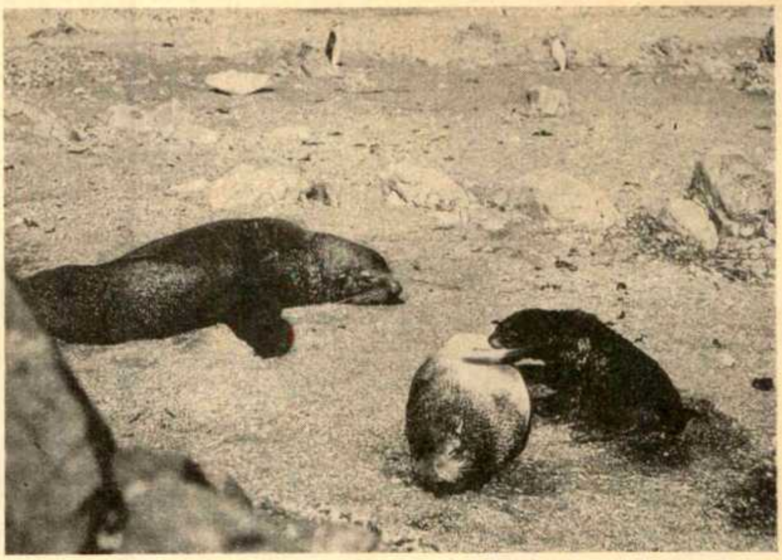

Fig. 1. Fur-seal family on Livingston Island, South Shetland Islands, February 2, 1959

In 1959 the Cape Shirreff colony, obviously at its earliest stage, consisted of one adult female and her male pup and seven adult males. A dead pup, also male, was found nearby. The seals were hauled out on a small windswept gravel beach frequented by groups of moulting elephant seals (*Mirounga leonina*), small numbers of Weddell seals (*Leptonychotes weddelli*) and numerous chinstrap (*Pygoscelis antarctica*) and gentoo penguins (*P. papua*), none of which had any apparent effect on the behaviour of the fur-seal group. The colony was watched intensively during January 31–February 4. On February 3 a second female, possibly the mother of the dead pup, arrived; no further change in the size of the colony occurred during this period. Some harem maintenance was being carried out by the dominant bull, but not very energetically. Quite often the female sparred and played with one or two of the younger males present, sometimes chasing each other in and out of the sea. The live pup measured 2 ft. 4 in. (71·1 cm.) in length and weighed approximately 15 lb. (6·8 kgm.) which, when compared with figures on the growth-rate in *Arctocephalus tropicalis* in South Georgia[18], would suggest that the pup was born about January 27. The pup was tagged with a monel metal cattle ear tag in the axilla of the right fore-flipper. On March 20 thirty-two fur seals were counted in the vicinity.

Unfortunately, it has not been possible to procure any further information on the subsequent development of this group, despite its considerable biological interest as one of the few mammal colonies where the natural growth could be studied from its inception.

The species has been identified, from a skull and skeleton, by Miss J. King of the British Museum (Natural History), as *Arctocephalus tropicalis gazella* (Peters, 1875); the same as that which occurs in South Georgia[19,20] and the South Orkneys.

The South Sandwich Islands, the most eastern islands of the Scotia Arc have never been renowned for fur seals. There are apparently no records of them having been laid waste in the 1820's as were those of the South Shetlands. Morrell, whose account of his voyages is generally discredited[21], found none in 1822, but Captain Buddington[9] states that in the 1875–76 season 2,000 were taken at the northernmost island (Zavodovski) and that, in 1876–77, 4,000 were taken by six vessels. In the following year the catch did not exceed 100. In 1880–81 no seals were found and in 1891–92 400 were obtained and a further 200 seen. Larsen visited several of the islands in 1908 but failed to find any[11]. These appear to be the only records of fur seals until the visit in 1960 of the Governor of the Falkland Islands, Sir Edwin Arrowsmith, on board H.M.S. *Protector* (Captain Forbes, R.N.). The only group of fur seals was found at the south-east corner of Visokoi Island on a beach which consisted of volcanic gravel and isolated outcrops of rock over which the seals sprawled. Sir Edwin Arrowsmith and Captain Forbes estimated the numbers to be in the region of 400. Photographs of the colony show a number of harem bulls and a loosely knit harem system. Several black pups can also be seen. None of the other islands in the group was visited. However, during January 7–11, 1961, the Falkland Islands Dependencies Survey vessel R.R.S. *Shackleton* visited the group to carry out geomagnetic observations, but only one fur seal was found on Visokoi Island and none was seen on the Islands of Zavodovski, Leskov, Candlemas, Vindication or Saunders. It must be emphasized that no special effort was made to locate fur seals. Though there were no skins or skulls collected from the South Sandwich fur seals, from their appearance they would seem to be similar to those of the South Shetland and South Georgia and are presumably the same species. Fur seals were also found on Clerke Rocks (55° 01′ S., 34° 41′ W.) on January 6 [22,23].

The discovery of these colonies and of the one on Powell Island in the South Orkneys in 1960 [24] demonstrates the rapid recolonization of this section of the Antarctic by the fur seals, with South Georgia presumably acting as the reservoir for this expansion.

Although no positive anatomical proof is available, it is reasonable to suppose that the fur seals of the South Shetland and the South Sandwich Islands (and

South Orkneys) present in the 1820's were also *Arctocephalus tropicalis*, and not *A. australis* as has been assumed until quite recently. Poland[25], in 1892, seems to have been in little doubt as to the differences between the species of fur seal on the Falkland Islands and the mainland of South America and those on South Georgia, South Sandwich and South Shetland Islands, his conclusions being based on the characteristics of the pelt. A fur seal upper canine tooth dug up from among remains of an early nineteenth century sealing camp on Livingston Island is not, unfortunately, referable to either species, but a more detailed search at this site might reveal material specifically identifiable.

It is hoped that these colonies, especially those in the South Shetlands will be strictly protected in keeping with the resolution on conservation in Antarctica passed by the Special Committee on Antarctic Research of the International Council of Scientific Unions[26], and allowed to re-inhabit the areas where they were once so numerous and so wantonly destroyed.

I wish to thank Sir Vivian Fuchs for permission to publish this article and Sir Edwin Arrowsmith and Captain David Forbes for the photograph and information on the South Sandwich Islands.

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OBITUARIES

Mr. M. A. C. Hinton, F.R.S.

With the death on October 3 of Mr. Martin A. C. Hinton there passed one of the last great amateur naturalists of the Victorian era, whose contributions to geology and zoology earned him world-wide renown.

Martin Hinton was born in London on June 29, 1883, the eldest of three children. His formal education ended at the age of twelve when he left school to begin legal work in the City. His interests in natural history were already growing and he was a frequent visitor to the Museum of Practical Geology, then in Jermyn Street, where handling the collections and talking with the curators he rapidly acquired an extensive knowledge of the subject. After eleven years as a 'voluntary worker' at the British Museum (Natural History), he joined the staff as an assistant in the Zoological Department in 1921; he was made deputy keeper in 1927 and keeper in 1936, a post which he held until his retirement in 1945.

In 1899, when only sixteen years old, he read his first paper to the Geologists' Association on the Pleistocene deposits around Ilford and Wanstead. This was the beginning of a series of papers on the Pleistocene geology of the Thames valley which continued for a quarter of a century. He did pioneer work in recording sections in the Thames gravels, he collected and identified the fauna they contained and gave stimulating if sometimes unorthodox interpretations of the Pleistocene history. His views are summed up in a paper read to the Yorkshire Geological Society in 1926.

On joining the staff of the British Museum (Natural History), Hinton directed his attention to faunal studies, especially of rodents, in which group he made major contributions. His painstaking work on these small and difficult mammals includes taxonomic investigations on voles and lemmings, British fossil rodents and lagomorphs; he was early to see the potential use of rodents for stratigraphical correlation of Pleistocene deposits, a technique which has become increasingly valuable. But he was not to be limited to the dry bones of the Museum collections; he took a keen naturalist's interest in the living rodents, and his British Museum booklet on *Rats and Mice as the Enemies of Mankind* stresses cogently the immense destructive potential of these pests. In the 1930's Hinton was largely responsible for the success of the Ministry of Agriculture's campaign to exterminate the muskrat in Britain, which had at that time become a major hazard, breeding very rapidly and undermining river and canal banks and even gravel railway embankments.

At the opposite end of the size-range in mammals, Hinton devoted his attention to whales, contributing reports on antarctic whales for the Colonial Office and collecting a school of false-killer whales stranded on the Dornoch Firth; he clearly appreciated the importance of studying populations as opposed to individuals, and his salvage of 127 whales from a remote part of Scotland and transporting them to London was a triumph of organization and perseverance.

Hinton carried out a large measure of administrative duties both within the British Museum and in various learned societies; for some years he was an editor of the *Zoological Record*, and zoological secretary of the Linnean Society; he served on the councils and was a vice-president of both the Zoological Society and the Linnean Society. He was elected to fellowship of the Royal Society in 1934.

Hinton was by nature shy, but in the company of friends he was unsurpassed as a raconteur. He was blessed with robust health throughout life, and possessed a remarkably retentive memory, failing eyesight in later years virtually prevented him continuing with scientific pursuits His first wife, by whom he had three children, predeceased him, and shortly after his retirement he married Dr Dina Dobson and went to live in Somerset close to the Mendip Hills, where his early interests in Pleistocene cave faunas had grown He rarely went to London in later years, but all who visited him, professional or amateur, novice or doyen, were assured of a very warm reception, and the benefit of his sage advice.

R. J G. Savage

Dr. G. H Young

Dr. George H Young, whose sudden death on October 10, 1961, at the age of fifty-two is reported from Pittsburgh, was a chemist known principally in the United States for his work in the field of organic polymers, resinous coatings, corrosion protection and industrial and marine paints.

During the Second World War he was a specialist consultant on corrosion and protective coatings to the U S War and Navy Departments, as a result of which he became known internationally for some joint studies with Drs. Seagren and Zehner on the interaction with steel of anti-fouling paints and he received the U S Navy's Bureau of Ordnance Development Award in 1945

He served as secretary to the technical committees which initiated the war-time synthetic rubber programme and was continuously associated informally with this major U S. Government undertaking until its termination in 1955 He had been a special consultant to the Department of Defense and was a member of the National Research Council's Material Advisory Board He also served as technical consultant to the Pennsylvania General State Authority

Since 1951 Dr. Young had been a director of research at the Mellon Institute, where he had been active in many spheres of administration and consultation, as well as patent adviser to the Institute's scientific personnel, having been admitted to practice before the United States Patent Office in 1940

To his many activities Dr Young added membership of numerous scientific and technological societies, including some in Britain and Germany, and in 1949 he served as U S delegate to the United Nations Conference on the Conservation and Development of Natural Resources

O D Hunt

Prof. W. N. Bailey

Prof Wilfrid Norman Bailey, who died at Eastbourne on October 23, was born at Consett, County Durham, on September 5, 1893 He soon displayed his mathematical abilities, and after completing a brilliant undergraduate career at Trinity College, Cambridge, becoming a Wrangler in 1915, he resigned a Research Exhibition to take up war service During 1915–19 he was an instructor lieutenant in the Royal Navy in which capacity he witnessed the Battle of Jutland. In later years he looked back on this period of his life with a certain nostalgia. When war service was finished, he held a number of university appointments at Birmingham and Manchester, and in 1944 he was appointed to the chair of mathematics at Bedford College, University of London, from which he retired in 1958

Bailey's interest in mathematics was focused on the theory of hypergeometric series and Bessel functions, and on these topics he published some fifty papers in addition to the well-known Cambridge Tract which he wrote on *The Hypergeometric Series* During the whole of his life these subjects retained their fascination for him and he had, to an exceptional degree, the capacity for conveying his excitement to others He was normally of rather a placid demeanour, but it was a revelation to hear him lecture on any topic which had aroused his interest

Prof Bailey took a full part in the mathematical life of the country, and was at various times librarian, editor, secretary and vice-president of the London Mathematical Society. Although he found committee membership somewhat tedious, he always accepted any work of this nature which he felt it was his duty to undertake.

Prof Bailey is survived by a widow, a son and a daughter He will be remembered by those who knew him both for his intense enthusiasm for mathematics and for his genial sociability

H. G Eggleston

Dr. Marian W. Smith

Dr Marian W Smith (Mrs. H F Akehurst), honorary secretary of the Royal Anthropological Institute since 1956, died on May 2, after a long illness

Marian Wesley Smith was born on May 10, 1907, in New York, and gained her master's degree at Columbia University in 1934, proceeding to the doctorate in 1938 Between 1937 and 1947 she taught in a number of American universities, and held office in several learned societies, including the presidency of the American Ethnographic Society during 1944–45 During this period too she carried out extensive field work on the Indians of Washington State and British Columbia, resulting in monographs of the Pyallup-Nisqually (1940) and the archæology of the Columbia-Fraser River (1950) as well as a number of papers in learned journals She later did work in Pakistan, but owing to her untimely death this was never brought to the stage of publication

She was already known as a leading anthropologist, notable for the breadth of her view, when, in 1952, she married an Englishman, Mr H Farrant Akehurst, and made her home in Great Britain She undertook part-time teaching work at the London School of Economics, and in 1956 she accepted the honorary secretaryship of the Royal Anthropological Institute. It was this part of her work which made her known to and loved by practically all British anthropologists. She devoted a great deal of time to the affairs of the Institute, including a much-needed overhaul of its finances, which was carried out in close collaboration with the honorary treasurer, Sir George Beresford-Stooke, and helped by the magnificent generosity of Mrs. Brenda Seligman She initiated an excellent tradition by organizing a succession of symposia on subjects marginal to anthropology, namely, race problems, primitive music and the domestication of cattle, thereby making use of the specialized knowledge of many experts in other fields of investigation.

Her ability, her devotion to the Institute, and above all the warmth of her personality were such that every regular attendant at the meetings of the Institute will feel the loss of a personal friend

A. E. Mourant

NEWS and VIEWS

The Royal Society: Officers for 1962

AT the anniversary meeting of the Royal Society on November 30, Sir Howard Florey, professor of pathology in the University of Oxford, was re-elected president. The other officers re-elected for the ensuing year were: Treasurer, Lord Fleck, formerly chairman of Imperial Chemical Industries, Ltd.; Biological Secretary, Sir Lindor Brown, Waynflete professor of physiology in the University of Oxford; Physical Secretary, Sir William Hodge, Lowndean professor of astronomy and geometry in the University of Cambridge; and Foreign Secretary, Sir Patrick Linstead, rector of the Imperial College of Science and Technology. Other members of Council elected (or re-elected) were: Mr. F. C. Bawden, director of Rothamsted Experimental Station; Mr. E. J. H. Corner, reader in plant taxonomy in the University of Cambridge; Prof. J. E. Harris, professor of zoology in the University of Bristol; Sir Harold Himsworth, secretary, Medical Research Council, consulting physician to University College Hospital, London; Prof. A. F. Huxley, Jodrell professor of physiology at University College in the University of London; Sir Christopher Ingold, emeritus professor of chemistry at University College in the University of London; Prof. W. T. J. Morgan, deputy director of the Lister Institute and professor of biochemistry in the University of London; Prof. C. F. Powell, Melville Wills professor of physics in the University of Bristol; Prof. O. A. Saunders, professor of mechanical engineering at the Imperial College of Science and Technology in the University of London; Dr. C. J. Stubblefield, director of the Geological Survey of Great Britain and the Museum of Practical Geology; Sir Gordon Sutherland, director of the National Physical Laboratory; Sir Graham Sutton, director-general of the Meteorological Office; Dr. H. W. Thompson, reader in infra-red spectroscopy, University of Oxford; Prof. C. H. Waddington, professor of animal genetics in the University of Edinburgh; Prof. A. G. Walker, professor of pure mathematics in the University of Liverpool; Prof. F. C. Williams, professor of electrical engineering in the University of Manchester.

Psychology at Cardiff: Prof. G. Westby

MR. G. WESTBY, who has been appointed to be the first holder of the new chair of psychology in the University College of South Wales and Monmouthshire, is at present head of the Department of Psychology at Hull. He is well known by reason of his outstanding success in developing his present department during the past twelve years. Starting almost from scratch, its activities now include a large and flourishing honours school, the high standards of which are widely admired and a varied and enthusiastically pursued programme of research. The broad basis, coupled with the strong integration, of the Department have enabled it also to make a full contribution to interdisciplinary courses at the honours level and to the training of graduate students in the social sciences.

Mr. Westby, after reading the Honours School of Philosophy, Politics and Economics, acquired considerable experience in the education and treatment of maladjusted children before returning to Oxford after the War to gain a distinction in the diploma in psychology in 1946. Soon after he went to Hull it became apparent that he commanded the wide understanding of issues in the many diverse aspects presented by contemporary psychology, and the power to attract to his staff people of ability and initiative, without which all effort to build a living department must be in vain. These talents will find full scope at Cardiff, where exceptional possibilities exist, not only because of the keenness for a department of psychology among all the faculties concerned, but also by reason of the proximity and interest of the Welsh National School of Medicine. When difficulties inseparable from starting almost at the beginning have been surmounted, and the advantages exploited, we may confidently look forward to the establishment of a notable new centre of psychological studies.

Atlas Computer Laboratory, National Institute for Research in Nuclear Science: Dr. J. Howlett

DR. J. HOWLETT has been appointed head of the *Atlas* Computer Laboratory of the National Institute for Research in Nuclear Science. The appointment is effective from December 1. This Laboratory, to be built adjacent to the Institute's Rutherford High Energy Laboratory at Harwell, will house an *Atlas* electronic digital computer. Dr. Howlett, born in 1912, was educated at Stand Grammar School, Manchester, and the University of Manchester, where he read mathematics. Following an initial period in the Research Department of the London, Midland and Scottish Railway Co., Dr. Howlett joined the Ministry of Supply in 1940, where he was engaged in mathematical and computational work on a variety of defence problems. Since 1948 he has been head of the Computing Group of the Theoretical Physics Division at the Atomic Energy Research Establishment, Harwell.

Courtaulds, Ltd.: Appointments

SIR ALAN WILSON, a deputy chairman of Courtaulds, Ltd., has been appointed chairman of the company in succession to Sir John Hanbury-Williams who retires next summer. Sir Alan was elected to the Royal Society in 1942 in recognition of his work on the electronic theory of solids and on the properties of metals, and joined Courtaulds in 1945. Mr. C. F. Kearton and Mr. H. R. Mathys have been appointed deputy chairmen of Courtaulds. Mr. Kearton has been with the firm since 1946, and was elected to the Royal Society in 1961 for his work on the chemical engineering characteristics of volatile uranium products and technological developments in the textile industry. Mr. Mathys is a chartered patent agent and was in general practice until the outbreak of war in 1939; he joined Courtaulds in 1946 to take charge of the Patent Department at Coventry, and was appointed a director in 1954.

Nora: a New Zero Power Reactor in Norway

THE zero power reactor, *Nora*, of the Norwegian Atomic Energy Institute (IFA), which will serve in connexion with the joint research programme in reactor physics of the International Atomic Energy Agency (IAEA) and Norway, was inaugurated at

Kjeller, near Oslo, on November 29. *Nora*, intended for the establishment of various physics data of importance for the development of power reactors, has been so constructed as to be very flexible and to permit a wide range of experiments to be carried out with it. One of its important features is the supporting device for the fuel elements which permits the fuel lattices to be changed readily. The reactor will operate at a power-level of only a few watts. The financing of *Nora* was made possible by a grant of 350,000 dollars from the U.S. Atomic Energy Commission under the Atoms for Peace programme in addition to the funds contributed by the Government of Norway.

The programme aims at obtaining precise and widely applicable reactor physics data on cores with mixed and variable lattices moderated by light water, by heavy water and by mixed heavy and light water. In addition to the critical assembly *Nora* itself, Norway is making available two reactor cores, one consisting of natural uranium and the other of slightly enriched uranium. A third core, provided under the supply agreement between IAEA and the United States, consists of fuel enriched to 3 per cent in the radio-isotope uranium-235. This core was originally used in development work for the nuclear merchant ship *Savannah* and contains approximately 42 kgm. of uranium-235. This is the first lease of special nuclear material through the Agency to one of its Member States. The Joint Scientific Programme Committee which is to conduct the *Nora* research project is headed by Dr. Raja Ramanna (India). The other committee members are Mr. Olav R. Kåsa and Dr. Henrik Ager-Hanssen, both from IFA, and Dr. Pierre Balligand, deputy director-general, and Dr. Carlo Salvetti, director of the Division of Research and Laboratories, both of IAEA.

Diamond Jubilee of Trans-Atlantic Radio

On December 12, 1901, Guglielmo Marconi became the first to send a wireless signal across the Atlantic. This remarkable achievement with such primitive equipment marked the birth of world-wide communication. During the spring of 1900, Marconi had succeeded in sending reliable signals from St. Catherine's in the Isle of Wight to the Lizard in Cornwall, a distance of 186 miles. This encouraged his belief that by using larger aerials and far more powerful transmitters he would be able to achieve trans-Atlantic distances. Scientists were highly sceptical, many said it was impossible because of the curvature of the Earth. Marconi was determined to make the attempt and a transmitting station nearly one hundred times more powerful than any previously constructed was built at Poldhu, near Mullion, in Cornwall. Enormous aerials were erected at Poldhu and at Cape Cod in Massachusetts, but both were wrecked in severe gales. Another, less ambitious in design, was put up at Poldhu, while Marconi and his two assistants sailed to Newfoundland where, from the top of Signal Hill, a receiving aerial was hoisted, at the third attempt, by means of a kite. At 12.30 p.m. (Newfoundland time) on December 12, 1901, Marconi and his assistant, G. S. Kemp, using one of the primitive receivers of the period with a telephone earpiece, heard a faint succession of S's in Morse code. Signals from Poldhu, 2,200 miles away, had crossed the Atlantic.

To commemorate this historic achievement, a Special Exhibition will be displayed at the Science Museum, South Kensington, during December 13–January 25.

Research in Shipbuilding

In a written answer in the House of Commons on November 21, the Parliamentary Secretary for Science, Mr. Denzil Freeth, stated that the shipbuilding and marine engineering industries in Britain have set up an executive committee, with the encouragement of the Department of Scientific and Industrial Research, to form a new association for ship research, and the proposals agreed within the industries were to be announced. Discussions with the Department on constitutional matters were well advanced, and those on details on the final financial arrangements are to follow.

U.S. Research Activities in the Antarctic

The U.S. National Science Foundation has announced a further allocation of approximately 1,500,000 dollars for research work in the Antarctic. The largest single grant among the twenty-seven grants which this sum comprises is for the Antarctic Meteorological Research Program of the U.S. Weather Bureau. The total grant for this programme is now nearly 400,000 dollars. A further sum, exceeding 100,000 dollars, has been allocated to a series of meteorological rocket firings. These firings will reach heights up to 200,000 ft. to investigate winds and temperatures. There has previously been no systematic measurements of these elements in high southern latitudes. The firings will be co-ordinated with similar soundings in other parts of the world through the Meteorological Rocket Network, and the results will be correlated with world-wide stratospheric wind measurements. The U.S. Weather Bureau will also conduct an ice survey of antarctic waters with the assistance of U.S. Navy aircraft. This is intended to assist in the analysis of data to be received from projected polar satellites. A biologist from Ohio State University will carry out research while being based for sixteen months at the main U.S.S.R. base at Mirny. The main objective is to study invertebrate land animals and correlate the results with ecological studies which have been made at United States stations. This arrangement will continue the practice whereby the United States and U.S.S.R. exchange scientists at antarctic bases through agreements reached between the U.S. National Academy of Sciences and the Academy of Sciences of the U.S.S.R.

The Institute of Reprographic Technology

The Institute of Reprographic Technology was inaugurated at a reception held at St. Ermin's Hotel, Westminster, on November 23. The Institute will function as a professional body with entrance by examination, and its aims will be to increase the efficiency and technical ability of those practising the art of reprographic technology. This will be achieved by providing a forum for the exchange of ideas and a continuing programme for the dissemination of the scientific knowledge related to the profession. It is hoped that as the Institute grows regional organizations will be developed based on the industrial centres. Mr. Charles Hanrott has been elected chairman of the Institute. Further information can be obtained from the Honorary Press Officer, Institute of Reprographic Technology, 157 Victoria Street, London, S.W.1.

Impact of Science on Society

Impact of Science on Society, No. 3, 1961 (Vol. 11), includes several particularly interesting articles. An editorial note, "Mankind and Outer Space", and an article by Prof R Courant, "General Problems confronting Computing Centres", both stress the opportunities and the importance of international co-operation in these fields Dr G H O Burgess, of the Torry Research Station, reviews the contribution which fish technology can make to the food supply of the world and stresses the need for a much greater research effort and more attention to the dissemination of the results of research Dr G. Charlesworth, now head of the London Traffic Management Unit, Ministry of Transport, writing on "Science and the Road Traffic Problem", describes some of the contributions which research has made in this field, and the opportunities which the scientific study of roads and traffic presents both in the prevention of accidents and improving the efficiency of transport

Education and Training in France

In May 1960 nine members of the West Regional Group of the British Association for Commercial and Industrial Education paid a visit to France to study the French system of education and training with special reference to the needs of industry Their report contains a comprehensive description of the present system of education and training in France, as well as the changes in the system which will take place during the next few years as a result of legislation enacted in 1959 (Pp v+51 London British Association for Commercial and Industrial Education, 1961 10*s*) When reforms are completely integrated, all children will remain at school until sixteen years of age and selection for vocational training will commence well before the end of the normal school career A large section of the report is devoted to the various types of vocational institutions which exist, the certificates and diplomas awarded and educational practice in the vocational schools There is also a detailed description of education and training at the three main factories of the Renault Works

Television and School Teaching

In a booklet on schools television, the National Union of Teachers suggests that television should not be considered as a substitute for the teacher but as an addition to teaching aids already available (*Schools Television · a Discussion Document* Pp 18 London National Union of Teachers, 1961 1*s*), that if television is to be regarded as an integral part of the apparatus of education, it should be the responsibility of local education authorities to provide sets; and that particular attention should be directed to the need for the provision of more television sets in primary schools The booklet quotes some of the findings from the Union's inquiry on schools television among 111 schools, distributed over the areas of 71 local education authorities, and compares them with similar findings in the B B C report The National Union of Teachers welcomes the setting up by the B B C and commercial companies of special bodies with responsibility for the form and content of the schools programmes, and comments favourably on the "degree of independence of the parent body" which the Schools Broadcasting Department of the B B C and its counterparts in commercial television enjoy The booklet examines the constitution of these bodies, the scope of their work, the special role of the Union therein, and their relationships with the schools The pamphlet states that "the principal limitations of the television programme compared with the film are that it is not under the control of the teacher to the same extent either before, during or after showing, and that it has not the same resources of colour presentation" On the other hand, it points out that "television has a great impact on account of its immediacy" The booklet also touches on the general impact of television viewing on children and the special position of Wales with regard to television

The British Broadcasting Corporation

In the annual report and accounts of the British Broadcasting Corporation for 1960–61 it is recommended that the higher-frequency bands, Bands IV and V, should be used for television with a change of line standard from 405 to 625 lines, as proposed by the Postmaster-General's Television Advisory Committee, and that two of the four channels available in the higher-frequency bands should be allotted to the B B C One channel would be used for an entirely new programme service so that the B B C could give viewers a genuine choice of balanced alternative programmes, while a second channel would carry the existing Band I service, duplicated on the 625-line standard The B B C recommends that the channels in Band III, not at present allocated to either B B C or the Independent Television Authority, should be used to improve the coverage of existing services in areas not well served at present, and, in particular, to provide an improved and distinctive service for Wales The B B C is carrying on with its experimental work on colour television with the approval of the Post Office and with support from the radio industry The experiments will include test transmissions in colour on 625 lines in Bands IV and V, thus extending the work already done by the B B C in the experimental transmissions on 625 lines in black and white On sound broadcasting, the report states that there is need for an earlier start to the day's programmes a similar extension is needed at the other end of the day, after midnight, for late-night workers Over and above the provision of three programme services, the B B C sees the need and the opportunity now for a supplementary fourth service in the form of local sound broadcasting on very high frequencies

Licentiateships in Printing and Furniture

For some time both the printing and furniture industries, in association with the City and Guilds of London Institute, have been developing advanced courses at a level beyond the full technological certificate to meet specific requirements for high-level qualifications for which, at present, there are no recognized awards The Institute has announced recently a new award, the licentiateship of the City and Guilds of London Institute, intended to provide that kind of recognition in selected fields where such a need exists The Institute has approved two such fields, printing and furniture, and the awards will be known as the Licentiateship in Printing of the City and Guilds of London Institute and the Licentiateship in Furniture of the City and Guilds of London Institute Candidates for either award must have had at least five years approved industrial experience after completion of apprenticeship. In printing, a candidate will be required to have obtained one of the Institute's craft certificates, have obtained the full technological certificate and have passed the examination for advanced qualifications In furniture, a candidate must have obtained a craft course certificate, the full technological certificate and passes in two of the advanced courses

The Dunfermline Museum

The Dunfermline Museum was opened in June by Dr Douglas Allan, lately director of the Royal Scottish Museum, Edinburgh This new museum has been established by the Carnegie Dunfermline Trust and will serve as a local museum of natural history and folk material The collections came mainly from the old museum at Pittencrieff House, which has now been closed The conversion of a private house has provided two main galleries which have been equipped with modern 'shop window' cases One of the galleries is given over to a display of the birds and animals of West Fife in their natural habitats, and the other shows material relating to Dunfermline from the stone age to the present day A smaller room is devoted to linen, for many years the staple industry of Dunfermline

South Australian Museum

The *Records of the South Australian Museum*, **14**, No 1 (1961), maintains the high standard of this publication and includes papers on the Aboriginal hammer-stones of South Australia, Acarina fauna, Central Australian mammals, the stratigraphy and vertebrate fauna of the Tirari Desert, South Australia, and a report on the archæological excavation of the Noola rock shelter.

Experimental Alteration of the Speech-rate

It is known from the work of Dr Wilder Penfield and his associates in Montreal that speech may be arrested or otherwise disturbed by electrical stimulation of certain areas of the cerebral cortex. In a recent paper from the Neurosurgical Service of the Foch Hospital (Suresnes-Paris), entitled "Arrest of Acceleration of Speech evoked by Thalamic Stimulation in the Course of Stereotaxic Procedures for Parkinsonism" (*Brain*, **84**, 363, 1961), Dr G. Guiot and his collaborators have been able to show that striking changes in rate of speaking may be produced by thalamic stimulation in the course of stereotaxic operations for Parkinsonism Although arrest of speech is stated to be the more common phenomenon, striking acceleration in the speech-rate was noted in certain cases and changes also occurred in the strength and modulation of the voice The locus of stimulation is reported to be the ventrolateral nucleus of the thalamus Although these observations call for repetition on the more adequate scale, they would appear to throw an interesting light on the part played by subcortical mechanisms in the delivery and control of speech

Mineral Resources of Uganda

The important part played by geological surveys in the economic advancement of under-developed territories is brought to mind by a recent publication on *The Mineral Resources of Uganda* (Bulletin No. 4 Compiled and edited by Dr. J. W. Barnes Pp v+89+2 plates Entebbe: Geological Survey, 1961. Shs 25) During 1960 the mineral commodities exported from Uganda reached a record value of £3 5 million, equal to almost a quarter of the aggregate output since mining first began in 1927, and in addition industrial minerals exceeding £1 million in value were produced for local use, and cobalt concentrates valued at £1 million were stockpiled The principal products are blister copper and ores of tin, tungsten and beryllium, but within the next year there will be a large output of superphosphate (estimated at £325,000 per annum) for East African use and possibly of niobium ores for export Since the Geological Survey began drilling for water in 1931 some 3,000 producing boreholes have been completed, yielding water supplies that have supplanted the disease-laden and ephemeral surface sources on which much of the population used to depend In addition to reviewing resources now being exploited, the publication records various mineral potentialities of possible future interest, one of the more unusual of these being heavy water, which forms 8–12 per cent of the water in some volcanic lakes in the Ankole district. Although much of the country has still to be thoroughly prospected and, particularly in the north, to be mapped geologically, the report is a striking refutation of the view, once frequently voiced by Colonial administrators, that Geological Surveys are not revenue-producing departments

Chagas' Disease

Preliminary surveys on the incidence of Chagas' disease in the Matalaque region, Southern Peru, by Eleazar Cordova, have shown that housing conditions favour the development of *Triatoma infestans*, the only vector found in this zone Domiciliary infection shows a high incidence (89 02 per cent), the rate of trypanosome infection in triatomides being 51·28 per cent (41 97 per cent in bedrooms), a fact that suggests the almost certain existence of human cases of Chagas' disease. Domestic animals are frequently kept and raised within houses; such is the case, for example, of guinea pigs (84 per cent), where *T. cruzi* was found in 4 out of 32 specimens examined. Felix Naquira, Eleazar Cordova, Cesar Naquira and Enrique Rubin de Celis (*Bulletin Chileno de Parasitologia*, **16**, No 3, July–September 1961) have also carried out epidemiological surveys on Chagas' disease in the village of Matalaque (Tambo Valley, Southern Peru) The application of xenodiagnosis revealed an incidence of 34 67 per cent (43 out of 124 individuals surveyed) of *Trypanosoma cruzi* infection, whereas complement fixation procedures yielded 32·23 per cent positive cases (30 out of 93 individuals) Xenodiagnosis was also positive in four guinea pigs and one dog The results obtained with the two methods were not entirely concurrent.

Fellowships in Engineering Study and Fundamental Research in Plastics

Princeton University is offering a number of fellowships in engineering study and fundamental research in plastics leading to the M Sc and Ph D degrees, particularly suited to chemical, electrical and mechanical engineers, and to chemists and physicists Instruction covers basic theory and properties, and principles of evaluation, production, fabrication, design and application of materials, as well as the chemistry of plastics The programme includes lecture and laboratory classes, and contact with industrial plants representing various interests of the plastics industry Fellowships with stipends valued at 1,500 dollars–2,100 dollars are available to applicants holding a first degree in engineering or physical science from a recognized institution. Further information can be obtained from Prof. Louis F. Rahm, Plastics Laboratory, Princeton University, Princeton, N J.

The Manchester College of Science and Technology

The annual report of the Principal of the Manchester College of Science and Technology for 1960–61 gives an impressive picture of expansion, achieved and projected, which demonstrates how important a contribution the colleges of technology are making to Britain's scientific and technological strength. The number of students increased by 164 to 1,983, of whom 1,535 were studying for the B.Sc. (Tech.), 192 for the M.Sc. (Tech.) and 129 for the Ph.D.; 5,408 students were also enrolled for non-university courses, and of these only 333 were full time. The teaching staff now numbers 287. A list of research projects in progress during the year, arranged under Departments, appended to the report, gives an impressive picture of the research effort, and there is also a list of publications during the year by members of the staff. No information as to expenditure on research is included other than the statement that 9 per cent of the income of £1,580,523 was in support of research. Expenditure rose by £167,562 to £1,574,826. Students obtaining the B.Sc. (Tech.) with honours numbered 281, compared with 180 for the period 1959–60 and 96 for 1956–57; for B.Sc. (Tech.), ordinary, the corresponding figures are 178, 128 and 77; for M.Sc. (Tech.), 71, 59 and 33; for Ph.D., 25, 27 and 32; and for the postgraduate diploma, 22, 24 and 22.

University News.

Cambridge

Corpus Christi College is planning a graduate extension of the College at Leckhampton House, Cambridge. It is intended to provide public rooms, and accommodation for a few Fellows, in the existing house, and to house research students and other Fellows in a new building close by. With this additional accommodation the College will be able to increase the number of its active Fellows from 22 to 37 or more, and its research students from 40 to at least 60. Prof. H. C. Longuet-Higgins, professor of theoretical chemistry in the University, has been appointed warden.

Edinburgh

Dr. M. E. Davies has been appointed as lecturer in the Department of Veterinary Bacteriology, and B. F. Leek has been appointed as lecturer in the Department of Veterinary Physiology.

London

Dr. B. W. Lacey, reader in bacteriology in Westminster Medical School, has been appointed to a chair of bacteriology at that School. Mr. D. G. MacRae, reader in sociology in the London School of Economics and Political Science, has been appointed to the chair of sociology tenable at that School. Dr. E. A. Power, lecturer in mathematics in University College, has been appointed to the readership in applied mathematics tenable at that College. Mr. J. L. Stollery, lecturer in the Imperial College of Science and Technology, has been appointed to the readership in aeronautics tenable at that College. The title of professor of molecular spectroscopy has been conferred on Dr. A. G. Gaydon, honorary lecturer in the Imperial College of Science and Technology.

Prof. J. T. Randall, Wheatstone professor of physics at King's College since 1946, has been transferred by the University of London to the chair of biophysics at the College. The lease of premises in Drury Lane, W.C.2, some 40,000 sq. ft. in extent, has been acquired to house the new Department of Biophysics. After conversion, the premises will be known as the Wellcome Biophysics Laboratories in recognition of the grant of £120,000 made by the Wellcome Trust in 1960 towards the purchase of the lease. Prof. Randall will assume the directorship of the Laboratories on their completion in 1963. The new premises will also house the Medical Research Council's Biophysics Research Unit, which has been an integral and important part of the biophysics activities of King's College since 1947.

Massachusetts

A high-speed digital computer has been given to the Massachusetts Institute of Technology by the Digital Equipment Corporation of Maynard, Massachusetts. The computer is a "Programmed Data Processor", manufactured by the Digital Equipment Corporation, and will be used in teaching students the principles and operation of modern electronic data-processing equipment.

Rhodesia and Nyasaland

Dr. A. D. Kemp, senior lecturer in chemistry in the University College of Fort Hare, has been appointed lecturer in organic chemistry in the University College of Rhodesia and Nyasaland.

Announcements

Sir Douglas Logan, principal of the University of London, has been appointed vice-chairman of the Association of Universities of the British Commonwealth in succession to Sir Eric Ashby. The chairman for the year 1961–62 is Mr. K. J. Maidment, vice-chancellor of the University of Auckland, New Zealand, and the honorary treasurer of the Association is Prof. R. Ogilvie Buchanan, of the London School of Economics.

Dr. Randal M. Robertson has been appointed as associate director (research) of the National Science Foundation, Washington. Dr. Robertson has been assistant director for mathematical, physical and engineering sciences at the Foundation since July 1958, a post which now passes to Dr. Geoffrey Keller.

Mr. R. M. Arnold, until June of this year chief veterinary research officer in Tanganyika, has been appointed deputy head of the Wellcome Veterinary Research Station. Mr. Arnold became assistant veterinary investigation officer at Reading in 1942. Later he was appointed as veterinary investigation officer, Jamaica, and became deputy director of agriculture (veterinary and livestock) and superintendent of livestock services in that Colony.

The 1962 International Conference on "Precision Electromagnetic Measurements" (formerly Conference on "Standards and Electronic Measurements") will be held during August 14–16 at the Boulder Laboratories of the National Bureau of Standards, Boulder, Colorado. Further information can be obtained from Dr. James F. Brockman, National Bureau of Standards, Boulder, Colorado.

The International Society of Soil Science Joint Meeting will be held at Massey Agricultural College, New Zealand, during November 13–22, 1962, with pre-meeting tours during November 2–12 and post-meeting tours during November 23–December 1. The Joint Meeting is one of Commissions IV (Soil Fertility) and V (Pedology) of the International Society. Further information, together with an attractive brochure, can be obtained from the Secretary-General, P.O. Box 8001, Wellington, New Zealand.

THE TROPICAL PRODUCTS INSTITUTE

THE Tropical Products Institute of the Department of Scientific and Industrial Research held its first open days during October 19–20 when more than 500 people accepted invitations to be present. These included representatives of diplomatic missions; scientists from other Government institutions, universities and industry; representatives of commercial firms dealing in tropical products; and visitors from tropical countries. During the open days most of the laboratories were open to visitors and special displays were arranged to describe work in progress. This varied from the short-term advisory work to long-term research projects.

The Tropical Products Institute began as a part of the Scientific and Technical Department of the Imperial Institute and, therefore, has a history going back nearly seventy years. During most of the post-war years it was attached to the Colonial Office, but in 1959 was transferred to the Department of Scientific and Industrial Research, primarily in order that its services could continue to be available to Colonial territories after they had attained independence. In 1960 a total of nearly 1,000 inquiries was received from 62 Colonial, Commonwealth and foreign countries. These covered aspects of all the subjects on which specialists' advice is available at the Institute, including foods, fruits, essential oils, plant drugs, pesticides, oilseeds, fats, waxes, leather production, fibres, pulp and paper-making, board-making and solar energy. A comparatively new section at the Institute, the importance of which is growing considerably, is that dealing with inquiries on the economics of the production and marketing of tropical products; it also advises on suitable small industries for under-developed areas.

A research project with which the Institute has been concerned for many years is that of the pyrethrum industry in Kenya. Scientists at the Imperial Institute were instrumental in introducing this crop into Kenya in 1928 and it is now a very important export. One of the lines of work at present in progress is directed to improving the strains of the plant by breeding, and investigations are going on with Rothamsted Experimental Station to estimate the individual toxicities of the four esters responsible for the insecticidal activity of pyrethrum. These are separated by two-stage chromatography and sent to Rothamsted for testing. Another part of the Institute's work on pyrethrum is the investigation of the biosynthesis of pyrethrins in the plant. In particular, work has been directed at finding the site of synthesis and, for example, the effect of fertilization of the flower head. For this purpose acetic acid or mevalonic acid labelled with radioactive carbon is fed to the plant, the pyrethrins are isolated and, if radioactive, the site of labelling is determined. From this work it has been found that the synthesis occurs in the flower head and that fertilization of the flower does not influence the production of the insecticide.

One of the important projects in the Oils, Fats and Waxes Section has been an investigation into the factors which make Nigerian palm oil difficult to bleach. As a large part of the palm oil produced is used for margarine it is essential that it should be easily bleached to remove the reddish coloration which is normally present. Malayan palm oil has always been more easily bleached, and therefore has fetched a higher price, than palm oil from Nigeria. Work at the Institute has shown that the poor bleaching qualities of the Nigerian oil are due to the high initial carotene content and to oxidation caused by delays in processing which occur when primitive methods of collection and extraction are used. Oxidation by lipoxidase has been shown to take place when unsterilized and bruised fruit is left for long periods before extraction, and atmospheric oxidation during processing also takes place, usually due to iron contamination, where the iron acts as a catalyst. As in much of the Institute's work a fundamental chemical investigation has been followed by practical recommendations, and the small-scale producers of palm oil in Nigeria are now being encouraged to give more attention to the preparation and processing of the oil.

The accommodation at the Tropical Products Institute includes a pilot-plant laboratory which was on show to visitors during the open days. Here wax was being extracted from banana skins on a fairly large scale in a continuous process using about 7 l. of solvent. Heat is supplied by a steam-heated glass coil. The wax, which is removed by this method, constitutes 8–9 per cent by weight of the banana skin and may prove to be of use in the formulation of polishes. Investigations are also being carried out of the esters of a triterpenoid alcohol present in the wax. Also in the pilot plant laboratory are stills for the hundredweight-scale extraction of essential oils from bark, leaves and other plant materials. These provide very useful information for overseas growers who wish to produce these oils under larger-scale conditions.

In the Fibres Laboratory visitors were shown methods used for the physical testing of fibres, including a hybrid sisal which has been developed in Tanganyika. This has a much greater yield than the normal variety, and at the Institute the physical characteristics of the two types are being compared. Initial tests have shown that the hybrid gives a finer fibre and compares favourably in other respects with ordinary sisal. Other work in this Laboratory involves a study of the structure of fibres using methods similar to those used in electron microscopy. A cellulose acetate replica of the surface of a longitudinally cut fibre bundle is made, and this is metal-shadowed with chromium under high vacuum. The preparation is then examined using a light microscope and magnification of about 1,000.

The Pesticides Information Service of the Department of Technical Co-operation is also housed at the Tropical Products Institute and its work was shown to visitors during the open days. Much of the work in London is in providing information and abstracts, but there is also a laboratory where the control of bilharzia disease is being studied. This disease is caused by a parasitic worm and spread by various tropical snails. A colony of one of these, *Australorbis glabratus*, is maintained at the Institute so that promising chemicals can be tested as molluscicides.

Among the many other exhibits of work in progress, visitors were able to see types of building board made of waste materials such as groundnut shells, work on the formulation of insecticides for the malaria control campaign of the World Health Organization, the study of the flavouring constituents of pineapple using gas chromatography, and the analysis of the amino-acid content of animal feeding stuffs.

The open days gave many people an opportunity to see something of the wide range of scientific work which the Tropical Products Institute is doing on the renewable resources of the less-developed countries of the world, for producers and users alike.

B. R. SMITH

NEW INSTRUMENT ASSEMBLY WING OF THE CAMBRIDGE INSTRUMENT CO., LTD.

A NUMBER of scientists, industrialists, and well-known scientific and engineering journalists were among the 150 guests who attended a luncheon on October 23 to mark the opening of a new wing at the Cambridge Works of the Cambridge Instrument Co., Ltd. The guests were met by Dr. P. Dunsheath, chairman of the company's Board of Directors, and Mr. H. C. Pritchard, managing director, and the opening ceremony was performed by Sir Keith Joseph, M.P., Minister of State, Board of Trade.

After luncheon they toured the new wing, the Research Block which has been in use for two years, and Production Departments producing instruments for use in medicine and gas analysers for research and industry. In addition, a number of research projects and special displays of instruments made by the Company's two London factories were exhibited.

The new wing—which is 150 ft. long, 50 ft. wide and provides, on two floors, a working area of approximately 15,000 sq. ft.—is primarily intended to accommodate production departments for the final testing and assembling of instruments prior to dispatch. Immediately obvious features are the clean functional sweep of its two raised storeys, the ground-floor-level car-park and loading bay beneath, and the harmonious way it blends with the research laboratories, opened two years ago (see *Nature*, 184, 1364; 1959), to which it has been planned as a logical architectural extension, and to which one end is joined by connecting doors. The other end of the wing is connected to other works buildings and the loading bay by a link block containing stairs and toilet facilities.

Structurally, the reinforced concrete frame of the raised building rests on a solid concrete slab supported by two rows of circular columns along its length, over which the slab is cantilevered out about 8 ft. front and back. The second floor and roof are mounted on pre-cast pre-stressed concrete beams spanning the full width of the building, so that the floor areas of both storeys are uninterrupted by intermediate columns. Windows are double glazed and the infill panels below faced with golden buff bricks. Air supplied throughout the building is conditioned and filtered by plant contained in two penthouses on the roof. Essential services, ventilating and heating inlets, gas, compressed air, water, electrical supplies, telephones, etc., are laid in horizontal ducts running the full length of the building below the windows and, to reduce fire risks, all work benches are wired with mineral-insulated metal-sheathed conductors.

Two-thirds of the first-floor area, adjacent to the entrance hall, is used as Production Offices and the remainder is occupied by the Microanalyser Department. This Department is responsible for assembling and testing 'Microscan' X-ray analysers and for the production of sealed and flow-type proportional counters. It is also concerned with the commissioning and servicing of 'Microscans' and operates an advisory service, including the analysis of samples provided by prospective customers. Complete installations are assembled in specially designed cubicles, each of which is supplied with its own special services. Conditioned air supplied to this Department undergoes an additional degree of filtration in view of the need for absolute cleanliness during final assembly of a 'Microscan'.

The whole of the second floor is devoted to final assembling, wiring and testing of a wide range of galvanometric indicators, recorders and controllers. Here again a dust-free atmosphere, so necessary for the assembly of intricate precision instruments and components, is provided. The functional interior design with its modern décor makes the new building a pleasant and comfortable place in which to work.

The new wing is the latest illustration of the considerable

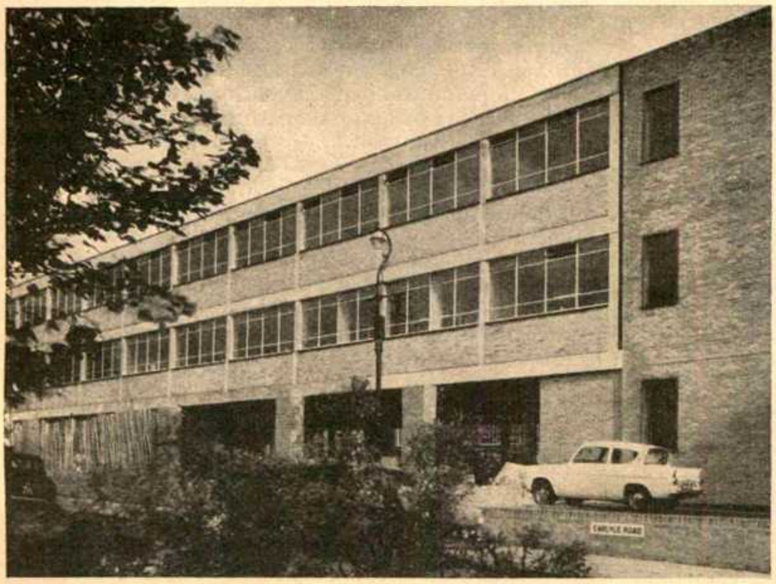

Fig. 1. Instrument assembly wing of the Cambridge Instrument Co., Ltd.

development of the Company in recent years and has been made necessary by the increasing numbers of new and improved instruments developed by the Research Department The building, which was designed by Edward D Mills and Partners, architects, was completed in less than a year by the contractors, J Jarvis and Sons, Ltd

J G HAMMOND

LIFE AND ITS ENVIRONMENT IN ANCIENT SEAS

DURING the British Association meeting at Norwich, the Geology and Zoology Sections (C and D) held a joint session on "Life and its Environment in Ancient Seas".

Prof C M Yonge, president of Section D, was in the chair and about 150 persons attended The programme was designed as a general session covering a wide range of palæoecological topics, but unfortunately the opening address by Prof Kaare Strøm (professor of limnology, University of Oslo), entitled, "In Quest of Ancient Seas", and the paper by Dr R H Cummings (Department of Geology, University of Glasgow) on the "Palæoecology of Foraminifera in Reef Limestones" were both cancelled, and so the scope of the meeting was more restricted than originally planned

The paper by Dr K A Joysey (Department of Zoology, University of Cambridge) on "The Life of Cephalopods in Ancient Seas" was a discussion of existing views on the mode of life of fossil forms, considered in the light of new information about living cephalopods Dr Anna Bidder (Department of Zoology, Cambridge) showed a series of excellent colour slides of the living *Nautilus* and gave an account of her observations, largely based on freshly captured specimens living in the aquarium at Noumea, New Caledonia The paper by Mr R W Sheldon and Dr F M Broadhurst (Department of Geology, University of Manchester) and Dr J G Potts (United Steel Companies, Ltd) on "Aspects of the Palæoecology of Marine Faunas in the Upper Carboniferous of the Southern Pennines", was a preliminary account of work still in progress seeking to elucidate the relationship between certain fossil faunas (mostly goniatites, lamellibranchs and brachiopods) and the petrology of the enclosing sediment Hence the session as a whole had an emphasis on molluscs, particularly cephalopods. Although unplanned, this bias came as a natural sequel to the molluscan theme of Prof. Yonge's presidential address

Many 'palæoecological' publications (including some by the present author), are merely records of association and distribution, and offer little interpretation of the relationship between the fauna and the environment The appreciation of a fossil biological system demands not only the reconstruction of the animals themselves but also of how they lived, what they did, their effects on other animals and on the environment. The Norwich session served well to illustrate this palæobiological approach to palæoecology

In surveying the problems of buoyancy control, Dr Joysey noted that the majority of living cephalopods are more dense than sea-water and support themselves by swimming, but *Sepia*, *Spirula* and *Nautilus* have a density near that of sea-water, owing to gas enclosed in rigid-walled chambers Unlike the swim-bladder of a fish, the volume of the cephalopod float is not affected by changes in pressure Hence, so long as some mechanism prevents the hydrostatic pressure from forcing liquid into the chambers through the porous siphuncular tube, changes in depth should not affect the buoyancy Denton and Gilpin-Brown have recently shown that *Sepia* can adjust its buoyancy by pumping liquid in and out of the chambers, like a submarine altering ballast This liquid has a lower salt concentration than the body fluids, and so osmotic pressure helps to counteract hydrostatic pressure Dr. Joysey observed that as *Spirula* and possibly *Nautilus* are recorded from depths below that at which an osmotic mechanism would fail, some other mechanism must be sought if the gas is to be maintained in the chambers An increase in the gas pressure would prevent liquid from flowing into the chambers and also prevent implosion of the shell, but any other mechanism whereby the siphuncular tube might become temporarily impermeable to liquid must be accompanied by a shell sufficiently strong to withstand the external pressure

Dr Bidder showed some photographs of *Nautilus* in a state of nearly neutral buoyancy swimming in mid-water or resting on the bottom, and others of it floating with part of the shell breaking the surface of the water She had also observed under-buoyant animals hanging by their tentacles, and had found that individual specimens could adjust their buoyancy

Dr Bidder stated that the gas in the chambers was not under pressure in a specimen just hauled from a depth of nearly 200 metres She had also extracted quantities of liquid from the gas chambers of *Nautilus* and using X-ray photographs she demonstrated that when a specimen had been turned upside down the shadows which were presumed to be bodies of liquid had indeed run to the opposite side of the chambers She found no evidence of living tissue in the gas chambers of *Nautilus*

In reviewing present-day interpretations of the posture of various fossil cephalopods, Dr Joysey noted that the position of the gas chambers in orthoconic shells at first sight suggested that they hovered vertically in the water, or rested on the sea-floor like church steeples Alternatively, if a load were present at the apical end, such as liquid in the apical chambers, they could have adopted a horizontal posture It is generally accepted that, in some forms, this apical counterbalance was provided by solid deposits within the siphuncular tube. Other forms have deposits within the apical gas chambers, but there has been some controversy as to whether these were laid down by a cameral mantle or whether they are post-mortem mineral infillings In view of the recent findings on living forms, Dr Joysey suggested that serious consideration should be given to the possibility that these deposits might have been precipitated during life from solutions transported through the walls of the siphuncular tube

By analogy with orthoconic nautiloids, Dr. Joysey pointed out that one of the functions of the belemnite guard was to act as a counterbalance. It lay at the posterior end of the main gas chambers while the bulk of the animal was at the anterior end, so giving a 'see-saw' type of stability. The morphological series from the belemnite to *Sepia* is well known. Functionally, this change involved sliding the body mass under the gas chambers, giving an 'air-ship' type of stability. In consequence, the counterbalance was progressively reduced and hence the volume of gas required to provide support could also be reduced.

Dr. Joysey referred to some palæozoic nautiloids (for example, *Ascoceras*) in which the juvenile was orthoconic, but by the development of highly oblique septa the adult achieved a horizontal posture, without resort to a counterbalance. Coiling of the shell produces a similar result as the body chamber comes to lie more or less horizontally below the float. Dr. Joysey briefly described Trueman's elegant investigation on the buoyancy and posture of ammonites. Trueman showed that an uncoiled form such as *Lytocrioceras* possessed greater stability than its tightly coiled counterpart. In functional terms such a shell has achieved the 'air-ship' type of stability, independently attained in such distantly related forms as *Ascoceras* and *Sepia*.

Dr. Bidder stated that *Nautilus* appears somewhat clumsy in side view but when seen from above, below or behind, its outline is remarkably streamlined. Its movement is slow in the encumbered environment of the aquarium, but more rapid in open water. Although it frequently swam near the bottom of the aquarium with its tentacles 'searching' the substratum, it had never been observed to crawl. Some of the photographs clearly showed the remarkable flexibility of the tip of the funnel, which can send the jet in almost any direction, thus giving considerable manœuvrability.

Dr. Bidder stated that the density of the body of *Nautilus* appears to be somewhat lower than Trueman's calculated value, and she suggested that it might prove necessary to modify his conclusion that the majority of ammonites were under-buoyant. Furthermore, his interpretations of the posture of ammonites are amenable to modification if the chambers contained unequally distributed fluid ballast.

Dr. Broadhurst outlined the work of Dr. J. G. Potts on the *Gastrioceras cancellatum* marine band near the top of the Millstone Grit Series, the work of Mr. R. W. Sheldon on another marine band in the Edale Shales towards the base of the Millstone Grit, and his own work in the Coal Measures.

In Dr. Pott's marine band some of the goniatites are preserved flat on their sides, whereas others are crushed in a vertical position, just as they settled on the sea-floor, leaving impressions of the venter on the bedding planes. It was suggested that a high proportion of vertically preserved goniatites probably indicated quiet conditions, and this was found to be at a maximum near the middle of the band, where the goniatites themselves were most abundant. The lamellibranchs had two periods of abundance, one above and the other below the goniatite maximum. The grain size and the amount of coarse detritus both had high values at the top and the bottom of the band, prior to the first and after the second lamellibranch peak. The marine band as a whole was described as a 'palæontological sandwich', the goniatites being the filling, the lamellibranchs the butter, and the coarse detritus the bread. It appeared that the absence of fauna in the sediments above and below the marine band could be attributed to turbulence and the greater rate of terrigenous sedimentation.

Mr. Sheldon's band contained fewer goniatites and lamellibranchs but abundant brachiopods. The amount of detrital material was low, most of the sediment being ankerite and silica. The brachiopods *Chonetes* and *Martinia*, the goniatite *Eumorphoceras* and the gasteropods in the fauna were abundant in the upper and lower parts of the band and sparse in the middle, analogous in pattern to the lamellibranch distribution present in Dr. Pott's marine band. In contrast the brachiopod *Productus* was abundant near the middle of the band and diminished upwards and downwards, this pattern corresponding with the abundance of ankerite.

Dr. Broadhurst was largely concerned with the conditions of sedimentation between marine bands. He showed photographs of a tree-cast 38 ft. high, standing vertically in non-marine sediments, its base rooted above a coal seam and its top in mudstone. He stressed that vertical preservation is not to be regarded as a freak occurrence, for he has records of many such trees at several horizons. Such preservation can only be interpreted as indicative of rapid sedimentation and it is significant in this respect that these vertical trees never cut through marine bands. Viewing their work as a whole Dr. Broadhurst suggested that a slow rate of deposition of terrigenous material appears to have been an important controlling factor during the deposition of the relatively thin marine bands, whereas more rapid sedimentation appears to have characterized the thicker intervening beds.

In the discussion which followed the papers Dr. Broadhurst and his colleagues indicated that the onset and the ending of marine band conditions were transitional rather than abrupt. Lateral changes could be detected at some horizons, for some *Lingula* beds pass laterally into true marine bands in other regions.

Dr. Joysey agreed that partially uncoiled ammonites like *Lytocrioceras* must have adopted different postures at various stages during growth, and commented that these might reflect changes in its feeding requirements. He noted that it was widely stated in the palæontological literature that *Nautilus* could adjust its buoyancy by moving in or out of its shell. Changes in shape of the body mass, however, would not affect its volume and so the density of the body would remain constant. Assuming that any space vacated within the body chamber would be filled with sea-water, then movements of the body would not affect its overall density. It appeared to him that this mechanism could not produce changes in density unless there was an unsealed gas bubble within the body chamber. Dr. Bidder stated that there was no gas bubble between the animal and the last septum, and that although small bubbles were sometimes present in the mantle cavity, these could be attributed to the aquarium aeration system.

The session as a whole provided useful liaison between geologist and zoologist, and each was able to gain a greater appreciation of some of the practical difficulties faced by the other in obtaining sufficient information to give an unequivocal answer to an apparently simple question. It also served to illustrate that one class of animals alone could provide abundant material for discussion, and that there remained plenty of scope for future joint sessions.

K. A. JOYSEY

THIRD AUSTRALIAN SPECTROSCOPY CONFERENCE

THE third Australian Spectroscopy Conference was held in the School of Chemistry at the University of Sydney during August 22–24 under the sponsorship of the Australian Academy of Science Spectroscopy Committee. A varied and interesting programme, consisting of 5 invited review papers and 51 research papers, was provided for the 227 participants. In addition, six exhibitors displayed some of the latest developments in spectroscopic equipment. The papers were arranged into sessions on vibrational, theoretical, molecular electronic and resonance spectroscopy with some parallel sessions concerned with instruments and analytical applications, including atomic absorption spectroscopy. After Prof R J W Le Fèvre had briefly welcomed the participants of the Conference to the School of Chemistry, Prof N S Bayliss officially opened the proceedings on behalf of the Australian Academy of Science and welcomed to the conference the overseas guests, Prof W C Price (King's College, London) and Mr J E Allan (Rukuhia Soil Research Station, Hamilton, New Zealand).

The conference opened with a session on vibrational spectroscopy, and Prof W C Price commenced with a stimulating review of recent developments in the far infra-red. After briefly referring to the experimental difficulties associated with investigations in the range 1–500 cm^{-1}, he then proceeded to discuss the selection of sources, dispersing materials and detectors, and concluded by giving examples of the use of this region to obtain information on rotational energy-level in gases, lattice vibrations in crystalline and molecular crystals, and impurity energy-levels in semi-conductors.

Three research papers then followed dealing with infra-red studies on hydrogen bonding. Prof A N Hambly (Department of Chemistry, School of General Studies, Australian National University) discussed the infra-red evidence for hydrogen-bonding in amino-sulphones, and concluded that stronger interaction occurs between the amino and sulphone groups on adjacent carbon atoms than occurs in the corresponding nitro-amines. This was attributed to the difference in the geometry of the interacting groups. Mr P. E Rogasch (Weapons Research Establishment, Salisbury) produced evidence to show that proton transfer can occur in systems that are strongly hydrogen-bonded. Mr I H Reece and Prof R L Werner (Department of Physical Chemistry, University of New South Wales) dealt with the intermolecular interaction between carbonyl and hydroxyl groups in solution, and showed how this interaction can be used to deduce *pKa* values for the hydroxyl compounds from the infra-red spectrum.

The session continued with papers on analysis of the vibrational spectrum on mono-methyl-arsine (Dr V Cranmer, Imperial Chemical Industries, Australia and New Zealand, Ltd , Central Research Laboratories, Ascot Vale), of dichlorofluoromethane-sulphenyl chloride (Dr N S Ham, Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) and tri-methyl borate (Prof R L Werner, Department of Physical Chemistry, University of New South Wales). Dr E Spinner (Department of Medical Chemistry, Australian National University, Canberra) discussed the vibrational spectra and structures of the hydrochlorides of the aminopyridines. The Raman and infra-red spectra suggest that whereas the positive charge resides on the ring nitrogen in 3-aminopyridine it is located on the amino-nitrogen in 2- and 4-aminopyridine. Mr R H Laby and Dr D J Greenland (Waite Agricultural Research Institute, University of Adelaide) were concerned with the implications of the infra-red spectra of molecules of amino-acids, simple peptides, alcohols and glycols adsorbed by montmorillonite. Methods for increasing the discrimination between similar infra-red spectra were described by Dr J Tregellas-Williams (Defence Standards Laboratories, Maribyrnong). Progress being made in the development of improved micro-wave-powered Raman sources was summarized by Dr N S Ham and Dr A Walsh (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization). The session on vibrational spectroscopy was concluded with a paper by Dr P Marmet (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) dealing with the problem of producing mono-energetic electrons in a mass-spectrometer and their use in obtaining details of the vibrational structure in the energy states of simple molecular ions.

Dr N S Ham (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization), in the session on theoretical and molecular electronic spectroscopy, reviewed some theoretical and experimental aspects of the Jahn–Teller theorem. He pointed out how the experimental manifestations of the theorem depended on the magnitude of the distorting forces. Examples showing orbital and/or spin degenerate ground and excited states were discussed. Mr E F. McCoy and Dr I G Ross (Department of Physical Chemistry, University of Sydney) considered the change of shape in electronic transitions of polycyclic aromatic hydrocarbons. They showed that encouraging agreement is obtained with experiment if calculations of the overall width and intensity distribution in the corresponding emission or absorption spectrum are based on the sum of the squares of the bond-length changes that accompany the electronic transition. Mr J C Mackie and Dr L E Lyons, from the same department, outlined theoretical methods which enable the accurate calculation of the ionization potential of a molecular crystal from a knowledge of the ionization potential of the free molecule, its polarizability and the crystal structure. Prof W. C Price discussed the ionization potentials of some simple radicals and molecules in terms of their electronic structure. It was shown that the π electron orbitals of the halogen substituted CH_3, CH_2 and CH radicals account for the magnitudes of the observed ionization potential and stability of parent molecules and ions. The effects of progressive substitution on the ionization potential of ethylene were also explained.

Dr E G McRae spoke on the theoretical relationship between fluorescence and vibrational structure and Davydov splitting in molecular crystals. Application of his theoretical treatment to the available information on the crystal fluorescence spectrum of anthracene leads to a prediction of a Davydov splitting of the order of 500 cm $^{-1}$ It is interesting to compare this with a later paper in the conference in which Mr A. R. Lacey and Dr L E Lyons (Department of Physical Chemistry, University of Sydney) described experimental investigations of the Davydov splitting in the spectrum of anthracene crystals which yielded an estimate of ~ 330 cm $^{-1}$ for this splitting, the much lower values (0–60 cm $^{-1}$) usually reported are attributed to the combined effect of incomplete polarization of the incident radiation and the thickness of the crystals used by other investigators

Mr M Batley and Dr L E Lyons (Department of Physical Chemistry, University of Sydney) discussed refinements in the quantitative prediction of the crystal spectrum of phthalocyanine In a paper by Dr E. A Magnusson and Dr H Shull (Australian Missionary College, Cooranbong) it was suggested that the barrier to internal rotation about a single bond may be explained both with regard to height and sense, in terms of interactions between the electrons in the terminal bonds and also to the possibility that the distribution of the electrons in the single bond itself is not cylindrically symmetrical Mr G L White (Defence Standards Laboratories, Maribyrnong) presented a theoretical paper on polarization in Raman spectra Dr A D Buckingham (Chemistry Department, Harvard) spoke on spectroscopy with polarized radiation He discussed the existing methods of determining the direction of the transition dipole moment with respect to molecular axes and outlined a new approach involving the measurement of the Kerr constant through absorption bands.

A review by Dr. I G Ross (Department of Physical Chemistry, University of Sydney) on optical masers preceded the session on molecular electronic spectroscopy After explaining the basic principles of optical masers, Dr Ross considered some of the general and research applications possible for the monochromatic high-intensity beams that can be produced Dr T M Spotswood (Division of Coal Research, Commonwealth Scientific and Industrial Research Organization) presented evidence for the formation of charge transfer complexes during the bromination of forms of carbon known to contain relatively high proportions of aromatic structures The existence of charge-transfer complexes between brominated polycyclic hydrocarbons and halogens in solution was also established, and the spectra of these were compared with those for the corresponding complexes for the corresponding unsubstituted hydrocarbons In a contribution from Dr A. V Bree (University College, London) the fluorescence and absorption spectra in the near ultra-violet of crystals of 9,10-dichloroanthracene, measured at room temperature, were discussed and shown to confirm that the corresponding transition in crystalline anthracene is short-axis polarized Dr Bree showed that photo-produced impurities accounted for fluorescence and photo conductivity observations In another paper from the same department, Dr T M Dunn presented the results of a rotational analysis of the 2700 Å system of *para*-difluorobenzene which confirm that a $B_{3u} \leftarrow A_g$ transition (polarized along short axis, that is, in plane of benzene ring and perpendicular to F–F direction) is responsible

Three papers from the Weapons Research Establishment, Salisbury, were concerned with investigations of the character of the emission associated with various electronic transition processes in flames and in the upper atmosphere Dr C L Cook and Mr J A McFarlane described the spectral characteristics of the emission from an ammonia/bromine pentafluoride diffusion flame Mr L J Drummond and Mr P E Rogasch did the same for the active nitrogen–chloroform and active nitrogen–methylene chloride flames Dr J C Burns outlined some of the difficulties associated with attempts to measure temperatures from the AlO-radical emission which accompanies the re-entry of the main body of a rocket research vehicle into Earth's atmosphere Vibrational equilibrium appears to exist and temperatures in the range 1,600°–2,000° K were obtained

During the session on analytical applications on emission spectroscopy Mr L S Dale and Mr R N Whittem (Australian Atomic Energy Commission Research Establishment) described the spectrographic techniques in use in health surveys of beryllium contamination In a second paper, Mr Whittem discussed the spectrochemical analysis of toxic and α-active materials. Mr A B Timms (Australian Mineral Development Laboratories, Adelaide) spoke on the spectrographic determination of hafnium/zirconium ratios Mr J M Nobbs (Defence Standards Laboratories, Finsbury) presented evidence to show that while the input of electrical energy to an arc gap between metal electrodes can be controlled to within very fine limits, the output of radiation from the arc column varies randomly from pulse to pulse by factors as large as 200, and is caused primarily by fluctuations in the evaporation of electrode material The theory of the photographic characteristic and its use in plate calibration formed the subject of a paper by Mr C Candler (South Australian Institute of Technology) The analysis of lubricating oils for the additive elements barium, phosphorus, calcium and zinc with a direct-reading spectrograph was outlined by Dr. R A. Cummins (Defence Standards Laboratories, Maribyrnong) Mr I Collison and Mr T Scott (Broken Hill Pty , Newcastle, N S W) spoke on their experiences with the calibration of an A R L. 'Quantovac' for the analysis of sulphur and phosphorus in carbon and low alloy steels

In a session on instruments and techniques the following papers were presented Mr J J McNeill (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) discussed factors influencing the design of an inexpensive general-purpose monochromator for use above 2000 Å The construction and performance of an Ebert scanning spectrometer was discussed by Dr S C Baker (Physics Department, Newcastle University College, N S W) A photoelectric device to prevent slow image shifts during long exposures in a high-resolution échelle spectrograph was described by Dr G R. Hercus (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) Outstanding problems of instrumentation in the development of the full potentials of X-ray fluorescence spectroscopy as an analytical tool were briefly considered by Mr J E Maloney (Philips Electrical Industries, Sydney) Mr J F Horwood and Mr D A Forss (Dairy Research Section, Commonwealth Scientific and Industrial Research Organization) described methods used for handling micro-quantities of samples for absorption spectrometry Mr F B. Wunderlich (John Lysaght Pty , Ltd , Newcastle, N S W) indicated how low-resolution infra-red spectra could be used for the identification of constituents in paints and plastics Mr A T Martin (C S R Chemicals, Rhodes) spoke on the reflectance of opaque materials as a function of pigment concentration Finally, Dr N Kelso King (Division of Physical Chemistry, Commonwealth Scientific and Industrial Research Organization) discussed the significance of isosbestic points in studying reaction mechanisms

The session on atomic absorption spectroscopy, which was held on the final morning, proved to be one of the most successful sessions of the conference It was very gratifying to witness the obvious intense interest in this subject, which was pioneered in Australia and New Zealand Mr J E Allan (Rukuhia Soil Research Station, New Zealand) opened this session by reviewing recent developments in the technique The progress made since the second Australian Spectroscopy Conference in 1959 was amply illustrated by the increase in published research papers from 5 to 50 at present Dr B G Davey (School of Agriculture, University of Sydney) described the application of atomic absorption spectroscopy to the analysis of agricultural materials It is now possible to determine rapidly many elements in soil and plant material, up to 170 samples an hour can be analysed for one element with a coefficient of variation less than ± 5 per cent using very simple equipment Mr D J David (Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization) discussed the determination of molybdenum by atomic absorption Molybdenum is believed to be present in a flame as the oxide under normal conditions, but in an acetylene–air flame operated under reducing conditions the molybdenum trioxide dissociates to atomic molybdenum which can be detected by absorption Mr David suggested that compound formation under flame conditions may account for many of the interferences that can occur in analysis by atomic absorption The research in atomic absorption being carried out at present at the Rukuhia Soil Research Station was surveyed by Mr J E Allan Extension of the range of elements accessible to the technique by the use of nitrous oxide–acetylene flames is being investigated. The determination of cobalt and nickel in agricultural materials has been studied in detail, and procedures established for the analysis of fertilizers Dr J B Willis (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) showed how atomic absorption was being used to determine traces of heavy metals such as lead, mercury, bismuth, nickel, cadmium and zinc in biological materials. The sample preparation procedures are much simpler than those involved in most existing methods, cadmium and zinc can be determined directly in urine and preliminary experiments suggest that zinc may be determined in blood serum and whole blood by spraying the diluted material directly into the flame Cathodic sputtering appears to be an attractive method for atomizing those elements (for example, aluminium, strontium and titanium) which form refractory compounds under flame conditions, and Drs B M Gatehouse, J V Sullivan and A Walsh (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) discussed the factors affecting the cathodic sputtering of various metals. In some instances it has been possible to establish sputtering conditions which permit analytical determinations, but the method is still unsatisfactory for aluminium.

The final session of the conference was devoted to resonance spectroscopy Dr G S Bogle (Division of Physics, Commonwealth Scientific and Industrial Research Organization) reviewed the development of the theory of electron paramagnetic resonance Mr A J Harle, Dr. L E Lyons and Mr B K Selinger (Department of Physical Chemistry, University of Sydney) summarized the results of a preliminary survey of the electron-spin resonance spectra of some charge-transfer complexes between chloranil and aluminium oxide, hexamethyl benzene and tetracyanoethylene, dibenzacridine and tetracyanoethylene or chloranil Dr G S Bogle (Division of Physics, Commonwealth Scientific and Industrial Research Organization), Drs W F Forbes and W E Savige (Division of Protein Chemistry, Commonwealth Scientific and Industrial Research Organization) reported some investigations of the electron paramagnetic resonance properties of cystine and related compounds after irradiation with mercury 2537 Å. radiation and a sun-lamp (peak ~ 3100 Å) Similar spectra are obtained for all the compounds examined, indicating that the same trapped free radical RS ($R = \mathrm{CH_3\,\underset{|}{CH}\,(NH)_2{\cdot}COOH}$) is formed in each case on irradiation Mr. T. J Seed (Department of Physics, University of Canterbury) discussed the paramagnetic spectra of manganous ions in undiluted crystals of the solid-solution ammonium chloride–manganous chloride–water.

In the final paper of the conference Dr C K Coogan (Division of Chemical Physics) and Prof H S Gutowsky (Chemistry Department, University of Illinois) reported on nuclear magnetic resonance in sodium hydrosulphide Observations on the hydrogen-1 and sodium-23 resonances in polycrystalline samples of sodium hydrosulphide tend to confirm Pauling's hypothesis that the hydrosulphide ion is tumbling very rapidly in the crystal lattice and thus presents an outward spherical form to the surrounding ions This occurs above 80° C, and it was also observed that SH⁻ ions can diffuse through the lattice

R A Durie

COLLOIDAL SYSTEMS

An informal discussion on colloids, under the auspices of the Faraday Society, was held in the Royal College of Science and Technology, Glasgow, on June 27 The meeting was held to mark the centenary of the appearance of Thomas Graham's paper, "Liquid Diffusion applied to Analysis", in which a clear definition of colloids and crystalloids was first given An attempt was made to cover as many colloidal topics as possible, in the event, this did not prove feasible and the subjects tended to be somewhat biased towards association colloids About seventy people attended the meeting, including several from Holland

The chairman, for the morning session, was Prof P L Pauson, who welcomed the visitors to the Royal College and indicated the reasons for holding the discussion at this time and place (Graham held his first chair at the College) The first speaker was Dr A J Hyde (Glasgow), who gave a brief biography of Graham with rather greater emphasis on the private and non-scientific side of his life than on his scientific achievements, which are reasonably well known

Sir Eric Rideal (London) then gave an address on "Colloid Science a Century of Progress" Sir Eric reviewed the development of colloid chemistry since 1861, commencing with Graham's work on peptization, gels and sols He then discussed work on coagulation, flocculation and peptization of sols, drawing on the work of Von Weimarn, Hardy and Perrin for examples A review of optical investigations on colloidal solutions was then given, reference being made to the work of Faraday and Tyndall, and finally to the ultramicroscope of Zsigmondy and Siedentopf Gels and adsorption were dealt with rather more briefly, mention being made of the work of Freundlich and the swelling experiments of van Bemmelen

The final speaker in the morning session was Dr A S C Lawrence (Sheffield), who gave a talk describing the work of his group at Sheffield on soap–water–amphipile systems Graham did not carry out any investigations on soap solutions, but they come within his classification of colloids Dr Lawrence commenced with a general description of the colloidal and other phenomena encountered, and of the phase diagrams of the systems He continued with a more detailed account of the liquid crystalline forms and myelinic figures which are obtained on adding soap solutions to crystals of a polar additive, or solutions of additive to a soap crystal These were interpreted in terms of adsorption of soap and additive at the solid-liquid interface In conclusion, mention was made of the effect of organic and inorganic salts on the systems

For the afternoon session, the chairman was Sir Eric Rideal and the first speaker, Dr A P Prosser (London) Dr Prosser gave a talk on the forces which, in conjunction with electrostatic forces, stabilize all colloidal systems Beginning with a general account of van der Waals forces and London dispersion forces he then went on to deal with their origin and the differences which exist when bodies are very close together (< 250 Å apart, the unretarded case) or relatively far apart (> 2000 Å, the retarded case) He then gave a brief outline of the theory of the forces and the problem of evaluating them from the theory in the retarded and unretarded cases The experimental determinations of attractive forces by the groups led by Bradley, Derjaguin, Overbeek and Kitchener were then discussed, along with some recent experiments on flocculation in colloidal systems

The second paper in the afternoon, entitled "Polyelectrolytes", was by Prof J A V Butler and Dr D J R Laurence (London) and was read by Dr Laurence The subject-matter is directly connected with Graham's work on caramel and gelatin Dr Laurence mentioned the general properties of polyelectrolyte solutions such as high viscosity and gel formation He then went on to discuss more specialized properties such as titration curves, conductivity curves and transport properties which give rise to the picture of a polyelectrolyte molecule in solution as a charged random coil with small gegenions, some of which are 'free' and some 'trapped' inside the coil The last point is particularly well illustrated by measurements of transport number The spatial configuration of the coils was then discussed in terms of the information obtainable from sedimentation, viscosity and light-scattering measurements Finally, deoxyribonucleic acid was treated in rather more detail, followed by a mention of proteins, including ribonuclease

The final speaker, Prof J T G Overbeek (Utrecht), spoke on the drainage of soap films He began with a few general remarks on the structure of soap films, their thickness and colours. Then followed a description of the stretching of films, with the formation of new film, and the drainage of films Finally, the nature of the black film was discussed, with reference to the discontinuity in thickness at the edge of the black area, the forces giving rise to the black film and the ultimate breaking stability of the film when electrostatic and van der Waals forces are opposed to one another Prof Overbeek then presented a colour ciné film of the drainage of soap films illustrative of all the points he had mentioned, and containing, in addition, several beautiful illustrations of moving colour effects obtainable in soap films

A J Hyde

THE LIBRARY ASSOCIATION

ANNUAL CONFERENCE

THE papers presented at the annual conference of the Library Association at Hastings during September 19–22 were of general rather than specific interest to the scientist Mr Leslie Wilson and Viscount Caldecote made some reference to the National Reference Library of Science and Invention and the National Lending Library for Science and Technology and to the proposals for the re-organization of the Library Association, particularly as they seek to establish the Association as the professional association of librarians, and their papers are of direct consequence to scientists and technologists Sir Charles Snow's lively presidential address, with its plea for a wide view of the functions of the public library in the Welfare State, should also commend itself to them Sir Charles believes that the public libraries must in future assume some of the functions of the great private lending libraries and that, in particular, they have a real responsibility to contemporary literature which some of them have yet to recognize Mr K J Lea, county librarian of Essex, on the other hand, while not discounting this amenity aspect, to which the smaller library authorities are virtually limited, urged that more attention should be given to the educational and specialized services, if necessary by the development of a two-tier system based on co-operation between boroughs and counties, the possibility of which is already being demonstrated by the county libraries of Britain

Mr Leslie Wilson's paper, on "Libraries and Information Services the Challenge of the Future", was equally concerned with co-operation, alike between such bodies as the Library Association and the Association of Special Libraries and Information Bureaux and between these and institutions concerned with the use of information He stressed first the desirability of library and information work being recognized generally as a single distinctive professional activity and then the relevance of such an activity to the fragmentation of knowledge Effective special library work in any one field depends, he said, on co-operation between librarian and information officer and the specialist working in that field, and he suggested we have still far to go before the idea was generally accepted that accumulated knowledge, with the librarian as its vehicle, is as essential to industrial prosperity as capital investment, production and sales Viscount Caldecote indeed went some way in this direction when, in his paper earlier that day, he suggested that the research worker can reasonably expect library staff to be responsible for two-thirds of his library searching Mr Wilson thought, moreover, that we must expect a much greater participation of the Government in library affairs, and for all these reasons it would be wise for librarians and their organizations to clarify their ideas as to the form of partnership in which they could function most effectively

Mr Wilson cited the plans for the new National Reference Library of Science and Invention as a glaring example of the confusion and frustration likely to result from failure to achieve such co-operation and consultation between user and librarian at the earliest stage, but he also viewed with concern the proliferation of professional bodies concerned with such a relatively small field as library and information work Formal federation is probably impractical at present, but he suggested that some form of consultative committee or a series of consultative committees, each dealing with a special interest such as education or research, might help to achieve the closely knit community that is needed The Aslib Groups, he suggested, seem to be a reasonably successful means of dealing with the problem, but of the seventeen organizations listed in the handbook of special librarianship and information work as active in this field in the United Kingdom, at least the Library Association, Aslib, the Circle of State Librarians, the Standing Conference of National and University Libraries, the United Kingdom branch of the International Association of Music Libraries, the Association of British Theological and Philosophical Libraries and the Institute of Information Scientists should come closer together

Viscount Caldecote's paper expressed an industrialist's view and criticism of library and information services He thought that the main industrial library problem centre around the small firm He suggested first that the larger firms might make their own extensive library facilities available to these smaller firms, and that by doing so the trained library staff would enable the smaller firm to tap also the resources of the national libraries Viscount Caldecote repeatedly stressed the importance of quality of trained library staff, and he also suggested that member firms could do more to strengthen the library resources and services of the Research Associations He was impressed by the value of the area co-operative schemes formed in Sheffield, Newcastle upon Tyne, Liverpool and elsewhere, and in this connexion he thought the liaison thus established with educational bodies is of vital importance for the pooling of resources and knowledge Finally, in discussing briefly the functions and staffing of industrial libraries, themselves, Viscount Caldecote suggested that the Association might form a committee to study the automation of routine library services

Other papers presented at the Conference, by Dr C Bibby and by Mr A E Sanderson, dealt with the functions of training-college libraries, Mr B Williams firmly challenged the exercise of censorship in reading by a local authority or other body In his Library Association Annual Lecture, "The Fourth *R*", Dr Lincoln Ralph reviewed the limitations of an education confined to reading, writing and arithmetic in coping with the modern world, he urged that it is necessary not simply to instruct but also to inspire Education should impart a sense of values, of the purpose of life, and lead to a re-birth

THE AUSTRALIAN ATOMIC ENERGY COMMISSION

THE eighth annual report of the Australian Atomic Energy Commission*, which covers the financial year ended June 30, 1960, records a year of substantial progress in the high-temperature gas-cooled reactor project, in the production and application of isotopes, and in the installation and commissioning of new equipment

The report consists of nine sections dealing respectively with an outline of the activities of the Commission, the search for uranium, uranium mining, raw materials, including thorium and beryllium, research activities, isotopes, nuclear power, international agreements and co-operation with the International Atomic Energy Agency, and general activities Details of research contracts, postgraduate research studentships and undergraduate scholarships, together with the financial accounts and the names of the staff of the Commission, are given in appendixes to the report

Although the world's annual uranium production potential at present exceeds the demand, steadily increasing requirements for nuclear power stations are expected to develop from the 1970's onwards, and the Commission, through the Bureau of Mineral Resources, has continued its surveys for new deposits of uranium Except for one area stated to show anomalous radioactivity, no discoveries of radioactive materials were reported to the Commission during the year, but a number of further monetary reward instalments were paid for earlier discoveries

For the first time, complete production figures are given in the report for operations at Rum Jungle since the start-up in 1954 A record tonnage of ore was treated during 1959–60, and all the uranium oxide produced was delivered to the Combined Development Agency under the terms of the Rum Jungle Agreement, which expires on January 6, 1963. No mining was carried out during the year, and the treatment plant continued to operate on stockpiled ore

Full-power routine operation of the reactor *Hifar* began in February 1960 after a prolonged period of testing and calibration The reactor has given little trouble, and major irradiation work has been performed for the Commission and under contracts with the United Kingdom Atomic Energy Authority and the General Electric Company. The materials irradiated included prototype fuels, beryllium and beryllium oxide samples, and cobalt for the production of cobalt-60

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* Australian Atomic Energy Commission Eighth Annual Report for the year ended 30th June, 1960 Pp 63 (Coogee, N S W Australian Atomic Energy Commission, 1960)

New buildings in the Research Establishment were officially opened on May 27, by the Minister for National Development, the Hon. W. H Spooner They consisted of the canteen, adjoining the building provided by the Commonwealth Government as the headquarters of the Australian Institute of Nuclear Science and Engineering, and a building devoted to isotopes and technical physics A 10-kW graphite moderated reactor, named *Moata*, ordered from the United States of America, is to be installed in the new reactor physics building to be finished this year *Moata*, which will have a maximum thermal neutron flux of 10^{11} neutrons cm $^{-2}$ sec $^{-1}$, is designed for flexibility of core and reflector arrangement, and will be used to measure thermal and resonance neutron cross-sections, to study slowing-down properties of moderators such as beryllium, and to investigate long-term changes in fuels and moderators Studies of the feasibility of a reactor system require intensive investigation into nuclear materials, and the various aspects of the research into the fabrication of graphite, beryllium, beryllium oxide, and sample fuel elements, the effects of irradiation on selected materials, and the compatibility of each material with the others, are described in some detail in the annual report For the study of beryllium and for work on solid-state physics in co-operation with the Commonwealth Scientific and Industrial Research Organization and Australian universities, two neutron collimators are in operation on *Hifar* for the production of neutron beams, a long wave-length neutron spectrometer has been installed, and two other spectrometers are being constructed

During the period under review, Australian industry showed increasing interest in the adoption of radioisotope techniques for the solution of its problems, but the field for potential industrial applications is still largely unexplored A second detailed survey of the extent of the applications of radioisotopes in Australian industry is being prepared, and information already available shows that a substantial increase since the first Commission survey in June, 1958, has occurred Two changes were made in the Advisory Service a direct consulting service to industry was initiated on a limited scale, and early in 1960 the Isotopes Section commenced making direct approaches to various firms Seventy technical discussions were held with inquirers at the Research Establishment and members of the Advisory Service made ninety visits to factories and laboratories In consultation with the Isotopes Section, the Commission's Engineering Services Design Group undertook the design of a cobalt-60 γ-irradiation unit for the study of plant mutations, and the long-term research programme includes the technical uses of radiation for insect pest control, sterilization of medical supplies and equipment, and food preservation by radiation At the end of May 1960 the Commission sponsored a conference on the "Technological Use of Radiation" The three-day conference was attended by 165 delegates, and sixteen papers were presented and discussed (the proceedings are now available in book form from the Melbourne University Press, Parkville, Melbourne, N 2, or from the Commission)

The number of staff employed at the Establishment increased by 189 to 673 during the year A few members of the Commission's scientific staff were stationed in the United Kingdom, United States of America and Canada, and an atomic energy attaché was appointed to the staff of the Australian Ambassador in Washington for closer working contact The Commission provided £60,000 for the building housing the Australian Institute of Nuclear Science and Engineering which was opened in May, and gives £50,000 annually for Institute research and training Although no undergraduate scholarships have been awarded since 1957, ten students are still undergoing training in geology, geophysics, metallurgy and chemical engineering The students graduate this year Forty-three research contracts have been awarded to eight universities and two hospitals

MENTAL HEALTH

THE tenth report of the World Health Organization Expert Committee on Mental Health is devoted to a review of present mental health facilities and resources and to the establishment of priorities for a mental health programme on a world-wide scale* Essentially it is based on a summary of work carried out in the field of mental health by the World Health Organization during 1949–59, and on the responses to an inquiry circulated to specialists in many countries in an attempt to ascertain what is being done and what needs to be done in that field

The report first examines trends in mental-health work during the past decade, covering such subjects as mental health aspects of public health, psychiatry of childhood, treatment methods and facilities, organization of psychiatric services, education and training, and research. Under each heading attention is directed to the contribution made by investigations sponsored by the World Health Organization, particularly those reflected in the reports of previous Expert Committees and Study Groups

An examination of the aims of mental-health work and of the ways of achieving them is followed by a discussion of psychiatric and mental-health services, their organization, planning and priorities Emphasis is placed on the importance of training programmes, since "even the most efficient organization and the best physical facilities will not prevent a badly staffed service from soon becoming unsatisfactory" The training of each category of mental-health worker is dealt with in turn. In addition, an idea is given of the kind of education in mental health required for personnel from the other health professions, as well as for community leaders and the general public, so that all may help to promote the mental health of the community

The report touches briefly on team work and describes the attitudes required in a team leader and qualities desirable in team members Considerable space is given to research in the field of mental health and it is pointed out that, although investigations undertaken in various countries over recent years show encouraging results, the complexity of the problem makes progress slow The areas of research considered of foremost importance in mental-health work are enumerated and discussed in detail Among the many subjects recommended for investigation are studies on brain function, epidemiology, health and social studies of communities undergoing rapid change, the hospital *milieu*, ecology of mental illness, problems of ageing, the effect of nutrition on mental health, and genetics

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* World Health Organization Technical Report Series No 223 *Programme Development in the Mental Health Field* Tenth Report of the Expert Committee on Mental Health Pp 56 (Geneva World Health Organization, London H M S O , 1961) 2 Swiss francs, 3*s* 6*d* , 0 60 dollars

The report concludes with a consideration of the vital part that the World Health Organization can play in future planning for mental health, by fostering international co-operation and exchange of information, and by facilitating and stimulating research

APPLICATION OF PALÆOMAGNETISM TO THRUST MECHANICS

By D K. NORRIS and R F BLACK

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THE surface trace of the Laramide Lewis thrust fault in the south-eastern Cordillera of Canada exhibits a change in direction of approximately 35° from a north-west trend east of the Clark and Lewis Ranges to a north trend east of the Flathead Range (Fig 1) The nature and genesis of this change of direction has been the subject of much controversy in recent years, and explanations have included differential erosion of the thrust plate and differential rotation of the plate The purpose of this article is to show that palæomagnetic directions in conjunction with surface geological data suggest that the Lewis thrust plate moved as a coherent tectonic unit and that the gross geometry of the plate may have been established at the time thrusting was initiated

For convenience in reference, the Lewis thrust plate will be divided geographically into two parts, termed respectively the Flathead Range segment and the Clark and Lewis Ranges segment

These two parts are structurally and stratigraphically continuous with one another at their common boundary in the vicinity of North Kootenay Pass Precambrian rocks of the Purcell system have been mapped without interruption between them, and no major transverse faults are there to suggest that the two segments of the Lewis thrust plate operated as independent tectonic units[1] The continuity as well as the simplicity of the structure between the two segments would suggest rather that the plate moved as a single structural unit

The change in trend of the surface trace of the Lewis thrust therefore must be due either to differential erosion of the plate, such that the Flathead Range segment has been eroded to some considerable degree farther west than the Clark and Lewis Ranges segment, or to the possibility that the latter segment was always structurally farther east than the former The fact, however, that the fold and thrust fault elements in the Clark Range do not end abruptly at the margin of the plate where the surface trace of the Lewis thrust swings west but rather swing sub-parallel to it suggests that the re-entrant at North Kootenay Pass is not due to differential erosion of the Lewis thrust plate The possibility of differential rotation, therefore, is especially impor-

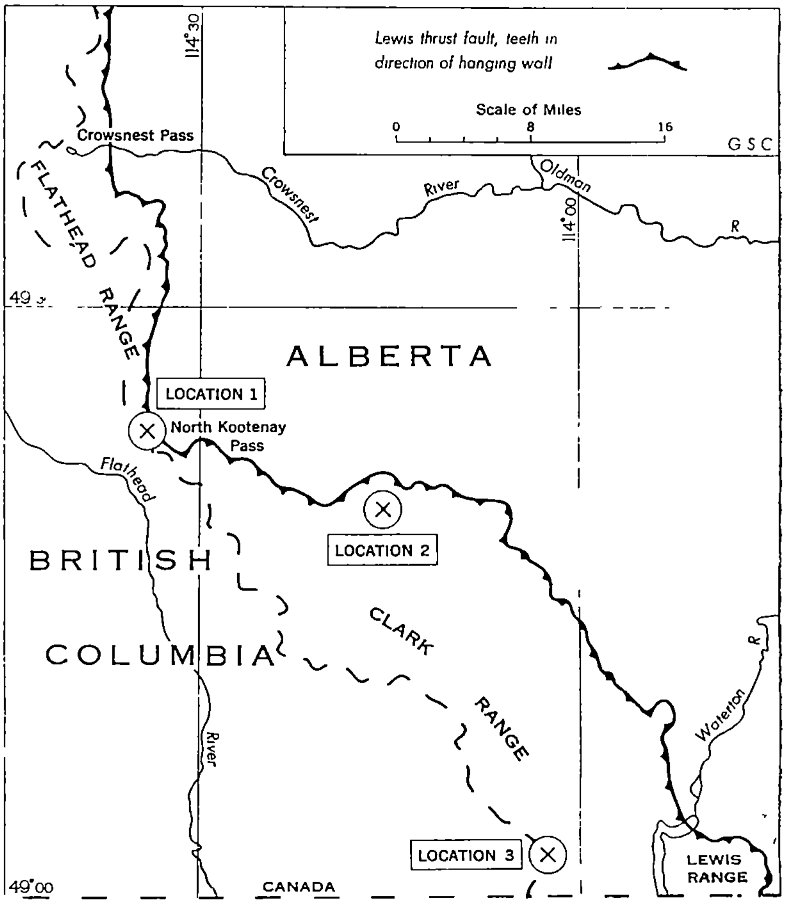


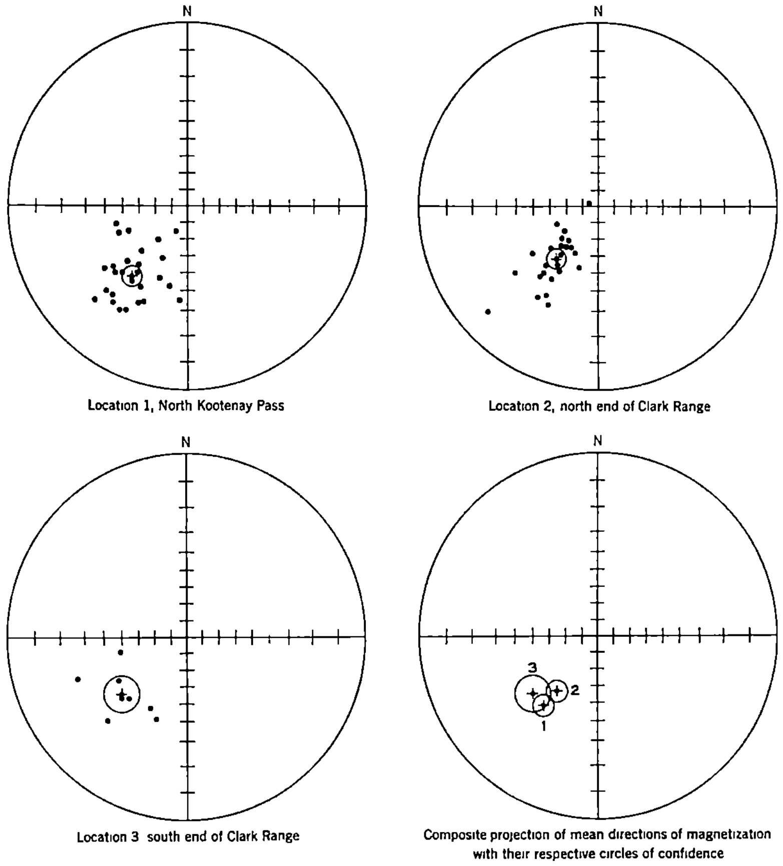
Fig 1 Location map

tant in the interpretation of the mechanics of emplacement of the plate.

If differential rotation has occurred it should be possible to determine the approximate amount of relative motion by a comparison of the directions of the remanent magnetic vectors obtained from Purcell samples collected in the two segments A necessary condition, however, is that the time of magnetization of the rocks making up the plate be pre-Laramide (older than Early Tertiary) in order that the results obtained can be applied to this problem

Fifty-nine samples of unweathered, highly quartzose, fine-grained, red sandstone were collected from the Precambrian Purcell system at intervals normal to the strike at three locations in the Lewis thrust plate (Fig 1) They were from member *A* of the Kintla formation in the Flathead Range where the strata strike north, and, from members *A* and *C* of the same formation, at two localities in the Clark Range where the strata strike north-west

The remanent magnetization in each of two cubes from each sample of Kintla formation was determined with a sensitive astatic magnetometer in the laboratories of the Dominion Observatory of Canada From the measurements of two cubes the mean attitude of the remanent magnetic moment was computed for each sample



Location 1, North Kootenay Pass

Location 2, north end of Clark Range

Location 3 south end of Clark Range

Composite projection of mean directions of magnetization with their respective circles of confidence

Fig 2 Stereographic projections of the directions of magnetization for the three locations and composite projection of their respective mean directions and circles of confidence ●, vectors pointing downwards, +, mean direction of magnetization

All cubes were subjected to decreasing-amplitude-alternating-field demagnetization and the maximum amplitude of the field was progressively increased after each set of measurements until no significant change (less than 5°) occurred in the magnetization direction in the cubes This approximately stable state of magnetization was reached at 156 œrsteds

Results from this demagnetization treatment indicate that the Kintla formation, where sampled, is magnetically stable, as the directions of magnetization for the majority of cubes were not significantly altered After demagnetization the directions in the remainder became consistent with those on which demagnetization had little or no effect

The mean palæomagnetic results for the three localities sampled are indicated in Table 1 and plotted in Fig 2 Solid circles plotted on the Wulff stereographic projections in Fig 2 represent the directions of the magnetic vectors with their positive pole pointing downwards The mean direction for each group of samples is represented by a solid circle centred by a cross The statistical parameters computed for the three groups of measurements are N, θ, and K as defined by Fisher[2], where N is the number of samples, θ is the radius of the circle of confidence calculated at the 95 per cent level of confidence (represented in Fig 2 as open circles on the lower hemisphere of the stereographic projection), and K is a measure of the dispersion All measurements are with respect to bedding, which was assumed to be horizontal at the time of deposition

The fact that the calculated pole positions included in Table 1 agree well with those pole positions determined from Purcell and other Late Precambrian North American rocks[3,4] would suggest that the remanent magnetization has been preserved since Precambrian times The results may therefore be applied with reasonable assurance to test for the possibility of differential rotation in the Lewis thrust plate in the early tertiary

The directions of the magnetic vectors of samples from member *A* were in all cases found to point upwards and have been adjusted (sign of the inclination changed from negative to positive and 180° added

Table 1 MEAN PALÆOMAGNETIC RESULTS FOR INDIVIDUAL COLLECTING SITES

		Location 1	Location 2	Location 3
Collecting site		North Kootenay Pass	North end of Clark Range	South end of Clark Range
Formation and member		Kintla *A*	Kintla *A* and *C*	Kintla *A*
Polarity *		–	– and +	–
Number of samples		27	24	8
Mean declination		218 5°	218 0°	230 0°
Mean inclination		+39 5°	+49 0°	+38 0°
θ		5 5°	6 5°	9 5°
K		23	24	40
Ancient pole position	Lat	10 5° S	3 0° S	6 5° S
	Long	150 5° W	146 5° W	159 0° W

* For comparative purposes all vectors have been adjusted to point downward

to the declination) to point downward for comparison of results from members A and C

It is evident from the composite projection in Fig. 2 that the circle of confidence for any one group of observations is intersected by those of the other two groups This would suggest that within the limits of error of collection and measurement, which is between 5° and 10°, little if any relative rotation of the Kintla formation has occurred between any of the three collection sites The differences in the mean declination angles are negligible between Flathead Range and the north end of the Clark Range and has a maximum of 12° between the latter and the south end of the Clark Range It should be recalled that if the change in trend of the surface trace of the Lewis thrust were due entirely to differential rotation a disparity in the declination of the order of 35° should be indicated

In summary, the continuity of surface structural and stratigraphic units throughout the length of the Lewis thrust plate in the area under investigation would suggest that the plate moved as a single, coherent, tectonic unit with no major transcurrent or transverse normal faulting Palæomagnetic data, moreover, support this contention as there was, within the limits of error, no measurable differential rotation of the plate between the Flathead and the Clark and Lewis Ranges It appears, therefore, that the Clark and Lewis Ranges were always structurally farther east than the Flathead Range The present configuration of the eastern limit of the Lewis thrust plate is apparently genetically related to the geometry of the thrust surface established during the initial stages of movement rather than to differential erosion along the eastern margin of the plate

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[1] Price, R A , Geol Surv , Canada, Map 1–1959 (1959)

[2] Fisher R A , *Proc Roy Soc Lond* , A, **217**, 295 (1953)

[3] Black, R F , *Geol Surv , Canada, Bull* 84 (in the press)

[4] DuBois, P M *Geol Surv , Canada, Bull* 71 (in the press)

LOSS OF FLUORESCENT PARTICLES IN ATMOSPHERIC DIFFUSION EXPERIMENTS BY COMPARISON WITH RADIOXENON TRACER

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AND
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ZINC cadmium sulphide, disseminated in a finely ground particulate form, mainly in the size-range 1–5μ, has been widely used as a tracer in work on diffusion and atmospheric pollution This fluorescent tracer technique was described independently by Perkins *et al* [1] and Braham *et al* [2] in 1952 The various problems in which it has been used can be conveniently divided into two groups The first includes investigations of the spread of airborne substances released into the atmosphere Here, only relative measurements of the concentrations of the particles are important in a particular trial, and provided any comparison of these is made after equivalent times of travel, then any time-decay of the tracer in a physical or chemical sense is unlikely to introduce error. The second is of particular importance in air-pollution investigations and includes all problems where the tracer is used to estimate the absolute concentration at any position resulting from a release of known size Here, the decay characteristics of the tracer must be well defined

Earlier workers[1,3] considered that zinc cadmium sulphide was stable in the presence of normally occurring concentrations of atmospheric pollutants for at least the first few hours after release However, some work by one of the present authors (N T), to be reported elsewhere, in which vertical profiles of the particles were investigated some tens of kilometres down wind of releases on a long cross-wind line, showed an apparent decrease in the number of airborne particles with increasing distances of travel A more reliable estimate of the loss was required, and it was decided to compare the fluorescent tracer directly with an inert gaseous tracer Radioactive xenon-133 (half-life 5 27 days) was selected because of ease of sampling and detection

The xenon and zinc cadmium sulphide tracers were released simultaneously at a uniform rate and sampled simultaneously at two distances down-wind The total amount (number of particles or activity) of either tracer collected, divided by the sampling rate, is a measure of the dosage (time-integral of volumetric concentration) of that tracer at the point in question If D_1, D_2 are the dosages of xenon at the near and far sampling distances respectively (units, μμc min /m ^{3}) and d_1, d_2 the corresponding dosages of particles (units, particle min /m ^{3}) then L, the percentage loss of particles, is given by

$$L = \left(1 - \frac{D_1}{D_2}\bigg/\frac{d_1}{d_2}\right) \times 100$$

This result is independent of the position of either sampling station with respect to the plume axis

On each of three days, approximately 10 kgm of zinc cadmium sulphide (Derby Luminescents 1318/10) and 3 curies of xenon-133 were emitted from a 20-metre incinerator stack at the Atomic Energy Research Establishment, Harwell, over a period of about 2 hours beginning near noon Calculation showed that the health hazard to a person situated at the point of maximum ground-level concentration was negligible During the three trials reported here, the wind directions varied between south-west and west-north-west with mean speeds of between 4 and 7 m /sec Vigorous convection extended to heights of up to 1,700 m and there was about one-half cloud cover present

Sampling of both tracers took place at two stations placed one about 16 km and the other about 60 km

Table 1

	June 23, 1961		July 7, 1961		July 25, 1961	
Amount of tracer						
Fluorescent particles (gm.)	1.442×10^4		1.362×10^4		0.983×10^4	
Xenon (curies)	3.31		3.65		2.00	
Sampler distances (km.)	14.5	60.0	17.7	57.0	16.1	63.5
Mean period of travel of tracers (hr.)	1.0	3.2	1.0	3.1	0.7	2.5
Dosages						
Fluorescent particles (particle min./m.^{3})	38,000	3,700	55,100	12,100	(*a*) 29,700 (*b*) 36,050	(*a*) 4,500 (*b*) 5,850
Xenon ($\mu\mu$c. min./m.^{3})	1,010	188	1,600	730	601	163
Dosage ratios						
Florescent particles (d_1/d_2)	10.3		4.5		(*a*) 6.6 (*b*) 6.2	
Xenon (D_1/D_2)	5.4		2.2		3.7	
Loss of particles (per cent)	48		51		(*a*) 44 (*b*) 40	

(*a*), Assessment under 80 × microscope, (*b*), higher intensity ultra-violet illumination and 100 × microscope used in assessment.

from the source. A sampler was also operated about 1 km. up-wind of the source to measure the background activity of atmospheric xenon. Satisfactory positioning of the sampling stations in the plume of material was obtained through frequent pilot balloon measurements of the wind at Harwell, and by direct observation of the bearing of the plume axis a few kilometres from the source, using a mobile fluorescent particle sampler in conjunction with inspection equipment set up nearby.

The fluorescent particles were collected by drum impactors, through which air was drawn at a rate of about 20 litre/min. The drums were rotated through a small angle at 5-min. intervals to prevent a large accumulation of dust on the traces, which might have obscured a significant number of the impacted particles. In the subsequent assessment, the drums were illuminated with ultra-violet light and the particles counted through an 80 × microscope. The samples from the third trial were also assessed under ultra-violet light of higher intensity, with counting under a 100 × microscope.

The xenon-133 was collected by passing air from a petrol-driven compressor at a flow-rate of 100 litre/min. through a charcoal U-trap immersed in a dry-ice alcohol mixture at $-78°$ C. The trap contained 400 gm. of activated charcoal and was preceded by a soda-lime column to remove carbon dioxide, a magnesium perchlorate drying column and a pre-cooling coil. The trap was followed by a warming coil and a rotameter.

After sampling was completed, the charcoal trap was returned to the laboratory where a measured volume, approximately 10 ml., of inactive carrier xenon was added. The trap was then heated slowly to about 200° C. and the xenon, together with some oxygen, nitrogen and other gases, was flushed with purified hydrogen into a smaller charcoal chromatographic column immersed in liquid nitrogen. The xenon was separated from other gases by a gas chromatographic technique using hydrogen as the eluting gas. A measured volume of the xenon, together with diethyl ether vapour as a quench, was transferred to a 50-ml. Geiger counter. This was operated in a low-background assembly with an anti-coincidence ring. Counter backgrounds were about 1.5 c.p.m. and the minimum sample count-rate approximately 10 c.p.m. The chemical purity of the separated xenon was established by mass-spectrometric analyses. Radon was the principal radioactive impurity expected and its absence was demonstrated by operating the counter in the proportional region to enable any radon α-pulses to be distinguished from the xenon-133 β-pulses, and also by following the decay of each sample for at least one half-life. Count-rates were corrected for radioactive decay to a standard time and an allowance was made for the xenon lost in processing and transfer to the counter. The correction due to xenon background activity did not exceed 20 per cent of the sample activity. The collection efficiency of the field sampling equipment, determined in separate experiments, was better than 85 per cent for periods up to 4 hours, and a small correction was made, appropriate to the duration of sampling after arrival of the xenon-133 at the sampling station. A full description of the xenon sampling technique will be published elsewhere.

The results are summarized in Table 1.

The mean loss of particles in travelling between the two sampling stations is seen to be approximately 50 per cent. A several-fold increase in the intensity of ultra-violet light used in the assessment of the fluorescent particle samples resulted in an increase of about 25 per cent in the number of particles counted in the last trial. However, the dosage ratios from the two assessments were almost identical and the corresponding values for the loss of particles differed by less than 4 per cent. The explanation for the loss is unknown at present, but it is relevant that separate studies of the size spectrum of the particles after different distances of travel showed an apparent decrease in the proportion of small particles with increasing distances of travel. Measurements[4] on particles collected in these last experiments showed that the fluorescent brightness of particles less than 2.5μ diameter decreased with increasing time of travel. To account for the loss by deposition, a velocity of deposition of 1.5 cm./sec. would be required, which is about 60 times larger than the value determined in wind tunnel experiments (Chamberlain, A. C., personal communication). In any event, this process would lead to a preferential loss of the larger particles.

Our thanks are due to D. V. Booker (Atomic Energy Research Establishment) for advice and assistance in counting the xenon samples, also to G. F. Collins and R. A. Titt (Chemical Defence Experimental Establishment) for assistance with certain aspects of the fluorescent tracer technique.

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STRUCTURE OF THE 5,6-DIMETHYLBENZIMIDAZOLYLCOBAMIDE COENZYME

By Dr. P. GALEN LENHERT* and DOROTHY CROWFOOT HODGKIN, F.R.S.
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RECENTLY, H. A. Barker *et al.* reported the isolation of a crystalline 5,6-dimethylbenzimidazolylcobamide (DBC) coenzyme[1]. This is one of a series of coenzymes which were first recognized as catalysts in the conversion of glutamate to β-methylaspartate in certain bacteria. The coenzyme was shown to be active in systems requiring vitamin B_{12}, but it differed from cyanocobalamin in having an adenine nucleoside in place of the cyanide group. We have now carried out an X-ray analysis of the coenzyme structure, having for our use crystalline preparations kindly given to us by Dr. H. A. Barker, Dr. Karl Folkers and Prof. A. W. Johnson.

Part of Dr. Barker's preparation was recrystallized by slowly diffusing acetone into an aqueous coenzyme solution. Two freshly grown crystals prepared in thin-walled glass capillary tubes in dim light and kept in contact with their mother liquor were used to obtain the X-ray diffraction data for the structure analysis. Copper *K*α radiation was used throughout. The intensity measurements were made visually from multi-film equi-inclination Weissenberg photographs taken about the *b* and *c* axes. The Lorentz-polarization correction and a spot shape correction were applied. The scale factor for each layer was calculated from reflexions common to other layers and the absolute scale was obtained by Wilson's method. 3,145 independent reflexions extending out to a spacing limit of 0·9 Å. were observed. These included about 85 per cent of the reflexions with a spacing greater than 1·2 Å.

The unit cell dimensions characteristic of the wet crystal lattice, as measured from precession photographs, are $a = 27 \cdot 9_3$, $b = 21 \cdot 7_3$ and $c = 15 \cdot 3_4$ Å. The space group is $P2_12_12_1$ and the measured density of the fully solvated crystals, $1 \cdot 35_5 \pm 0 \cdot 01$ gm./cm.^{3}. The molecular weight, coenzyme plus solvent, calculated from this information is 1,899. This is larger, as would be expected, than the weight measured by Mattern on air-dried crystals[2], the difference corresponds to the weight of about 8 molecules of water of crystallization.

The structure analysis began with the calculation of a sharpened three-dimensional Patterson synthesis which clearly showed the Co—Co vectors. The first electron-density synthesis was computed using phases calculated from the cobalt positions and the observed *F* values weighted according to a method suggested by Sim[3]. The resulting electron-density map contained peaks representing most of the atoms in the structure, and in addition, spurious peaks, many of which were larger than those corresponding to atoms. The position of the cobalt atom ($z = 0 \cdot 02$) produced a pseudo-mirror plane which distorted the electron-density pattern representing the corrin nucleus and increased the number of spurious peaks. In spite of this, reasonably accurate positions were worked out for nearly all the atoms in the corrin nucleus, the 5,6-dimethylbenzimidazole, the ribose and the phosphate group.

A second electron-density synthesis, again using Sim's weighting scheme, was computed with phases calculated from the 54 atoms with known positions. This synthesis, containing less false symmetry and fewer spurious peaks, enabled us to place nearly all the remaining atoms of the coenzyme molecule. The electron-density in the region above the corrin nucleus, on the side occupied by cyanide in cyanocobalamin, is of particular interest (Fig. 1). The adenine moiety can be clearly seen among the spurious peaks. It is evident as a planar set of peaks with the 5- and 6-membered rings and the amino-group correctly related. Further, the five nitrogen atoms, with one exception, correspond to the peaks of greater electron-density. The sugar part of the nucleoside is not so easily seen, but on careful study it is no less convincing. This arrangement of peaks represents a *d* ribose unit linked directly to the cobalt atom at C 5′. No peak can be found which would correspond to the hydroxyl oxygen of C 5′. Most of the spurious peaks are in chemically inadmissible positions and have disappeared from subsequent electron density syntheses.

Any residual doubt about the correctness of the above interpretation was completely dispelled by the results of the next structure factor calculation and the appearance of the corresponding difference synthesis. All the atoms in the coenzyme molecule (except six in the vitamin B_{12} part) and three water molecules were included in the third structure factor calculation. The agreement factor, *R*, was thereby reduced from 0·44 to 0·28.

Agreement between the observed and calculated structure factors has been further improved by adding the remaining six atoms of the vitamin B_{12} part, by including additional solvent molecules and by making adjustments in the positional and thermal parameters

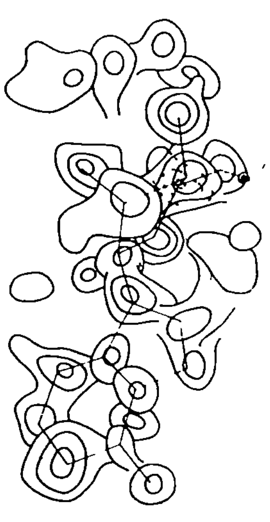

Fig. 1. Peaks in the second electron density synthesis in the region of the adenine and sugar groups. The contours are at levels corresponding approximately to 1·0, 1·5 and 2·0 $e/\text{Å}^3$. The distribution is shown viewed along *a* and the contours are drawn only near the level of the peak maxima

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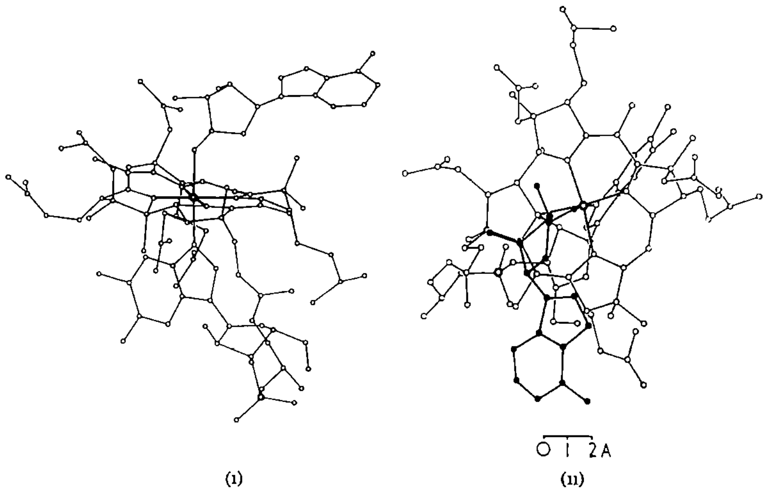
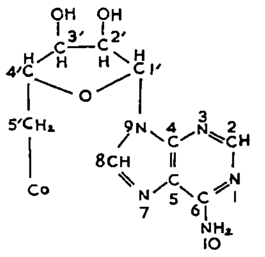


Fig. 2. The atomic positions in one molecule of the 5,6-dimethylbenzimidazolylcobamide coenzyme as found in the wet crystals and viewed along (i) the *b* axis, (ii) the *a* axis. In (ii) the atoms of the nucleoside unit are marked by filled circles

of the other atoms. All the atoms of the coenzyme molecule (excluding hydrogen) and contributions from 11 water molecules have been included in the latest structure factor calculation. The agreement factor is 0·173.

The structural formula of the nucleoside as it occurs in the intact coenzyme should almost certainly be written as in I, which represents it as an adenosine derivative.



(I)

The *d* ribose unit and the adenine are linked at C 1′ (β) and N 9 in accordance with the observations of Ladd *et al.*[4] The unsaturated sugar, $CH_2 = CH \cdot CHOH \cdot CHOH \cdot CHO$, which these authors obtained by the action of cyanide or mild acid hydrolysis, must be formed on degradation of the coenzyme following the rupture of the Co—C 5′ bond. The observed tetrahedral character of the atomic centres C 2′, C 3′ and C 4′ makes it unlikely that a double bond involving any of these atoms is initially present in the molecule, and very recently, adenosine derivatives in which the pentose ring is intact and the 5′ CH_2OH is replaced by COOH and CHO have been isolated from among the photolysis products of the coenzyme by Johnson and Shaw and by Barker *et al.*[5]

Fig. 2 shows the arrangement of the atoms in the coenzyme molecule as found in this crystal structure. All the atoms of the vitamin B_{12} molecule appear to be present except the cyanide group which has been replaced in the coenzyme by the adenosyl residue. The cobalamin nucleus and the nucleoside are connected by the C 5′—Co bond of 2·05 ± 0·05 Å, the Co—C 5′—C 4′ angle is rather large, 130°, which suggests that this bond may have partial ionic character. No other direct covalent or hydrogen bonds connect the corrin nucleus and the nucleoside. The closest non-bonded contact is 3·1 Å.

While several of the amide side-chains have adopted conformations different from those found in B_{12}, there is no evidence of any change in the corrin nucleus itself. Within the accuracy of the present partly refined co-ordinates, the corrin nucleus appears to be identical in the vitamin and coenzyme molecules. In the circumstances we could most easily formulate the cobalt atom as trivalent in the coenzyme molecule and write the structure in a similar way to that of vitamin B_{12}. The coenzyme molecule would then have a molecular formula of $C_{72}H_{100}O_{17}N_{18}PCo$ and a molecular weight of 1,580. The uncertainties here are in the number of hydrogen atoms, the valency of cobalt and the exact character of the bonding in the corrin nucleus, they may possibly be resolved by further refinement of the crystal structure, particularly if we can observe the hydrogen atom positions. Other evidence, notably on the magnetic character of the crystals, is very conflicting at present[6,7] and it seems likely that easy transformations occur within the molecule which involve the valency of cobalt and the corrin nucleus. These, combined with the unusual character of the carbon–cobalt bond, are presumably responsible for the catalytic activity of the coenzyme.

The crystal structure of the DBC coenzyme is, in detail, quite different from that found in the vitamin B_{12} crystal structures. Although the orientation of the corrin nucleus with respect to the crystal axes is similar, the relationship between the four molecules which make up the translational unit of the crystal has altered. In the new arrangement, very interesting intermolecular contacts arise which suggest methods of approach by different reagents in the neighbourhood of the Co—C 5′ bond. For example, the adenosyl 3′ OH is hydrogen bonded to the phosphate group of one neighbouring molecule while the NH_2 group of the adenine unit is in contact with the phosphate group of another. The geometrical relations strongly suggest easy pathways both for the formation of the coenzyme from adenosine triphosphate and for its possible intervention in nucleic acid synthesis.

We wish to thank Dr. J. S. Rollett for the use of his structure factor programme and for many helpful discussions. The Fourier series programme written by Dr. O. S. Mills and other programmes written by various members of this Laboratory have greatly facilitated the work. All the calculations were done on the Ferranti *Mercury* computer with the co-operation of the staff of the Computing Laboratory, Oxford. This investigation was carried out during the tenure by one of us (P. G. L.) of a postdoctoral fellowship from the Division of General Medical Sciences, United States Public Health Service.

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VARIANTS IN THE STRUCTURAL POLYSACCHARIDES OF ALGAL CELL WALLS

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ALTHOUGH the wall polysaccharides of the algae have been extensively investigated there are nevertheless many puzzling features which remain to be resolved. We have for this reason been making detailed examination of the walls of a wide variety of these plants, and it is the purpose of this article to present these newer results in the general context of the older, pending more extensive publication elsewhere.

It has long been recognized that the walls of plant cells consist of two phases, one a dispersed crystalline phase and the other a matrix which is commonly non-crystalline, both phases containing largely polysaccharides and/or polysaccharide derivatives. Since 1948 the dispersed phase has been identified in the electron microscope as consisting of long thin threads—the microfibrils—and it has become the custom, except in most fungi, to associate these with cellulose. In higher plants, the microfibrillar component usually occupies a large proportion of the wall volume so that the wall normally gives a characteristic X-ray diagram of cellulose I (native cellulose). In the algae, however, the relative volume of the crystalline wall material is often so small that the scatter from the amorphous incrusting substances may mask the X-ray diagram completely, and microfibrils may no longer be visible when chemically untreated material is examined in the electron microscope.

In 1952, Nicolai and Preston[1], using in the main the technique of X-ray diffraction analysis of untreated algae, divided the Chlorophyceae into three groups so far as wall structure is concerned. Group 1 consisted of the Valoniaceae and the Cladophoraceae (with the exception of the *Spongomorpha* group of the Cladophorae), algae containing highly crystalline cellulose I in abundance in the form of relatively straight and comparatively thick microfibrils. The bulk of the plants were placed in Group 2, characterized by X-ray diagrams resembling those of cellulose II (mercerized cellulose) and the rest, giving unidentifiable X-ray diagrams, made up Group 3. It was afterwards reported from this laboratory that the marine Phaeophyceae[2] and, with one exception, the Rhodophyceae[3] resemble in this regard the green algae of Group 2. The classification was regarded throughout as provisional because the interpretation of the X-ray diagrams of untreated algae in Groups 2 and 3 was scarcely ever unambiguous. This was due both to uncertainties inherent in the possible masking of reflexions by scatter from amorphous substances and to the presence in some diagrams of reflexions referring clearly to two different crystalline substances.

The major difficulties in the way of an identification of the crystalline component of algal cell walls lie partly in the need for careful removal of incrusting substances and partly in the invariable presence in marine algae of a deposit of micro-crystalline material on the plant surfaces[4]. This deposit, which is often present in large amounts, especially in filamentous algae, we have identified as a mixture of clay minerals. As it cannot be removed, even by the most severe washing, the X-ray diagram of untreated algae is often that of the clay minerals alone. It is undoubtedly because the strongest three reflexions of the mixture of minerals present lie very close to the strongest reflexions of cellulose II, both in disposition and in spacing, that cellulose II has been suspected to be the skeletal substance of Group 2 algae. Chemical treatment can reduce this contamination to an amount usually so small as to allow the diagram of the crystalline wall polysaccharides to be recognized. In particular, the treatment used by Jermyn and Isherwood[5] and adapted by Cronshaw, Myers and Preston[2] for the isolation of α-cellulose progressively removes the clay minerals, with a consequent progressive change in the X-ray diagram of the wall as reported and found confusing by the latter authors[2].

With these points in mind we have examined representative plants from most groups of the marine algae, using in the main the techniques of X-ray diffraction analysis and paper partition chromatography of hydrolysates of wall fractions, supplemented by electron microscopy and, very occasionally, polarization microscopy. The plants are dealt with in turn according to the nature of the crystalline wall substance present.

The X-ray diagrams of all the members of the Rhodophyceae we have examined, chosen to be representative of the group, are consistent with the presence of cellulose I as the skeletal substance, except in the Bangiales, a member of which will be discussed separately. The cellulose content of the wall and the degree of crystallinity of the cellulose are both rather low. The X-ray diagram is therefore revealed only after extensive removal of incrusting substances. For this removal, solutions of potassium hydroxide stronger than 10 per cent should not be used since with the red algae these solutions convert the cellulose I to cellulose II. The microfibrils contain a range of sugar residues other than glucose residues[2]; but it has recently been shown for one species at least that the central core of the microfibrils consists of cellulose I (ref. 6) alone. In all the Phaeophyceae we have examined, the X-ray diagrams again demonstrate clearly the presence of cellulose I as already reported for *Fucus*[7]. The clarity of these diagrams and the sharpness of the arcs distinguish them clearly from those of the red algae. Moreover, mercerization of the cellulose in the brown algae in potassium hydroxide solutions does not proceed at concentrations below 14 per cent. In the holocellulose of most of the Fucales examined an intense reflexion appears on the X-ray diagram corresponding to a spacing of about 14·1 Å. This indicates the presence of another crystalline wall substance, though we are not certain whether this is originally present in untreated material or whether crystallization is induced by the treatment involved. Untreated material from stipes of *Alaria* (but not from the laminæ) undoubtedly contains a second crystalline component in addition to the cellulose.

The Chlorophyceae are, however, by no means so uniform as regards the nature of the skeletal substance, though cellulose I is again to be found in many families. There is no need here to document the presence of highly organized and well-crystalline cellulose I in the algae of Group 1, though some remarkable features of some plants in this group have only recently been pointed out[8]. Other members of the Chlorophyceae are also now known to contain this substance, including *Nitella*[9], *Ulva*[9] and *Ulothrix*, and perhaps *Spirogyra*[10], though there are peculiar features in this last species. The condition of the cellulose resembles that in either the Phaeophyceae or the Rhodophyceae, and all these algae should clearly be separated from those of Group 1.

The incrusting substances of the algal walls containing cellulose I as the crystalline component are constructed from uronic acids and a wide variety of sugars[3]. Among the sugars, galactose, arabinose and xylose predominate.

Beyond these, one plant in particular, namely, *Halicystis* (Siphonales) was long ago reported to contain cellulose II in [place of cellulose I (ref. 11). Hydrolysates of the whole wall contain about equal quantities of glucose and xylose, and extraction of the wall with caustic soda solutions of increasing strength leaves unchanged both the X-ray diagram and the proportion of sugars in the hydrolysates of the residue[12]. The X-ray diagram contains arcs attributable to cellulose II together with a reflexion at about 13 Å. (12 Å. in our photographs) spacing which Roelofsen *et al.*[12], correctly in our view, refer to the large amount of xylan present. We have found that treatment of *Halicystis* holocellulose with caustic potash solutions of progressively increasing concentration causes a progressive decrease in the intensity of the arcs of long spacing, and that 4 *N* potassium hydroxide causes their final disappearance leaving the diagram of cellulose II. Hydrolysates of the very small residue (so-called α-cellulose) nevertheless still contain, besides glucose, about 40 per cent of xylose. Separation of the glucan and the xylan can, however, be achieved by treatment of the wall with cuprammonium. Most of the glucan dissolves and the precipitate gives an X-ray diagram of cellulose II. The residue insoluble in cuprammonium contains predominantly xylose residues and gives an X-ray diagram identical with that of the xylan to be discussed further on. We may compare the condition in *Halicystis* with that reported by Kreger[13] for *Hydrodictyon* (Chlorococcales), a fresh-water alga. The walls of these plants contain glucan and mannan only, but neither of these yields an X-ray diagram until the wall has been treated chemically with, for example, dilute mineral acid. A composite diagram of both cellulose II and mannan is then obtained, symptomatic of a crystallization of glucan and mannan chains which, in the fresh wall, were at the most in a paracrystalline state.

Halicystis and *Hydrodictyon* are very special cases since only two sugars are used in the construction of the wall, and the boundary between crystalline and para- or non-crystalline phases must be vague. In these algae, in addition to glucose, mannose and xylose respectively must play an important part in both phases. Among the Siphonales there are other algae in which xylose or mannose form the only crystalline wall component and we now discuss these in turn.

Untreated walls of *Bryopsis*, *Caulerpa*, *Penicillus*, *Udotea* and *Halimeda* (all Siphonales) yield a remarkably distinct X-ray diagram which is quite different from that of cellulose and the spacings of which are listed in Table 1. Treatment of the material with boiling water, followed by chlorination and extraction with dilute alkali solution, leaves the diagram unchanged. In stronger alkali (10–18 per cent potassium hydroxide according to species) the wall dissolves. Suspensions of cell wall material of any of these species dried down to a thin film on glass show uniplanar orientation, that is, certain planes in the crystal lattice tend to lie parallel to the film (and therefore the cell wall) surface. The inner wall layers of the large filaments of *Penicillus dumetosus* give an X-ray diagram, illustrated in Fig. 1, which demonstrates that the crystallites in the wall lie parallel to filament length. *Penicillus* walls so treated as to remove incrusting substances (containing mainly glucose residues) yield, on hydrolysis, xylose together with a little glucose. The X-ray diagram (Fig. 1) is quite certainly not that of the β-1,4-linked xylan which can be isolated from the incrusting substances of cellulosic walls (cf. col. 1 and col. 2 in Table 1). The diagram does, however, resemble in character though not in detail those obtained from yeast glucan[14] and certain preparations of laminarin (Nicolai, E., unpublished results), both recognized β-1,3-linked glucans. Mackie and Percival[15] and Iriki *et al.*[16] have shown that the xylan of *Caulerpa*

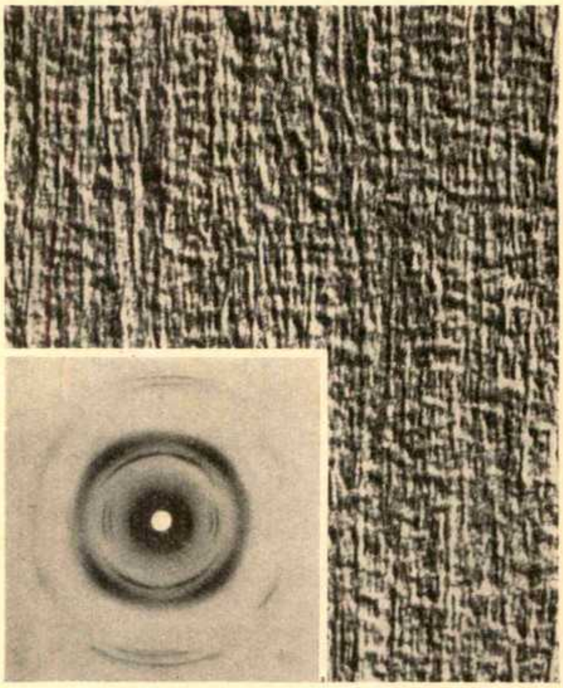

Fig. 1 Fig. 2

Fig. 1. X-ray diagram of a parallel bundle of inner wall layers of filaments of *Penicillus dumetosus*, filament length vertical. Copper K_α radiation

Fig. 2. Electron micrograph of wall lamella (untreated) from inner wall layer of a filament of *Penicillus dumetosus*, filament length vertical. (× 35,000)

Table 1. X-RAY SPACINGS OF XYLANS AND MANNANS FROM VARIOUS SOURCES

Xylans			Mannans		
Elm*	*Penicillus*†	*Porphyra*† rhizoids	Ivory nut endosperm	*Dasycladus* (untreated)	*Porphyra*† 'cuticle'
	11·93 *V.S.*	11·90 *V.S.*			
8·15 *M.*					
			7·80 *W.*		
7·05 *M.*	6·80 *W.*	6·70 *M.*			
			6·61 *M.W.*	6·65 *W.*	6·58 *W.*
	6·12 *W.*				
		5·95 *V.W.*			
	5·50 *W.*		5·50 *V.S.*	5·51 *V.S.*	5·52 *V.S.*
5·42 *W.*					
	5·12 *M.*	5·22 *W.*			
			4·85 *M.W.*	4·84 *M.*	4·86 *M.W.*
4·57 *S.*					
	4·45 *S.*	4·46 *S.* (broad)	4·42 *S.*	4·39 *S.*	4·43 *S.*
	4·27 *S.*				
	4·01 *M.*		4·02 *W.*	4·03 *W.*	4·01 *W.*
3·94 *W.*					
			3·78 *M.S.*	3·74 *M.*	3·76 *M.*
3·51 *W.*	3·52 *W.*	3·52 *M.W.*	3·51 *M.S.*	3·47 *M.*	3·52 *M.*
	3·38 *W.*		3·29 *M.*	3·30 *W.*	3·30 *M.W.*
				3·21 *V.W.*	
	3·00 *W.*		3·04 *W.*	3·01 *W.*	3·03 *W.*
2.87 *W.*		2·89 *W.*	2·88 *M.*	2·89 *W.*	2·90 *W.*
	2·78 *W.*				
	2·72 W.		2·70 *M.W.*	2·68 *M.W.*	2·69 *M.W.*
		2·65 *W.*			
2·57 *M.*				2·54 *W.*	
	2·42 *W.*	2·48 *W.*			
	2·25 *V.W.*	2·30 *W.*		2·32 *W.*	2·32 *W.*
				2·13 *W.*	
				1·92 *V.W.*	

* Alcohol-precipitated from a 4 per cent potassium hydroxide extract of holocellulose.

† After extraction in boiling water followed by chlorination.

V.S., very strong; *S.*, strong; *M.S.*, medium strong; *M.*, medium; *M.W.*, medium weak; *W.*, weak; *V.W.*, very weak, reflexions.

is β-1,3-linked, and the latter authors have demonstrated the same linkage also in the xylans of *Bryopsis*, *Udotea*, *Halimeda* and some others. The X-ray diagram is therefore certainly that of β-1,3-linked xylan, and this is the crystalline component of the walls of these plants.

In the electron microscope, the cell walls of all these 'xylan weeds' are seen to be microfibrillar, illustrated here by an electron micrograph of an inner wall lamella of *Penicillus* filaments (Fig. 2). Of the two kinds of fibrillar bodies visible in Fig. 2, one, running approximately parallel to filament length (and about 100 Å. wide), resembles closely the microfibrils common in cellulosic walls. The other, less-abundant kind, lying approximately at right angles to the first, are not true microfibrils. The fibre diagram of inner layers (Fig. 1) clearly has its origin in the longitudinally oriented microfibrils. The birefringence of the wall, both in face view and in optical section, is nevertheless negative. This of necessity implies that the longitudinally oriented microfibrils are negatively birefringent, that is, the refractive index is maximum for light vibrating perpendicular to microfibril length. Both the X-ray diagram (Fig. 1) and this negative birefringence suggest that the molecular chains in these xylan microfibrils are arranged helically. Since the crystalline component of the walls of all the algae in this group is β-1,3-linked xylan then the same considerations must apply to all of them and, indeed, wall sections are always negatively birefringent. It is not therefore safe to carry over principles which apply to cellulose walls, by assuming that the major extinction position defines the microfibril direction, as has recently been done for *Bryopsis*[17]; though in *Bryopsis* itself the situation is complicated by the fact that extraction of the wall with alkali may reverse the sign of birefringence of the wall as seen in face view (but not, oddly enough, in optical section).

Since whole-wall hydrolysates of these walls contain xylose and glucose with little trace of other sugars, glucans are here prominent among the incrusting substances. These glucans are known to be also β-13,-linked[15,16].

On the other hand, as pointed out by Iriki and Miwa[18], wall hydrolysates of *Codium*, *Acetabularia* and *Halicoryne* (again all members of the Siphonales), treated successively with dilute solutions of sodium hydroxide, sulphuric acid and sodium chlorite, reveal mannose as the main constituent of the preparations. This substantiates our own independent findings on *Codium*, *Acetabularia*, *Dasycladus* and *Batophora* inasmuch as mannose is by far the most abundant sugar in hydrolysates of the whole walls though, in the water extract, galactose and glucose residues occur with the mannose residues. Glucose remains present in trace amounts throughout all stages of Jermyn and Isherwood's[5] extraction, including 24 per cent alkali. This sugar cannot therefore be a contaminant derived from starch, but must form a small but perhaps significant part of the wall organization. Walls of all these plants yield good X-ray diagrams of mannan (Table 1) except in *Codium*, where the reflexions are very diffuse unless incrusting substances have been removed. The inner parts of the relatively large central siphons of *Batophora* plants, which in face-view are strongly positively birefringent, yield an X-ray diagram which shows true orientation, indicating that the crystallites lie longitudinally in agreement with the birefringence. When examined with the X-ray beam parallel to the flattened faces of the wall and perpendicular to the length of the siphon, an even more beautiful diagram is obtained, as illustrated in Fig. 3. This is, so far as we know, the first oriented diagram of mannan to be pub-

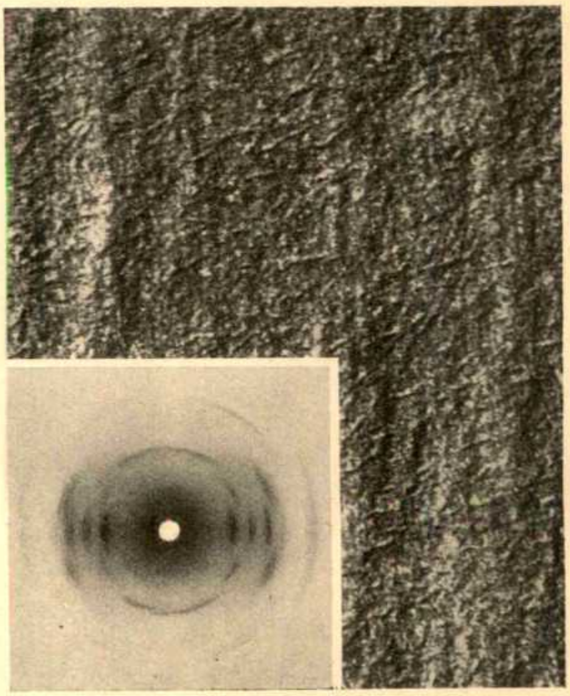

Fig. 3 Fig. 4

Fig. 3. X-ray diagram of walls of *Batophora oerstedi*; details as in Fig. 1

Fig. 4. Electron micrograph of wall of *Batophora oerstedi*; details as in Fig. 2. (× 47,000)

lished. The corresponding spacings are listed in Table 1 in comparison with those in the β-1,4-linked mannan of higher plants.

It is difficult to be certain of the form of the skeletal substance in these 'mannan weeds' as seen in the electron microscope, though the indications are that the mannan here consists of short fibrillar bodies of the order of 50 Å. in diameter (Fig. 4). In these walls, which contain no cellulose, we have been unable to find any true microfibrils such as are claimed to exist for the mannan in some higher plants[19]. This may be associated with short chain-length[18]. With all these mannan walls it is of interest to note that material treated with stronger alkali solutions (12–24 per cent potassium hydroxide) yield an X-ray diagram which is not that of the original mannan.

Now that it is established that xylan and mannan can individually form both the major fraction and the only crystalline component of cell walls, we have found it possible to resolve the condition in the red alga *Porphyra* (Bangiales) which has puzzled us for some time. The X-ray diagram of whole fronds of this plant (after chemical pre-treatment) is substantially that of mannan but shows additional reflexions of long spacing. This diagram turns out to be a composite one derived from both the cell walls proper and from a continuous surface sheath, a 'cuticle'. When cell walls alone are examined (as they may be *inter alia* with the rhizoids) the X-ray diagram is the same as those of the group discussed above in which the skeletal material is β-1,3-linked xylan. The corresponding spacings, listed in Table 1, are again unchanged after extraction of the material by boiling water, by chlorination and dilute alkali. The 'cuticle', on the other hand, yields an X-ray diagram of mannan. Both these diagrams conform with the fact that, after extraction of incrusting substances (which contain a range of sugar residues[2]) the cell walls hydrolyse to give xylose and the 'cuticle' to give mannose, each with small amounts of glucose. Cell wall hydrolysates contain no mannose and 'cuticle' hydrolysates no xylose. When chemically extracted cell walls are treated in a homogenizer, the homogenate can be seen in the electron microscope to contain a network of microfibrils indistinguishable from those found in, for example, the primary cell walls of higher plants. 'Cuticles' prepared in the same way show a granular structure. As in the mannan walls, the X-ray diagram of the 'cuticle' after treatment with stronger alkali solutions is not that of the original mannan.

Thus with *Porphyra* we find the odd circumstance that in different parts of the same plant xylose and mannose are used individually in the construction of the skeletal substance. For once the term 'cuticle', used loosely to describe the surface layer of many algae, has real meaning.

Cellulose no longer occupies a unique place as the skeletal substance in cell wall constitution as it was at one time thought to do. Even in the algae considered here, few indeed compared with the large groups which still await attention, we meet some forms in which the sole skeletal substance is a xylan and others in which mannan either forms the only crystalline material or (in *Hydrodictyon*) co-operates with a glucan in a wall which can be defined neither as a cellulose nor a mannan wall. These may be put alongside the greater number of algae in the walls of which the microfibrils, while certainly to be called cellulosic, nevertheless contain high proportions of xylan and the extreme case of the algae in Group 1 of Nicolai and Preston[1] in which the cellulose is pure. In all these forms, without any exception so far, some glucose at least is present in the skeletal material. Both cellulose and mannan are β-1,4-linked as is also xylan, according to X-ray evidence, when it is present in a cellulosic wall. The only skeletal xylan so far found, however, is β-1,3-linked, and it would seem to be significant that the glucans associated with it are also β-1,3-linked[15,16]. The situation in *Halicystis* appears to be an exception.

There is still some question whether the non-cellulosic skeletal substances form true microfibrils in the sense of the individual, easily separable bodies formed by cellulose. Even with the 'xylan weeds', what appear to be microfibrils cannot be separated, and torn edges of the wall never show the fringe of loose microfibrils typical of cellulose walls. This suggests that xylan microfibrils are interlinked. In the 'mannan weeds', separation of the wall into skeletal and incrusting substances seems pointless at the moment since here no microfibrils have yet been found. This fact, together with the observation that the walls of 'mannan weeds' are still coherent after treatment with strong alkali which leaves the only crystalline material in the form of granules, suggests that the role of microfibrils in strengthening a wall may have been over-emphasized.

The relative amount of skeletal material found in algal cells walls is in this respect not without interest. In the algae of Group 1, in which the skeletal material is pure cellulose, the microfibrillar component makes up between 30 and 75 per cent of the wall weight, a range which covers also most of the (cellulosic) secondary walls of higher plants. A somewhat similar figure holds also for the 'xylan weeds' ($\geqslant$ 50 per cent). With 'mannan weeds' the walls of which contain up to 90 per cent of mannose residues, no figure can be given on account of the difficulty of defining the skeletal component. In the remainder of the algae so far investigated, in which the microfibrils probably consist of a cellulose core with a hemicellulose cortex[6], the relative amount of skeletal material is low (2–20 per cent). All these walls are equally coherent whatever the proportion of the skeletal material.

Nicolai and Preston[1] made no mistake in concluding that among the Chlorophyceae variations occur in the construction of cell walls, though the basis on which their conclusions were founded was not completely reliable. Our newer observations show that while the bulk of the algae contain cellulose I, including most of those which Nicolai and Preston placed in Group 2, some make use for the same purpose of long-chain polymers built from sugars other than glucose. It is surely significant that this latter condition is almost exclusively confined to the Chlorophyceae. No plant so far investigated, with the exception of one (dubious) case, *Halicystis*, has been found to contain cellulose II. The algae of Group 1 still stand as unique among plants; the attempt to classify the rest of the algae into two groups must, however, be abandoned, and no new grouping is justified at this stage, particularly in view of complex forms like *Halicystis* and *Porphyra*.

For part of this investigation we received material from: Dr. R. P. Bengry, Institute of Jamaica; Dr. P. B. Tomlinson, Fairchild Tropical Garden, Florida; Dr. W. Eifion Jones, Marine Biological Station, Bangor; and Miss J. M. Kain, Marine

Biological Station, Port Erin To all of them our thanks are due

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PRODUCTION AND PROPERTIES OF DI-FLUOROCARBENE

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THE di-fluorocarbene molecule, CF_2, was first detected spectroscopically in 1950, when its emission[1] and absorption[2] spectra in the ultra-violet region were photographed following the decomposition of fluorocarbon vapours in an electrical discharge The observation that, provided the containing vessel were large enough, the absorption spectrum could still be detected seconds after the discharge was discontinued[2] was striking. it was inferred that CF_2 was a remarkably stable species, and that it disappeared after diffusion to the wall of the vessel

Since 1950, CF_2 has been produced (and detected spectroscopically) by such a variety of methods that it is now apparent that its formation must be a favoured process Table 1 lists some parent molecules which have been used and the methods of decomposition adopted A zero order for the rate of disappearance of CF_2 produced in flash photolysis has been reported, and its long life-time confirmed[3]

Table 1

Parent molecule	Method	Reference
Fluoro-carbons	Elec discharge	1, 2
CF_4	Furnace	4
CF_3COCF_3	Flash photolysis	5
CF_2Br_2	,, ,,	6
CF_2HBr	,, ,,	7
$CF_2ClCOCF_2Cl$	,, ,,	3
$CF_2\,CF_2$	,, ,,	8
CF_3COOH	,, ,	8

It has been suggested[9] that the production of CF_2 in the flash photolysis of hexa-fluoroacetone might follow the disproportionation

$$2CF_3 \rightarrow CF_4 + CF_2$$

However, when the photolysis of CF_3I is used as a source of CF_3 radicals, no CF_4 can be detected among the volatile reaction products although there is an abundance of C_2F_6 (ref 10) Further, although flash photolysis of CF_2Br_2 and CF_2HBr in the quartz ultra-violet region produces a high yield of CF_2, no CF_2 absorption could be detected following the flash photolysis of CF_3I (ref 11) and CF_3Br (ref 8) under the same conditions Indeed, only when the latter molecules were photolysed in a specially constructed flash discharge cell which was transparent to wave-lengths well below 2000 Å (see Fig 1) was it possible to observe a faint absorption spectrum of CF_2 It follows that a far greater energy input is required to obtain CF_2 from CF_3Br than from CF_2Br_2 or CF_2HBr, although the C—Br bond dissociation energies in the molecules would not differ very greatly. If the CF_2 production occurs through the disproportionation of substituted methyl radicals, then it is hard to understand why the disproportionation of CF_2Br and CF_2H should be so favoured as compared with CF_3

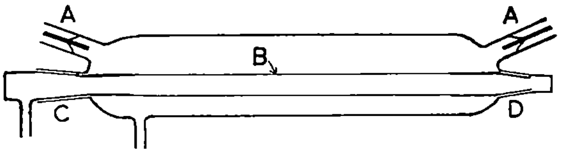

Fig 1 Silica cell for flash photolysis at wave-lengths < 2000 Å (Drawing not to scale) *A*, Electrodes, *B*, thin silica (thickness 0 5 mm), *C*, socket and cone, *B* 19, *D*, socket and cone, *B* 14

Finally, we have found that if CF_2Br_2 is flashed (under conventional conditions) in the gas phase in the presence of a large excess of oxygen, very few bromine atoms are produced, since the expected absorption spectrum of the BrO radical is barely detectable (Flash photolysis of CCl_2Br_2 under the same conditions resulted in a very intense transient spectrum of BrO) In particular, the yield of CF_2 is virtually independent of the oxygen pressure, which must exclude CF_2Br radicals as the precursors to CF_2 formation

The data presented are all consistent with the primary production of CF_2 in a molecular split, as.

$$CF_2Br_2 + h\nu \rightarrow CF_2 + Br_2$$
$$CF_2HBr + h\nu \rightarrow CF_2 + HBr$$
$$CF_3COCF_3 + h\nu \rightarrow CF_2 + CF_3COF$$

and so on A similar primary decomposition has been proposed in the xenon photosensitized decomposition of CF_4 (ref 12) Such modes of decomposition are not in conflict with the suggestion that CF_2 is a relatively stable species having the properties of a two-electron deficient molecule rather than a di-radical This hypothesis is favoured by (1) the observation of an intense absorption spectrum of CF_2 following flash photolysis of tetra-fluoroethylene in the gas phase[8], which demonstrates the relative weakness of the carbon–carbon bonding in the olefine, (2) the lack of reactivity of CF_2 towards molecular oxygen, and (3) its zero-order rate of decay, demonstrating its lack of reactivity to other molecules of the same kind

The difficulty in forming CF_2 following photolysis of CF_3Br is understandable in view of the energetics of the decomposition

$$CF_3Br \rightarrow CF_2 + BrF$$

Such difficulty would not be anticipated in the cases of CF_2Br_2 and CF_2HBr, since in the first case both the bonds broken are weak, and in the second there is a considerable energy 'rebate' in the formation of the HBr molecule.

The ready decomposition of tetra-fluoroethylene into two CF_2 molecules, taken together with the other experimental results presented, suggests that in the fluorocarbon series it is not unreasonable to regard di-fluorocarbene as the simplest of the olefines. The splitting out of CF_2 from the substituted methanes and ketones listed is then analogous to the production of ethylene from ethyl iodide[13], and the Norrish Type II split in ketones containing hydrogen. Indeed, it has been proposed very recently[14] that the primary steps in the pyrolysis of hexa-fluoroacetone include the molecular decomposition

$$CF_3COCF_3 \rightarrow CF_2 + CF_3COF$$

as well as the radical decomposition

$$CF_3COCF_3 \rightarrow 2CF_3 + CO$$

The striking stability and the lack of chemical reactivity in di-fluorocarbene may be attributed, no doubt, to the influence of the strongly electronegative fluorine atoms. These would tend to attract the two electrons residing in the most weakly bound of the molecular orbitals, away from the neighbourhood of the carbon atom, on which they would otherwise be localized. As a result, the possibility of electron exchange interaction with other species would be much reduced, since there would be less possibility of electron overlap. This effect would not be anticipated in other di-halogenocarbenes, such as CCl_2 and CBr_2, since the electronegativities of Cl and Br atoms are not so very different from that of carbon. On Pauling's electronegativity scale, F = 4.0, Cl = 3.0, Br = 2.8 and C = 2.5. Indeed, attempts to prepare CCl_2 and CBr_2 photochemically from parent molecules analogous with those used for CF_2 production have so far proved unsuccessful[7].

One of us (A. J. Y.) thanks the Department of Scientific and Industrial Research for the award of a maintenance grant.

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ACTION OF RADIATION ON SYNTHESIS OF DEOXYRIBONUCLEIC ACID IN NORMAL AND TUMOUR STRAIN CELLS

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HOWARD and Pelc[1,2] first showed that relatively small doses of radiation (50–200 r.) given during the deoxyribonucleic acid (DNA) pre-synthetic period, delayed the entry of cells into DNA synthesis, whereas cells already in DNA synthesis at the time of irradiation were unaffected by the doses given. These authors used phosphorus-32 uptake into DNA of bean root cells, and examined by autoradiography the number of cells synthesizing DNA over short periods up to 24 hr. during the interphase following irradiation. Holmes and Mee[3] and Kelly *et al.*[4], using regenerating liver and uptake of phosphorus-32 into DNA, again found that radiation doses of about 300 r. given during the pre-synthetic period delayed the onset of DNA synthesis; much larger doses (~2,000 r.) were required to depress DNA synthesis once it had commenced.

Lajtha *et al.*[5] found that doses of ~ 200 r. given to human bone marrow cells in culture during the pre-synthetic period delayed the entry of cells into DNA synthesis; the incorporation of ^{14}C-formate into DNA was observed by autoradiographic methods after various fractions of the interphase period following irradiation. On the other hand, it was found that a dose of 2,000–5,000 r. was required to reduce the rate of DNA synthesis to half, in cells which were already in synthesis.

In considering the effects of radiation on solid tumours *in vivo*, it is difficult to dissociate direct effects on the tumour from indirect effects acting through the surrounding host tissues[6]. In addition one such tumour usually represents a great variety of cell types; one way out of this difficulty is to use a tumour strain growing *in vitro*, and another possibility is to use an ascites tumour.

Painter and Robertson[7] used HeLa carcinoma strain cells in culture in conjunction with thymidine uptake and autoradiography, and found an increase in the number of cells in DNA synthesis at times up to 8 hr. after 500 r. of X-irradiation, and a decrease in the number of synthesizing cells after 17 hr. These results could partly be explained by inhibition of mitosis; first, an increase by retention in synthesis of cells which would normally have divided and, later, a decrease from the consequent depletion of mitosis; first, an increase by retention in synthesis. Robertson pointed out, the results are certainly inconsistent with a delay in onset of DNA synthesis induced by radiation in the pre-synthetic period[7]. Again, using HeLa cells with thymidine and autoradiography, Yamada and Puck[8] found that radiation doses of 34–135 rads produced mitotic inhibition, but not a delay in the onset of DNA synthesis in these cells; this was in contrast with their findings with normal human cells in culture which showed a pre-synthetic delay in addition to inhibition of mitosis.

Dickson, Paul and Davidson[9] used *L* mouse strain cells in culture in conjunction with incorporation of ^{14}C-formate into DNA. These authors concluded that the effect of X-irradiation (800 r.) was to inhibit division the total amounts of cell constituents were found to be almost the same in the irradiated cultures as in the controls, and no alteration of ^{14}C-formate incorporation into DNA bases could be detected at times up to 24 hr. (more than one interphase period) after a dose of 800 r. to the cultures.

Kelly *et al.*[10] irradiated Ehrlich ascites tumour cells with a dose of 800 r. and investigated subsequent DNA synthesis by uptake of phosphorus-32 into DNA, and also by Feulgen staining combined with photometry. These authors found that the uptake of phosphorus-32 into DNA remained unaffected until 20 hr. after irradiation, at which time the average DNA content per cell was found to have increased; after 20 hr., a decrease in uptake of phosphorus-32 was observed, because, according to these authors, the cells were prevented from going through mitosis by the irradiation dose given.

The purpose of this article is to point out that those experiments which have shown inhibition of the onset of DNA synthesis with radiation doses of the order of a few hundred rœntgens in the pre-synthetic period have used cells freshly prepared from normal tissues, whereas those experiments using similar radiation doses which have failed to show an effect on the onset of DNA synthesis in the pre-synthetic period have used tumour-strain cells.

In order to explain their radiation results, it has been suggested by Lajtha *et al.*[5] that in human bone marrow cells there exists a 'trigger mechanism' connected with the onset of DNA synthesis, which is more sensitive to radiation than the process of DNA synthesis itself. Because of the results reviewed above, it would seem that this applies to the normal cells but not to the tumour strains.

It was reported some years ago that an increase in ultra-violet absorption by the cytoplasm occurred in irradiated cells[11]. More recently it has been shown[12-14] that an accumulation of soluble deoxy-derivatives, including deoxyribonucleoside triphosphates, occurred in irradiated thymus; these results were interpreted as indicating that inhibition of DNA synthesis by radiation was not caused by lack of precursors, but by failure of polymerization or breakdown in energy-generating mechanisms in the nucleus[14]. In this connexion, Creasey and Stocken[15] found that nuclear phosphorylation of purine and pyrimidine monophosphates to triphosphates is completely inhibited by 100 r. and reduced by 50–80 per cent after 25 r. In addition, in regenerating liver an increase in thymidine kinase and DNA polymerase is stopped by irradiation in the early pre-synthetic period, but not by irradiation in the DNA synthetic period[16].

Recently it has been shown by me[17] that, in three types of freshly prepared normal cells grown in culture (monkey kidney, embryo human, embryo mouse), the processes of DNA and nuclear protein synthesis are initiated together after the same waiting time (the pre-synthetic period) has elapsed, and thereafter the syntheses proceed approximately together. However, in the continuous tumour strains HeLa and *L* mouse, it was found that nuclear protein synthesis proceeds continuously during interphase and its onset does not await the onset of DNA synthesis, which commences after a waiting time has elapsed as in other types of cells[17]. Supporting evidence for this time-disconnexion of nuclear protein and DNA synthesis in tumour strains comes from the distributions of DNA and dry mass values found in Krebs ascites tumour cells[18]. Because of the genetic role at present ascribed to DNA, I have suggested[17] that the difference in timing of the onset of nuclear protein synthesis reflects a fundamental difference in metabolism between normal and tumour strain cells. It would appear from the radiation work reviewed here[1-10], that the processes responsible for initiation of DNA synthesis in these cells are also different in character.

Allfrey *et al.*[19,20] found that, in isolated calf thymus nuclei, the incorporation of amino-acid into protein required the presence of DNA, and that one role of DNA was in the production of adenosine triphosphate. Inhibitors of ATP synthesis were found to inhibit incorporation of amino-acid[20], and also the addition of histones to the nuclei was found to inhibit strongly respiration and nuclear phosphorylation[21]. Davison and Butler[22] and Vendrely *et al.*[23] reported that the total amount of all the basic groups in the nucleohistone is approximately equal to the total amount of phosphate in the DNA.

It may therefore be permissible to suggest here that the simultaneous initiation of DNA and nuclear protein syntheses in normal cells[17] might reflect a mutual control system which would operate by regulating nuclear phosphorylation processes. The time-disconnexion of DNA and nuclear protein syntheses found in tumour strains[17,18] would imply dislocation of control of phosphorylation and would be consistent with the lack of radiosensitivity found in the DNA pre-synthetic period with these cells[7-10]. It is worth noting that Paul *et al.*[24] were unable to find evidence of any consistent radiation-induced depression of thymidine incorporation into thymidine triphosphate in either *L* mouse strain cells or in a lymphosarcoma cell strain, in contrast with the radiosensitivity of phosphorylation processes found in thymus nuclei[15].

This work was carried out while I was holder of the Royal Society Foulerton Gift Research Fellowship.

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SUBSTRATE SPECIFICITY AND INHIBITION OF NICOTINAMIDE MONONUCLEOTIDE-ADENYLYL TRANSFERASE OF LIVER NUCLEI: POSSIBLE MECHANISM OF EFFECT OF 6-MERCAPTOPURINE ON TUMOUR GROWTH

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SYNTHESIS of nicotinamide adenine dinucleotides (coenzymes I and II) in most animal tissues involves formation of dinucleotide in the cell nucleus[1,2] catalysed by nicotinamide mononucleotide–adenylyl transferase (formerly known as coenzyme I-pyrophosphorylase)[3], thus

nicotinamide mononucleotide + adenosine triphosphate ⇌ nicotinamide adenine dinucleotide + pyrophosphate

The biological significance of this reaction, which was discovered by Kornberg[4], has been discussed by Branster and Morton[2], and by Morton[5]. The work described here was carried out with enzyme obtained from isolated pig-liver nuclei[6], either purified (specific activity approx 3) or partly purified enzyme (specific activity, 0 7–1 μmole of nicotinamide adenine dinucleotide formed/min /mgm of protein at 37° C, *p*H 7 6) was used

Substrate specificity In work on dinucleotide synthesis, the reaction mixture (0 2 ml, *p*H 7 6) contained approximately 0 005 unit[6] of enzyme, 25 μmoles of glycylglycine, 3 7 μmoles of magnesium chloride, 1 μmole of adenosine triphosphate (Sigma Chemical Co, U S A, for purity, see ref 7) and approximately 1 μmole of nicotinamide mononucleotide[6], analogues replaced adenosine triphosphate or nicotinamide mononucleotide, as appropriate In work on pyrophosphorolysis of dinucleotides, the reaction mixture (0 2 ml, *p*H 7 6) contained similar amounts of enzyme, buffer and magnesium salts, 20 μmoles of pyrophosphate and 1 μmole of nicotinamide adenine dinucleotide or of appropriate analogue After incubation for 2½ hr at 37° C, an appropriate portion (0 02–0 05 ml) of the reaction mixture was applied to strips of filter paper (Whatman, 3 *MM*), nucleotides were separated by electrophoresis in 0 04 *M* sodium citrate, *p*H 4 2 for 3¼ hr at 400 V (approximately 14 V per cm) and detected by printing on reflex document paper with ultra-violet light[7] Quaternary nicotinamide compounds were detected by fluorescence after exposure to an atmosphere of methylethylketone–ammonia[8]

Table 1 shows the reactions investigated and the results obtained It was previously shown[9] that the α-anomer of nicotinamide mononucleotide is not a substrate of the enzyme, which is almost certainly specific towards the β-*N*-glycoside bond The pyridine mononucleotide (Table 1, reaction 7) probably contained both α- and β-anomers, but the proportions of these is not known, although unlikely, it is possible that the absence of a detectable reaction with this compound is due to a predominance of the α-anomer

A number of purine riboside triphosphates, in addition to adenosine triphosphate, act as nucleotidylyl donors to nicotinamide mononucleotide (Table 1) Of the dinucleotides so formed, only nicotinamide hypoxanthine dinucleotide has previously been described[11] Nicotinamide guanine dinucleotide was separated from the reaction products (Table 1, reaction 9) by chromatography on 'Dowex-2' formate with a gradient of formic acid, the dinucleotide was eluted between 1 and 1 5 *N* formic acid The properties of the dinucleotide are shown in Table 2

Table 1 shows that no enzymatic reaction could be detected with dinucleotides containing analogues of both nicotinamide and of adenine (such as 3-acetyl-pyridine hypoxanthine dinucleotide) With enzyme preparations obtained from acetone powders of pig-

Table 1 SUBSTRATE SPECIFICITY OF NICOTINAMIDE MONONUCLEOTIDE–ADENYLYL TRANSFERASE

Solid arrows indicate the reactions established All reactions were carried out at *p*H 7 6 and 37° C, in the presence of magnesium chloride

1	Nicotinamide mononucleotide	+ adenosine triphosphate	⇌	nicotinamide adenine dinucleotide	+ pyrophosphate
2	Nicotinic acid mononucleotide*	+ adenosine triphosphate	⇌	nicotinic acid adenine dinucleotide	+ pyrophosphate
3	Dihydro-nicotinamide mononucleotide	+ adenosine triphosphate	⇌	dihydro-nicotinamide adenine dinucleotide	+ pyrophosphate
4	Thio-nicotinamide mononucleotide†	+ adenosine triphosphate	⇌	thio-nicotinamide adenine dinucleotide††	+ pyrophosphate
5	3-Aldehyde-pyridine mononucleotide	+ adenosine triphosphate	←	3-aldehyde-pyridine adenine dinucleotide††	+ pyrophosphate
6	3-Acetyl-pyridine mononucleotide	+ adenosine triphosphate	←	3-acetyl-pyridine adenine dinucleotide††	+ pyrophosphate
7.	Pyridine mononucleotide†	+ adenosine triphosphate	⇢	(no reaction)	
8	Nicotinamide mononucleotide	+ hypoxanthosine triphosphate**	⇌	nicotinamide hypoxanthine dinucleotide††	+ pyrophosphate
9.	Nicotinamide mononucleotide	+ guanosine triphosphate**	→	nicotinamide guanine dinucleotide	+ pyrophosphate
10	Nicotinamide mononucleotide	+ 8-azaguanosine triphosphate***	→	nicotinamide 8-azaguanine dinucleotide	+ pyrophosphate
11	Nicotinamide mononucleotide	+ 6-mercaptopurine riboside triphosphate†††	→	nicotinamide 6-mercaptopurine dinucleotide	+ pyrophosphate
12	Nicotinamide mononucleotide	+ *deoxy*-adenosine triphosphate**	→	(*deoxy*)-nicotinamide adenine dinucleotide	+ pyrophosphate
13	Nicotinamide mononucleotide	+ uridine triphosphate**	⇢	(no reaction)	
14	Nicotinamide mononucleotide	+ cytidine triphosphate**	⇢	(no reaction)	
15		(no reaction)	⇠	3-aldehyde-pyridine hypoxanthine dinucleotide††	+ pyrophosphate
16		(no reaction)	⇠	3-acetyl-pyridine hypoxanthine dinucleotide††	+ pyrophosphate

* Prepared according to Atkinson and Morton (ref 10)
** Sigma Chemical Co, U S A
*** We are grateful to Dr R B Ross, National Institutes of Health, U S A, for a gift of this compound
† Atkinson, M R, Morton, R K, and Naylor, R (unpublished work)
†† Pabst Laboratories, U S A
††† Prepared according to Roy *et al* (ref 17)

Table 2 COMPARATIVE PROPERTIES OF β-NICOTINAMIDE ADENINE DINUCLEOTIDE (COENZYME I) AND OF β-NICOTINAMIDE GUANINE DINUCLEOTIDE

	NAD*	NGD*
Ascending chromatography in isobutyric acid–water–conc ammonia (33 66 1, v/v)	Single spot, R_F, 0 45	Single spot, R_F, 0 32
Treatment with methylethylketone–ammonia (ref 8)	Fluorescent spot	Fluorescent spot
Bases liberated after hydrolysis and chromatography (ref 12)	Adenine and nicotinic acid	Guanine and nicotinic acid
Absorption spectrum in 0 1 *N* HCl	λ_{max}, 260 mμ (see ref 13)	Similar to NAD, but inflexion near 270–280 mμ (cf other guanine nucleotides, ref 13)
Absorption spectrum in *M* KCN	λ_{max}, 260 mμ and 325 mμ, E_{260} mμ/E_{325} mμ, 1 6	λ_{max}, 266 mμ and 325 mμ, E_{266} mμ/E_{325} mμ, 1 96
Reaction with yeast alcohol dehydrogenase and ethanol at *p*H 10 in *tris* buffer*	K_m 1 6 × 10^{-4} *M*	K_m approx 2 × 10^{-3} *M*, ($V_{max.}$ of reaction, 0 01–0 02 that of $V_{max.}$ with NAD)

* Abbreviations used NAD, β-nicotinamide adenine dinucleotide, NGD, β-nicotinamide guanine dinucleotide, *tris*, 2-amino-2-hydroxy-methylpropane-1 3-diol

liver[4], Kornberg[4] earlier demonstrated reaction 3, and Preiss and Handler[14] reaction 2 of Table 1, Klenow and Andersen[15] previously demonstrated reaction 12 (Table 1) with an enzyme preparation from yeast[4]

Inhibition of synthesis of nicotinamide adenine dinucleotide Kinetic work was carried out at *p*H 7 4–7 6, the reaction mixtures contained at appropriate concentrations, enzyme, glycylglycine, adenosine triphosphate, nicotinamide mononucleotide, magnesium chloride, and inhibitor After incubation for 5 min at 37° C, the reaction was stopped with trichloroacetic acid and the amount of nicotinamide adenine dinucleotide was estimated enzymically[6] Determinations were made with several concentrations of inhibitor for each of two or more concentrations of the appropriate substrate (nicotinamide mononucleotide or adenosine triphosphate) Values of K_i (see Dixon and Webb[16], p 22 *et seq*) were estimated graphically In studies with hypoxanthosine triphosphate (inosine triphosphate), the concentration of magnesium chloride was twice that of the total concentration of the nucleoside triphosphate present in each reaction mixture

Values of K_m for several substrates were estimated similarly except that the rate of formation of nicotinic acid adenine dinucleotide was measured by estimation of pyrophosphate

With hypoxanthosine triphosphate and nicotinic acid mononucleotide, inhibition of adenylyl transfer was competitive, Table 3 shows the K_i values for these compounds as inhibitors, and also the K_m values for these compounds when used as substrates for the enzyme

Table 3 INHIBITOR CONSTANT (K_i) AND MICHAELIS CONSTANT (K_m) FOR SOME COMPETITIVE INHIBITORS OF SYNTHESIS OF NICOTINAMIDE ADENINE DINUCLEOTIDE (COENZYME I)

All values were determined at *p*H 7 4–7 6, and at 37° C

	Substrate*	Inhibitor	K_m (m*M*)	K_i† (m*M*)
1	Adenosine triphosphate		0 4	—
2	Hypoxanthosine triphosphate		1 8	—
3	Adenosine triphosphate	hypoxanthosine triphosphate	—	2 1
4	Nicotinamide mononucleotide		0 12	—
5	Nicotinic acid mononucleotide		0 4	—
6	Nicotinamide mononucleotide	nicotinic acid mononucleotide	—	0 38

* For 1–3, the co-substrate was nicotinamide mononucleotide, for 4–6, adenosine triphosphate

† See Dixon and Webb (ref 16, p 22)

6-Mercaptopurine riboside (Sigma Chemical Co, U S A) was converted to the corresponding 5′-monophosphate, which was treated with orthophosphate and dicyclohexylcarbodiimide to obtain the nucleoside triphosphate[17] The nucleotide products were adsorbed on to charcoal, and then washed, eluted and dried *in vacuo* (preparation *A*) Preparation *A* contained components which appeared to be the 5′-mono-, di- and tri-phosphates of 6-mercaptopurine riboside, since they migrated similarly to hypoxanthosine-5′-mono-, di- and tri-phosphates on paper electrophoresis Chromatography of preparation *A* on Ecteola' resin[17] gave four fractions, of which the last to be eluted (preparation *B*) was probably the 6-mercaptopurine riboside 5′-triphosphate, however, it was obtained in very low yield and in insufficient amount for chemical characterization

Adenylyl transfer to nicotinamide mononucleotide was inhibited competitively by both preparations *A* and *B* Separately, it was found that the 6-mercaptopurine itself, the riboside, and corresponding riboside 5′-phosphate do not inhibit adenylyl transfer at concentrations up to the concentration of the total 6-mercaptopurine nucleotide in preparation *A*

With preparation *A* and nicotinamide mononucleotide as substrates, nicotinamide 6-mercaptopurine dinucleotide was formed (Table 1), confirming that preparation *A* contained 6-mercaptopurine riboside triphosphate

From the kinetics of the competitive inhibition of adenylyl transfer by preparation *A*, and assuming that the only inhibitory material in preparation *A* was 6-mercaptopurine riboside triphosphate, K_i would be 0 3 m*M* if the yield of triphosphate were similar to that reported by Parks and co-workers[17]. Final K_i and K_m values for 6-mercaptopurine riboside triphosphate await preparation of larger amounts of material and adequate chemical characterization of the triphosphate

Possible mechanism of the selective cytotoxic effect of 6-mercaptopurine against tumours Branster and Morton[2] found that the activity per nucleus of the nicotinamide mononucleotide–adenylyl transferase of a number of tumours is considerably less than that of comparable normal tissues, the concentration of nicotinamide nucleotide coenzymes (coenzymes I and II) per nucleus (or per cell) is also considerably lower in tumours than in comparable normal tissues (see ref 5) Morton[5] therefore proposed that inhibition of the nicotinamide mononucleotide–adenylyl transferase of the cell nucleus would lower the level of the nicotinamide coenzymes of tumour cells below that necessary for survival of the tumour, and indicated the general characteristics required for a physiologically effective inhibitor

6-Mercaptopurine meets some of these criteria[5] for a compound selectively active against tumours. In susceptible cells, it is converted to 6-mercaptopurine riboside 5′-mono-, di- and tri-phosphates[18–20] 6-Mercaptopurine riboside triphosphate is an analogue of adenosine triphosphate and, like hypoxanthosine triphosphate, is a substrate for nicotinamide mononucleotide–adenylyl transferase (Table 1) Although the chemical characterization of the components of preparation *A* is incomplete, the potent inhibition by preparation *A* and preparation *B* is almost certainly due to 6-mercaptopurine riboside triphosphate

The selective toxicity of 6-mercaptopurine against tumour cells thus appears to be due to inhibition of the biosynthesis of nicotinamide nucleotide coenzymes (coenzymes I and II), this inhibition occurs after the enzymic conversion of the purine to the purine riboside triphosphate, which competitively acts on the nicotinamide mononucleotide–adenylyl transferase of the tumour nucleus and effectively limits dinucleotide formation Symptoms similar to those of nicotinamide deficiency have sometimes been observed in patients after prolonged treatment with 6-mercaptopurine[21]

Hypoxanthosine triphosphate is not sufficiently active to be an effective inhibitor of formation of coenzyme I *in vivo* (Table 3) Although nicotinic acid mononucleotide is strongly inhibitory (Table 3), the nicotinic acid adenine dinucleotide formed with substrate-levels of nicotinic acid mononucleotide may afterwards be converted to nicotinamide adenine dinucleotide When nicotinic acid is administered to rats together with nicotinamide, it causes a marked decrease in the rate of formation of nicotinamide adenine dinucleotide in the liver, as compared with rats receiving nicotinamide alone Table 3 shows that this effect is probably due to the inhibition of adenylyl transfer to nicotinamide mononucleotide by nicotinic acid mononucleotide

6-Mercaptopurine was introduced by Hitchings *et al* [22], as a potential antagonist of biosynthesis of nucleic acids, but recent findings[20,23] do not support this explanation of its action against certain human leukæmias and some transplanted tumours of animals Although the evidence presented here shows that 6-mercaptopurine probably acts on tumours via the effect on biosynthesis of nicotinamide nucleotide coenzymes, since 6-mercaptopurine riboside triphosphate is an analogue of adenosine triphosphate, it could possibly influence a wide range of enzymatic reactions The undesirable side-effects of 6-mercaptopurine may be partly associated with such multiple actions Moreover, certain tumours have a limited enzymatic capacity for converting the purine to the nucleotide[20,24] and so escape the lethal depression of coenzyme synthesis It thus seems preferable to inhibit formation of nicotinamide nucleotide coenzymes in tumours via analogues of nicotinamide mononucleotide, as previously suggested[5].

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MULTIPLE HÆMOGLOBINS AND TRANSFERRINS IN A MACAQUE SIBSHIP

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PHYSICO-CHEMICAL differences between embryonic, fœtal, and adult human hæmoglobins[1–4] and the occurrence of a number of transferrin phenotypes in man[5,6] have led several investigators to examine hæmoglobins and β-globulins in various Primates[7–13]. We report here on the hæmoglobins and β-globulins obtained from a related, inbred group of rhesus macaques (*Macaca mulatta*) Very young macaques have relatively high levels of alkali-resistant hæmoglobins, and the levels decrease as the animals grow older[8] The number of β-globulins reported for macaques (*M mulatta* and *M irus*) has been large[11,12]. The results presented here are consistent with earlier findings on alkali-resistant hæmoglobins[8] and with reports of transferrin polymorphism[11–15] in various Primates However, we

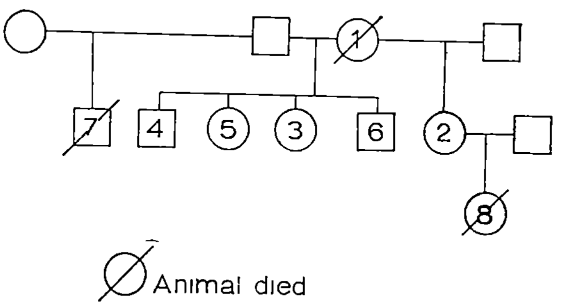

Fig 1 Pedigree of inbred macaque sibship Animal 1 died 14 days *post partum*, animals 7 and 8 were 90-day fœtuses taken by Cæsarean section The symbols which do not contain numbers represent animals not sampled for this work

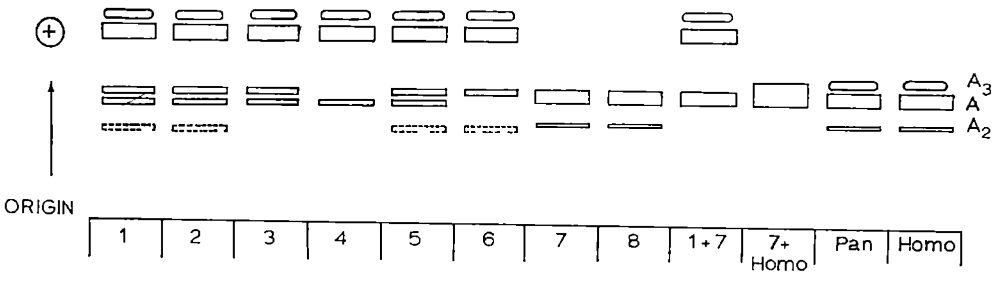

Fig 2 Diagram of a starch-gel after electrophoresis showing multiple hæmoglobins from macaques The human and chimpanzee (*Pan*) samples were run on this gel for comparison A, A_2, and A_3 refer to the known hæmoglobins in this human sample and do not refer to the macaque or chimpanzee samples When hæmoglobin from a fœtus (animal 7) was combined with either adult macaque or human hæmoglobin, the bands were incompletely resolved The bands represented with dotted lines are those which stained weakly with benzidine

find this macaque sibship contains individuals that unequivocally display multiple hæmoglobins

We received cord blood from two 90-day *M mulatta* fœtuses taken by Cæsarean section, and from one of these we obtained blood from the aorta as well Six animals related to the fœtuses in the same sibship were also sampled (Fig 1) In this sibship the normal gestation period is 168 days[16] Blood was drawn into tubes containing citric acid, sodium citrate, and dextrose Serum and cells were separated by centrifugation, and hæmolysates were prepared by the method of Jonxis and Huisman[17]. Alkali-resistant hæmoglobins were demonstrated by the method of Singer *et al* [18] The hæmoglobin components were resolved on horizontal starch-gels, 13 per cent starch in *tris*-citrate buffer at *p*H 8 6 The gels were run for 16 hr. at 6 V /cm. with borate buffer, 0 3 *M*, *p*H 8 6, as the bridge solution[19]. Gels were sliced in half, one section was stained with a benzidine reagent (200 ml of 50 per cent ethanol 0 1 ml. of glacial acetic acid 4 mgm. of benzidine , 0 4 ml. of hydrogen peroxide added immediately before use) The other section of the gel was stained with bromphenol blue or amido-black The β-globulins were resolved and identified on vertical starch-gels by a method previously described[14]

A diagram of a starch-gel electrophoretogram of the hæmoglobin samples is shown in Fig 2 Three of the samples, including the two fœtuses, show two distinct components In three of the samples there are 4 components, and two show 3 components , four of these five samples show an additional component ; this was a very slow-moving band that stained weakly with benzidine In order to obtain better resolution and a confirmation of these results, sample 1 was run for 24 hr. at 10 V /cm The mobility of the principal component was 4–5 times that of human hæmoglobin *A* on the same gel The several components seen in these animals have not been reported in other work on macaque hæmoglobins Our previous experience and that of other investigators[20] with macaque hæmoglobins showed that only the single component reported by Sen *et al* [8] appeared There is one report that agar-gel electrophoresis demonstrated differences between the cord blood of a new-born macaque and its mother , a second component was found in the cord blood of this new-born animal[7] In addition, Lie-Injo *et al* [10] and Tuttle *et al* [9], using paper electrophoresis and veronal buffers, demonstrated other components in the hæmoglobins of macaques They were able to resolve only 2 components in individual animals , we can show as many as 5 components in one of our animals, and all the animals in this sibship have at least 2 hæmoglobins

The transferrins of these animals are illustrated in Fig 3 The diagram shows the bands which appeared on the autoradiographs of the starch-gels in which the sera were run The half-gel which was stained with amido-black is illustrated in Fig. 4 It is clear to us that some of the bands in the β-globulin region on these gels are indeed transferrins In the fœtal sera (animals 7 and 8) there are two distinct bands of protein in the β-region which stain faintly with amido-black but do not appear on autoradiographs Their position corresponds to the 2–3 iron-binding bands in some of the adult sera However, it was not possible to demonstrate iron-binding by these bands in the samples from the cord or the aorta of the two fœtuses. In general, we accept as transferrin only those bands which clearly take up radioactive iron-59. This criterion must be applied with care, since it is possible that these iron-binding proteins are saturated *in vivo* at the time the blood is drawn[21] We have named the transferrin types according to the mobility of the iron-binding bands Thus, a 1–1 or a 2–2 type is a macaque with only one such band, and a 1-1 type has the most mobile band , a 2–3 type is an animal that has two iron-binding bands, the second and third in order of decreasing mobility

Tables 1 and 2 summarize the results obtained for the 8 samples under discussion here. It is important to note that the proportion of alkali-resistant hæmo-

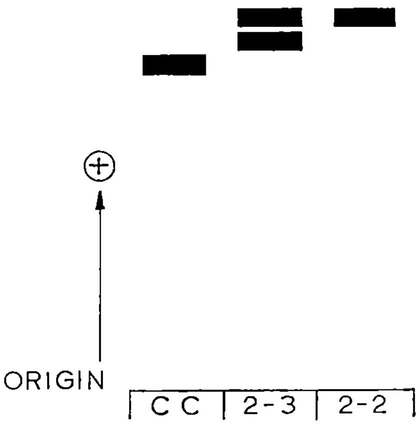

Fig 3. Diagram of autoradiographs showing transferrin types of 8 animals in the macaque sibship Three of the animals are type 2–2, 5, including the two fœtuses are type 2–3 A human homozygous CC phenotype is shown for comparison

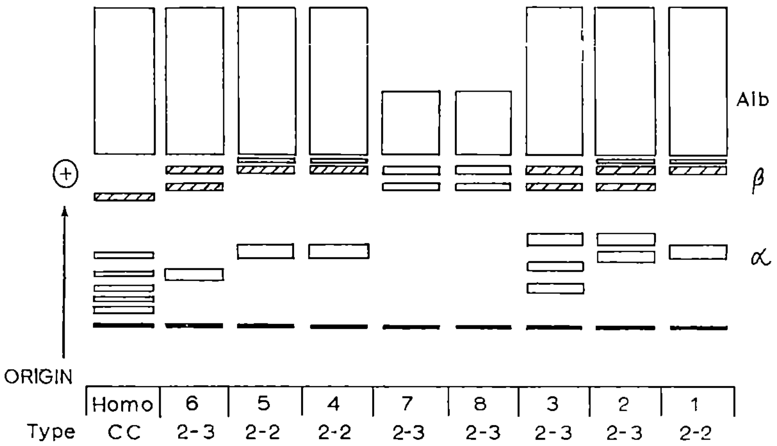

Fig 4 Diagram of a starch-gel electrophoretogram showing those bands which stained with amido-black Albumin, α- and β-globulin regions are noted In the β-region the hatched bands are those which show uptake of iron-59 on autoradiographs A human sample, run on the same gel, is for comparison

globin is very high in the samples from the 90-day fœtuses By the time an animal is one year old (animal 5) the level has dropped markedly In 4 of the 5 adult animals we were unable to demonstrate any alkali-resistant pigment, and in the fifth the amount was negligible. The ultra-violet absorption spectrum of the alkali-resistant hæmoglobin from a fœtal sample (animal 7) was identical with published spectra of alkali-resistant fractions prepared from human hæmoglobin[18]

The hæmoglobin components seem to be inherited as simple autosomal alleles The major problem here, as with human hæmoglobin *A* and A_2, is whether there are two loci involved or whether these hæmoglobins are allelic at the same locus It seems clear that the principal component (that producing the densest stain and the largest spot) and one analogous to A_2 (human) will prove to be inherited as simple autosomal traits The third component we definitely demonstrate may be analogous to human *F*, since it and the major band in macaque fœtuses correspond In the adult animal, it is a slow-moving band We found no alkali-resistant hæmoglobin in these adults, hence the analogy with human hæmoglobin may not hold The samples taken from the two fœtuses were so small that only alkali-denaturation studies and electrophoresis on starch-gels were possible

Table 1. HÆMOGLOBINS AND TRANSFERRINS

Animal	No. of hæmoglobins	Transferrin type
1	4 (5)	2-2
2	4 (5)	2-3
3	3 (4)	2-3
4	3	2-2
5	4 (5)	2-2
6	3 (4)	2-3
7	2	2-3 (?)
8	2	2-3 (?)

Table 2. ALKALI-RESISTANT HÆMOGLOBIN

Animal	Age	Sex	Alkali-resistant hæmoglobin (per cent)
1	Adult	♀	0
2	Adult	♀	1 2
3	2 years	♀	0
4	4 years	♂	0
5	1 year	♀	0
6	14 days	♂	50 0
7	Fœtus	♂	85 7 (cord)
8	Fœtus	♀	78 3 (cord) 75 6 (aorta)

The nomenclature used for the transferrin types implies that the inheritance of these β-globulins is due to simple non-dominant alleles, as is the case for the human transferrins[5] The pedigree (Fig 1) bears this out, but samples from the missing animals are necessary to demonstrate this conclusively

Sufficient data[11,12] have been reported so that it is reasonable to predict that transferrin polymorphism exhibited by macaques may be due to the action of natural selection on different populations of these animals Unfortunately, it is not possible to work out the inheritance of many of these transferrins until various different phenotypes have been cross-bred The number of hæmoglobins may not be explained so simply Our experience with a large number of macaque samples from animals captured in three different localities (India, Malaya, and the Philippines) indicated that no more than two hæmoglobins or components existed The results presented here on a group of related animals demonstrate that multiple hæmoglobins exist and imply that these hæmoglobins are inherited We also have unpublished data indicating that multiple hæmoglobins among the Prosimian Primates is the rule

A final statement about the mode of inheritance of these hæmoglobins cannot be made until additional data become available This problem of multiple hæmoglobins in the non-human Primates is just coming under investigation Thus, we are only starting on an evolutionary and genetic problem of great fascination

This work was supported by grant *RG* 6053 of the U S Public Health Service and a grant from the Department of Anthropology, Yale University, We wish to thank Vina Buettner-Janusch for determining ultra-violet spectra of several samples in a Cary spectrophotometer, model 14 The animals came from the Yale Obstetrics Monkey Colony

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EFFECT OF DIETARY ASCORBIC ACID ON THE SILKWORM, *BOMBYX MORI*

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THERE are a few papers suggesting the importance of ascorbic acid in the nutrition of the silkworm, *Bombyx mori*. Gamo[1] showed that the content of ascorbic acid in fresh mulberry leaves changed in relation to conditions influencing the food value of leaves, and that the amount of ascorbic acid in the larval body is dependent on that in the leaves. According to Murthy[2], ascorbic acid was effective for increasing survival of larvæ. From these papers, however, it may still be impossible to draw a conclusion as to the precise role of ascorbic acid in silkworm nutrition. Ascorbic acid was added to the diet for pink bollworm[3], but most artificial diets developed for other phytophagous insects do not contain ascorbic acid. The failure to rear these insects on such deficient diets has not, so far, been mentioned. On the other hand, Thorsteinson[4] indicated the phagostimulant action of ascorbic acid for several phytophagous insects, and Dadd[5] showed that the inclusion of ascorbic acid in synthetic diet was necessary for normal development to the adult stage in two species of locusts. In locusts, however, ascorbic acid was proved to have negligible effect on the feeding, but was considered to play some part in the processes occurring at the moult. It has long been felt that the role of ascorbic acid in silkworm nutrition, as suggested by previous authors, must be confirmed. The results presented in this article are based largely on observations on feeding responses of silkworm larvæ given ascorbic acid and on its growth-promoting effect, when added to artificial diets for this insect.

For feeding tests an agar diet was used. Its composition was similar in principle to that previously used to test the effect of sugars on silkworm feeding[6]. The diet was composed of agar, starch, sucrose, soybean casein, mulberry leaf powder, and cellulose powder. Twenty ml. water was added to 2.5 gm. of the dry diets. Where necessary, 1 part of soybean oil was added to 12.5 parts of the dry diets, and 30 mgm. of ascorbic acid was contained in 20 ml. of water.

Table 1

	Larval distribution (per cent)
Disk without ascorbic acid	8.5 ± 1.5
Disk with ascorbic acid	83.0 ± 7.2
Filter paper	8.5 ± 7.9

(Five replicates)

Two techniques were used to test the effect of ascorbic acid on feeding behaviour: (1) a two-choice design in which newly hatched, unfed larvæ were allowed to choose the dietary media on which to feed, (previously used by Beck[7] for the European corn borer) and (2) counting the number of fæces produced by newly hatched larvæ. The agar media were cut into disks and placed side by side on the filter paper in a Petri dish. Approximately 25 larvæ were used for each dish and dishes were kept at 25° C. in the dark for a few hours until counts were made on larval distribution resulting from feeding movement. In Table 1 the result on larval distribution is presented. Most of the larvæ (83 per cent) settled on disks containing ascorbic acid, and the remaining small number of larvæ were either on disks without ascorbic acid or on the filter paper. When disks were placed some distance apart, the result was not necessarily clear cut. Later, the newly hatched larvæ, ten in a group, were allowed to feed on the agar diets for 18 hr. at 25° C. At the end of that time, the larvæ were removed from the diet and, with the aid of a magnifying glass, counts were made of the number of fæces. As shown in Table 2, ascorbic acid had a high stimulative action on feeding, irrespective of the presence or absence of added soybean oil, with a high statistical significance. Further, a slight phagostimulant effect of either soybean oil[8,9] or plant sterols[9] was overshadowed by the strong phagostimulant action of ascorbic acid.

Table 2

Ascorbic acid	Soybean oil	Fæces count per larva
−	−	4.3 ± 2.0
+	−	28.3 ± 4.8
−	+	9.4 ± 1.7
+	+	30.2 ± 4.1

(Four replicates)

Since it became evident that ascorbic acid was a strong phagostimulant for the silkworm, an experiment was carried out using artificial diets to assess the effect of dietary ascorbic acid on growth. The diets used for this purpose were not agar-based ones, but culture media solidified with potato starch, as previously used for nutritional work on this insect[8–10]. The following materials were included for basal media: mulberry leaf powder (10 per cent on a basis of dry matter), potato starch, glucose, soybean casein, soybean oil, salt mixture, soybean sterols (or β-sitosterol), vitamin B mixture and cellulose powder. The amount of ascorbic acid to be added ranged from 0.2 to 40.0 mgm. per gm. dry diet. One set of results obtained is presented in Table 3, in which mean body-weight after a 20-day rearing period is shown. Even at a concentration of 0.2 mgm. ascorbic acid per gm. dry diet, good growth was obtained, and the growth was improved by increasing the amount of ascorbic acid in the diets. However, best growth and development were obtained with either 4.0 or 10.0 mgm. per gm. dry diet. Ascorbic acid was also very effective in decreasing the mortality at older larval stages.

Table 3

Ascorbic acid added (mgm./gm. dry diet)	Body-weight of individual after 20-day rearing (mean of 2 batches) (mgm.)
0	49.9
0.2	75.8
1.0	95.5
2.0	132.6
4.0	153.6
10.0	148.9
20.0	111.4
40.0	141.8

Thus ascorbic acid is necessary for better growth and development of the silkworm and should be added to artificial diets as an essential component, as in the locust[11]. The effects of ascorbic acid seem possibly to be due to its phagostimulant action. However, there is a possibility that the growth-promoting effect might be a combined one of those on feeding and nutrition. Further work is necessary on the physiology of ascorbic acid in the silkworm.

The details of this report will be published in the *Bulletin of the Sericultural Experiment Station*.

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VENTURICIDIN: A NEW ANTIFUNGAL ANTIBIOTIC OF POTENTIAL USE IN AGRICULTURE

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DURING 1957–58 we screened some 8,000 soil actinomycetes for ability to produce new antibiotics active against apple scab (*Venturia inaequalis*). Here we summarize work which terminated in the isolation and biological evaluation of a new antibiotic, 'venturicidin', now being investigated for use in agriculture.

Isolation from Soils

Actinomycetes were isolated by inoculating serial dilutions, from a suspension of soil in water, into agar media containing different carbon sources. After one week's incubation, the 'pin-head' colonies were transferred to potato-dextrose agar slopes in small cylindrical glass bottles.

The isolated strains were tested for antibiotic production in surface culture by removing 'plugs' from well-grown cultures on potato-dextrose agar and determining the inhibition zones produced when the agar 'plugs' were placed on microbiological assay plates seeded with homogenized mycelium of *V. inaequalis*. Strains producing inhibition zones were afterwards tested for antibiotic production in submerged culture on three different fermentation media by 'cup-plate' assay of the fermentation broth against *V. inaequalis*, on the 2nd, 3rd and 4th days after inoculation. Antibiotics giving zones of inhibition greater than 20 mm. diameter were again produced by fermentation, but this time on what had been found the best of the three submerged fermentation media; the antibiotic production was assayed by incorporating serial dilutions of homogenized broth into agar slopes and then inoculating the surface with an homogenized culture of *V. inaequalis*. Antibiotics that completely inhibited growth of *V. inaequalis* at broth dilutions in the range 1 in 80 to 1 in 1,280 were considered to be worth extraction.

The technique was to submit broths from completed fermentations to a number of different extraction procedures, with 'cup-plate' assay of extracts and spent broths. Similarly, adsorption or non-adsorption on ion-exchange resins was determined by 'cup-plate' assay of the resin filtrates and eluates. The results enabled a suitable procedure to be devised for preparing a crude extract of the antibiotic.

Fermentations with the selected strains were repeated under optimum conditions in submerged culture, but now in two 5-litre mechanically agitated and aerated laboratory fermenters[1], the bulked broth at the end of the fermentation was extracted by the selected method to give approximately 25 ml. of a highly concentrated solution in acetone. These 'first concentrates' were scrutinized in several ways (extraction pattern, ultra-violet absorption spectrum, chromatography and bio-autographs, microbiological 'spectrum' *in vitro*, phytotoxicity and antifungal foliage protection tests) so as to eliminate unwanted antibiotics (polyenes, antimycin complex, oligomycin complex and cycloheximide). It became clear, after considerable experience of this procedure, that an apparently new antibiotic, designated venturicidin, was being produced by several strains of streptomyces and that it was both non-phytotoxic and highly active against a wide range of plant pathogenic fungi.

In general, it was observed that strains producing venturicidin gave 'first concentrates' which completely inhibited *V. inaequalis* in tube assay down to dilutions of 1 in 100,000. Similarly, the 'first concentrates' usually gave inhibition zones against *Cercospora melonis* in 'cup-plate' assay down to dilutions of the order of 1 in 100,000. This plant pathogenic fungus being more suitable than *V. inaequalis* for use in assays of this type, it was selected as convenient for microbiological assay of solutions of venturicidin in 50 per cent acetone.

'First concentrates' containing venturicidin were obtained from several strains and gave good protection in greenhouse tests against *V. inaequalis* when applied at dilutions of 1 in 500 to 1 in 2,000 to the foliage of pot-grown apple root stocks (Malling/Merton 106 and Malling XVI). Similarly, excellent protection was given to barley seedlings against *Erysiphe graminis*. In no test did venturicidin show any signs of phytotoxicity.

Further extraction tests on fermentation broths accompanied by appropriate 'cup-plate' assays led to an improved technique for extraction, this was applied to the preparation in two 5-litre laboratory fermenters of an improved 'second concentrate' and then for provision of crystalline venturicidin from 225-litre stainless steel pilot-plant fermenters

The method of preparing crystalline venturicidin is summarized here, together with its main chemical and biological properties

Crystalline Venturicidin

Strains Venturicidin is produced by three distinct strains of *Streptomyces* having a general resemblance to *Streptomyces griseolus* and *S halstedii* and some affinity with *S xanthophaeus* They have been found in soils from many parts of England and also from New Zealand Typical members of these three strains have been deposited in the National Collection of Industrial Bacteria under Culture Nos N C I B 9198, N C I B 9199 and N C I B 9200

Fermentation Vegetative inoculum was grown in a 5-litre laboratory fermenter containing 'Sabouraud' and malt medium (2 per cent 'Oxoid' malt extract, 4 per cent glucose, 1 per cent 'Oxoid' peptone) and transferred at the 28th hr to a 225-litre pilot-plant fermenter containing the medium (3 per cent Rickard's oat-flour, 1 per cent light precipitated chalk, 0 75 per cent glucose). The fermentation temperature was 28° C and the broth was extracted after three days

Extraction. The broth was filtered at *p*H 6 and the filtrate discarded The mycelium was extracted at room temperature with acetone and the extract was freed from acetone by concentration under reduced pressure with addition of a little water, as necessary. The aqueous solution was extracted at neutrality with light petroleum (60–80°) to remove antifoam oil, and any venturicidin that had been inadvertently extracted was afterwards recoverd by re-extraction with 90 per cent methanol The aqueous methanol solution was freed from methanol under reduced pressure, and the aqueous residue was combined with the main aqueous solution before extraction with ethyl acetate The ethyl acetate extracts were dried over sodium sulphate and taken to dryness under reduced pressure to give an oily solid.

Crystallization A typical crystallization was carried out by the following procedure To 2 48 gm of crude oily solid, 25 ml of carbon tetrachloride were added The oil dissolved, but precipitation of semi-crystalline material occurred immediately, it was removed by filtration and washed with carbon tetrachloride to give 610 mgm of almost colourless solid This was dissolved in 7 ml of hot methanol, and 2 ml of water were added On cooling, 590 mgm of needle-shaped crystals (m p 128–132° C) separated

Alumina chromatography The procedure used in a typical purification was as follows A column was prepared with 15 gm of 'Woelm' alumina (neutral grade 1) 579 mgm of crude oily solid were added in 5 ml of chloroform Continued elution with chloroform eliminated 145 mgm of oily material, after washing the column with ethyl acetate, 95 mgm of a biologically active fraction were eluted with ethyl acetate containing 5 per cent methanol. This material was crystallized from methanol/water to give a solid (m p 135 5–137 5° C) and further purified by alternate re-crystallizations from ethyl acetate/light petroleum and methanol/water, to give crystalline material [m p 140–142° C (uncorrected), $(\alpha)_D$ = + 110° (*c* 1 per cent, chloroform)], containing only one biologically active substance, as shown by bio-autographs of chromatograms developed in several solvent systems.

Chemical properties Venturicidin is a neutral substance It is soluble in butanol, ethyl acetate or chloroform It is stable at room temperature at *p*H 7 It gives negative ferric chloride, ammoniacal silver nitrate and anthrone tests, but shows a red coloration with 2,4-dinitrophenylhydrazine

Prof B Lythgoe, of the University of Leeds, is now investigating the structure of venturicidin, and we are grateful to him for the following results

m p. 142–143° C , $[\alpha]_D$ = + 114° (*c* 1 per cent, chloroform)
Found C $\begin{matrix}64\ 7, & 65\ 1\\ 64\ 65, & 65\ 1\end{matrix}$, H $\begin{matrix}9\ 0, & 9\ 0,\\ 8\ 95, & 8\ 85\end{matrix}$, N 1 75, 1 75, 1 75,
O (Unterzaucher) 23 95, 23 5
Calculated for $C_{43}H_{71}O_{12}N$ C 65 0, H 9 0, N 1 75, O 24 2 per cent

Venturicidin can be conveniently prepared as a 10 per cent water-miscible solution in 'ethyl cellosolve' with addition of a suitable surface-active agent, on dilution with water this provides solutions of venturicidin, at concentrations between 200 and 25 μgm /ml , suitable for application to plant foliage

Biological activity of venturicidin in vitro Crystalline venturicidin is antifungal but not antibacterial, though when crude it is sometimes associated with antibacterial material Minimum concentrations in 50 per cent acetone producing inhibition zones in 'cup-plate' assays are given in Table 1.

Table 1 MINIMUM CONCENTRATIONS OF VENTURICIDIN NEEDED TO GIVE INHIBITION ZONES IN 'CUP-PLATE' ASSAYS AGAINST PLANT PATHOGENIC FUNGI

Fungus	Minimum inhibitory concentration (μgm /ml)
Ascochyta pisi	40 0
Aspergillus niger	5 0
Alternaria tenuis	5 0
Botrytis cinerea	2 5
Cercospora melonis	1 25
Didymella lycopersici	2 5
Fusarium nivale	2 5
Glomerella cingulata	10 0
Helminthosporium avenae	2 5
Phoma betae	5 0
Pythium de Baryanum	5 0
Penicillium expansum	10 0
Thielaviopsis basicola	20 0
Trichothecium roseum	5 0
Ustilago maydis	5 0
Verticillium albo-atrum	2 5

Tube-dilution assay (antibiotic incorporated in agar and test fungus inoculated to the surface) gave the results as follows

Venturia inaequalis complete inhibition at 1 μgm /ml and partial inhibition to 0 1 μgm /ml
Sclerotinia fructigena complete inhibition at 0 3 μgm /ml. and partial inhibition to 0 01 μgm /ml
Botrytis cinerea complete inhibition at 0 3 μgm /ml
Glomerella cingulata, *Cercospora melonis*, *Fusarium nivale* } partial inhibition to 0 3 μgm /ml

Venturicidin also exhibited exceptionally high activity against *Nocardia rubra* (complete inhibition by 0 08 μgm /ml after 7 days in liquid tube dilution assay) and markedly inhibited *Aspergillus fumigatus* and *Blastomyces dermatitidis* at concentrations between 20 and 1 25 μgm /ml

Greenhouse protection tests with venturicidin against plant pathogenic fungi Crystalline venturicidin gave excellent protection against a number of plant pathogenic fungi when used as a prophylactic foliage spray in greenhouse tests For example, at LD_{90-95}, 20 μgm /ml venturicidin was approximately as effective as 200 μgm /ml Captan against apple scab *V inaequalis*) and was about as good as 200 μgm /ml

Karathane against barley mildew (*E graminis*) Venturicidin is also exceptionally active against broad bean rust (Hunnam, D , and Smith, C B F , private communication) and has some activity at concentrations of 20–40 µgm /ml against cucumber mildew (*Erysiphe cichoracearum*), apple mildew (*Podosphaera leucotricha*) and grey mould (*B cinerea*) on tomatoes and vines Venturicidin showed local systemic action in apple foliage and some eradicant action against *V inaequalis*

Pharmacology of venturicidin Preliminary results on mice and dogs show venturicidin to be non-toxic orally, besides having low intraperitoneal toxicity and relatively low intravenous toxicity (Tomich, E G , private communication)

It is concluded that venturicidin is a new antibiotic with considerable potential use in agriculture It is now undergoing commercial evaluation as a fungicide against apple scab (*V inaequalis*)

We thank our colleagues, Mr W K Anslow and Mr D Fletcher, who were responsible for the 5-litre and pilot-plant fermentations

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STRUCTURE OF THE PLANT STEM IN RELATION TO ADVENTITIOUS ROOTING

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THE ability of one-year-old stems to form adventitious roots has been found, in a range of fruit plants, to be closely related to the anatomical structure of the primary phloem of the stem Shoots of shy-rooting varieties are frequently characterized, in this tissue, by a high degree of sclerification, that is, differentiation into fibres and sclereids In Conference pear, for example, an almost continuous cylinder of mature thick-walled fibres without living protoplasts encircles the secondary phloem and appears, in transverse section, as a ring of lignified tissue (Fig 1) In ready-rooting plants it is usual to find that most of the primary phloem parenchyma cells retain living protoplasts even when they are in a stage of transition to sclerotic parenchyma, or sclereids It follows, therefore, that in stems possessing a high rooting propensity many of the phloem rays abut, at their distal ends, on areas of living tissue (Fig 2) in contrast to the shy-rooters, where the phloem rays are frequently blocked by heavily lignified elements (Fig 1) The degree of continuity of the sclerenchymatous ring is compared, in Table 1, with ease of propagation as shown by the behaviour of stooled plants reported by Garner[1] As the propagation capacity falls, the continuity of the ring increases

A similar general relationship between the degree of sclerification of the primary phloem and rooting ability has been observed among rootstocks for stone fruits, and in a range of flowering shrubs from temperate regions Shoots of certain shy-rooting tropical plants, including *Hevea brasiliensis* sent from Malaya and *Acacia mearnsii* sent from Natal, have been found to possess, at the base of one-year-old stems, an almost unbroken cylinder of mature lignified elements, similar in continuity to that of the shy-rooting Conference pear, but differing in composition as regards type of element Working with stem cuttings of the olive in Italy, Ciampi and Gellini[2] noted a similar connexion between the absence of a sclerenchymatous ring and rooting potential Thus a relationship between structure and rooting capacity would appear to be applicable to a wide range of species That the presence of periphloic sclerenchyma is not the sole cause of the prevention of easy rooting is, however, shown by the following examples species of *Pittosporum* lacking such sclerenchyma are, nevertheless, difficult to root, as are also certain *Erica* spp where a phellogen is laid down on the inner side of the periphloic sclerenchyma (C R Metcalfe, private communication)

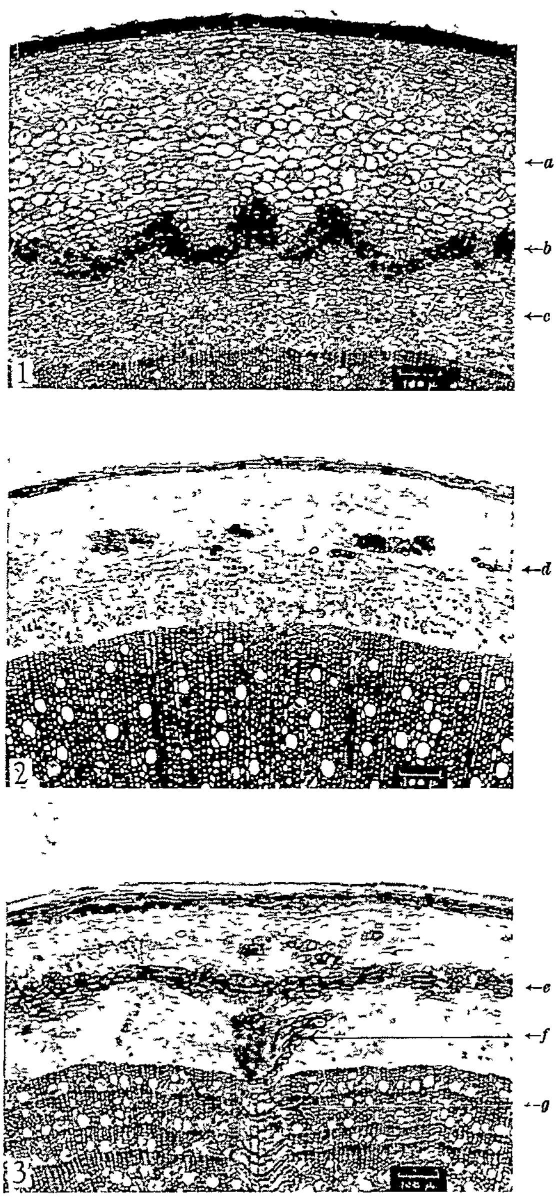
Inherent differences between clones in the degree of sclerification of the primary phloem have been found to be relatively stable in material grown under comparable conditions at East Malling Environmental effects may, however, be superimposed on the inherent structure, differentiation of the primary phloem parenchyma cells into fibres and sclereids being retarded, for example, by shading, and by partial etiolation as in the earthing up of stool shoots, or marcotting of aerial stems

Comparisons between shoots exhibiting juvenile characteristics and mature shoots, both derived from the same plant, have indicated that, in seedling apples, the basal juvenile shoots are relatively free from sclerotic cells in the primary phloem compared with lateral shoots from fruiting branches close to the apex of the maturing tree These observations provide indirect evidence of a relationship between

Table 1 RELATION BETWEEN PROPAGATION CAPACITY AND PRIMARY PHLOEM STRUCTURE IN STEMS

Variety	Propagation capacity	Continuity of fibre ring* Per cent	Continuity of fibre ring* Mean
Apple rootstocks			
M XIII	Excellent	28	41
M V		43	
M XI		52	
M.IV	Good	59	64
M XVI		64	
M II		69	
M XII	Fair	69	75
M XXV		71	
M VIII		85	
Crab C	Poor	82	84
Northern Spy		84	
Ivory's Double Vigour		86	
Scion varieties			
Apple			
Bramley's Seedling	Poor	86	80
Cox's Orange Pippin		83	
Lane's Prince Albert		70	
Pear			
Conference	Poor	99	98
Williams's Bon Chrétien		96	

* Proportion of radii through the primary phloem blocked by sclerenchyma



Figs 1-3 Structure of primary phloem (1) Conference pear, (2) Malling V apple rootstock, (3) *Fagus sylvatica* (beech) *a*, cortex, *b*, sclerenchymatous sheath, *c*, phloem, *d*, primary phloem fibres, *e* sclerenchymatous sheath, *f*, sclerosed phloem ray, *g*, multiseriate xylem ray

anatomy and rooting potential since loss of rooting ability over the period of change from juvenility to maturity, first reported in 1929 by Gardner[3], is now well established for a number of plants, including the apple Ready-rooting sphaeroblast shoots, artificially induced by continuous removal of normal bud meristems[4], have also been found to have fewer fibres and sclereids in the primary phloem than shy-rooting normal shoots from the same apple clone

Sphaeroblasts are small woody inclusions occasionally found in the bark of many tree species These nodules, which are separated from the central vascular cylinder, possess a cambium and may continue to grow over a period of years, forming annual rings and eventually protruding conspicuously from the trunk Following certain stimuli, including severe pruning accompanied by the removal of all lateral buds and of any subsequent shoots from dormant buds, young sphaeroblasts may grow out producing either rosettes of leaves or long shoots The work of Baldini and Mosse[7] on the inception of sphaeroblast primordia in apple has shown that sphaeroblast shoots arise adventitiously from a massed meristem originating in a primary tissue, thus providing anatomical evidence of juvenility, supporting the observations of Stoutemyer[5] and Wellensiek[6] that adventitious shoots from sphaeroblasts exhibit juvenile morphological characters If sphaeroblast shoots are in a more juvenile phase than those arising normally from a cambial meristem, the differences in anatomy between them provide a further example of the increasing sclerification of the primary phloem with advancing maturity of the tissue and they are in line with the suggestion made by Foster[8] that sclerification is an aspect of tissue senescence

It is obvious that if a correlation exists between the degree of sclerification of the primary phloem and rooting capacity, the rooting of difficult subjects might be facilitated by using very young shoots taken before the cells of the primary phloem lose their living protoplasts This method is, of course, exploited in propagation of softwood cuttings under mist where rapidly growing shoots are used At this stage of development, wall thickening may have begun in primary phloem cells destined to become fibres, or sclereids, but lignification is usually absent, or slight, and the cells retain a living protoplast in all except the most shy-rooting kinds In plants that are exceptionally difficult to root, even from softwood cuttings as, for example, beech, the onset of tissue senescence in the phloem appears to be very rapid, and, in addition to the formation of a sclerenchymatous cylinder in the primary phloem, the broad multiseriate secondary phloem rays may become lignified in quite young tissue close to the cambium (Fig 3)

The simplest explanation of the effect of a sheath of lignified tissue on rooting capacity would be a mechanical one, but this is probably not the sole—or even the major—cause of failure to root since, in many plants, root initials do not form at all readily within such a sheath Some alternative hypotheses are at present being investigated

Quite apart, however, from speculations on the mechanisms involved, it would appear that the observed relationship between anatomy and ease of rooting may have a practical application and that, within certain limits, it may be possible to forecast rooting capacity from the structure of young stems

Thanks are due to Dr C R Metcalfe, keeper of the Jodrell Laboratory, Royal Botanic Gardens, Kew, for having suggested this investigation, and to Mr R J Garner for providing much of the material and giving information on the rooting of a number of clones

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LETTERS TO THE EDITORS

GEOPHYSICS

A Morning Discontinuity in the Orientation of Quiet Auroral Arcs

From all-sky camera recordings taken at this Observatory (geographic co-ordinates 67·8° N., 20·4° E., geomagnetic co-ordinates 65·3° N., 115·5° E.) during the period December 1958 – March 1961 the directions of 366 quiet auroral arcs were measured. Only arcs extending from horizon to horizon, or at least covering an angle of view of about 100° for the most distant arcs and having an elevation angle of more than 15° for the highest point, were used in the analysis.

With the assumption of a constant height along the lower limit of the luminosity—for which there is good evidence (cf. Störmer[1])—the angle between a straight line connecting the points at which the arc intersects the horizon and a line through the midpoint of the chord, parallel to the meridian through Kiruna, was measured. Although this angle may differ slightly from that between the projection of the arc on Earth's surface and the local meridian through the midpoint of the arc—as measured by most investigators before the advent of all-sky cameras—the difference is estimated at less than one degree in the actual case. Since this difference is less than, or at the most equal to, the error introduced in measuring the angle of the chord, it has no appreciable influence on the results obtained. To the types of uncertainties mentioned should be added the possible error introduced in extrapolating some of the arcs to the horizon. Since this is estimated at 1–3°, the total error in the obtained direction of an arc will certainly not exceed 4°.

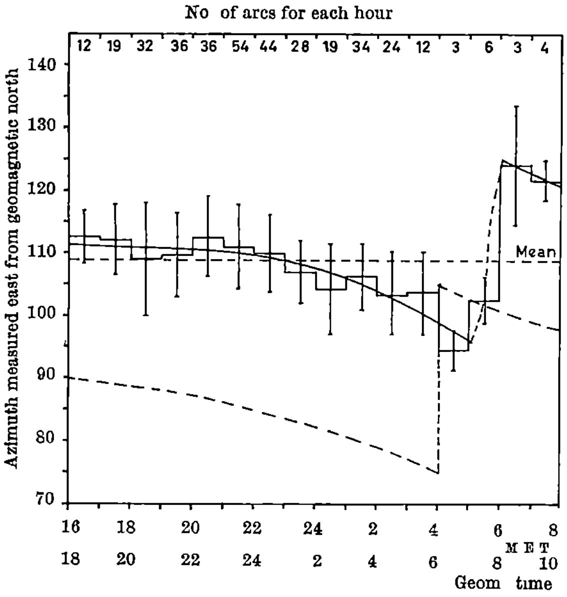

Fig. 1. Observed directions of quiet auroral arcs at Kiruna as a function of M.E.T. and of approximate geomagnetic time. The average azimuth and the standard deviation are given for each hour. The lower, dashed curve is Alfvén's theoretical diurnal variation curve

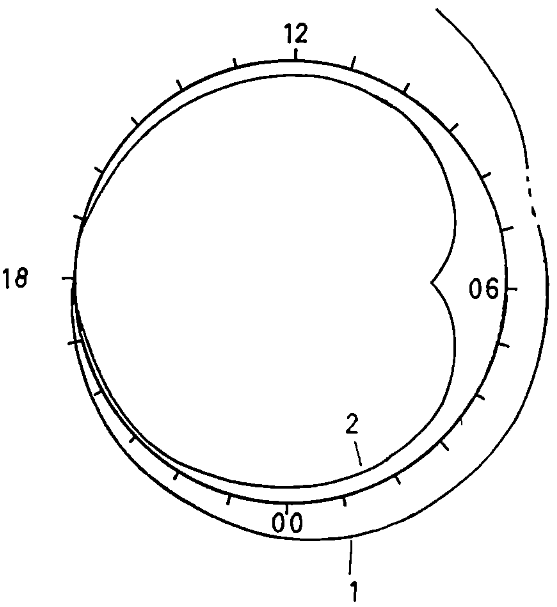

Fig. 2. Curve 1 is the auroral curve on Earth corresponding to Alfvén's I-curve, plotted from the diurnal variation in the orientation of quiet auroral arcs observed at Kiruna. It is corrected for the non-dipole part of the geomagnetic field. Curve 2 is Alfvén's theoretical I-curve

The result of the alignment investigation is shown in Fig. 1. For each hour of M.E.T. the average direction and the standard deviation were computed. In so doing, the direction value of each arc was weighted with the duration of the arc. The total average for the whole material was 109° and a typical standard deviation was 6°.

At the top of Fig. 1 the number is given of arcs used for each hour. The number has a maximum around geomagnetic midnight. In the morning hours the aurora as a rule is not suitable for direction determination, being usually diffuse and situated far to the north. It is not surprising, therefore, that the observational results are least for those hours. The morning arcs for which the direction was measurable were all of short duration.

Although the observational material is not extensive for the interval 0400–0800 M.E.T., it allows the conclusion that the very rapid increase of the azimuth occurring between 0500 and 0600 M.E.T. is significant.

The azimuth change in the morning is so rapid as to resemble a discontinuity of the type predicted by the Alfvén theory[2]. Such a marked 'discontinuity' has not, to our knowledge, been observed before. Lassen[3] observed a rapid change in direction at Godhavn at approximately the same local time, but the amplitude of the change was less (about 20° as compared with 30° in Kiruna).

In point of fact it should be possible to observe the discontinuity only in those longitudes in which geomagnetic time runs ahead of local time, since daylight will preclude auroral observation until 8 or 10 o'clock (geomagnetic time) in other longitudes.

From the smoothed diurnal variation curve of Fig. 1 the auroral occurrence curve—corrected for effects of the non-dipole part of the geomagnetic field—corresponding to the 'ideal auroral curve' (the I-curve) of Alfvén[2] was plotted and is shown in Fig. 2 together with Alfvén's theoretical I-curve. The entire experimental curve is situated outside the circle, with which it coincides at 1800, in contrast to the theoretical curve, which is inside the circle. The difference is attributable to the fact that the azimuth values of the experimental curve are 7–9° greater than the values of the theoretical curve even after correction for the effects of the non-dipole components of the geomagnetic field. Hence, the theoretical curve is situated outside the standard deviation 'region' of the observational material. The difference, therefore, is probably a real one, which is not surprising since the theoretical curve is based on a highly idealized model.

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ASTROPHYSICS

Lunar Dust and the Gegenschein

OF the four possible explanations of the gegenschein which have been proposed, not one so far has explained satisfactorily the meagre observational data concerning this phenomenon. The Glyden–Moulton hypothesis[1], which suggests a concentration of meteoric material at a libration point of the Sun–Earth system, seems incapable of explaining all the luminosity of the gegenschein. The zodiacal light hypothesis[2], which claims that the glow opposite the Sun results from a phase-function for the interplanetary dust layer which produces such a brightening, fails to explain the fact that the gegenschein usually lies approximately 3° west of the anti-solar point[3]. The gaseous tail hypothesis[4,5], in explaining the light as the result of excitation of Earth-escaped gases by the interplanetary plasma, does not account for the fact that the colour of the gegenschein is slightly redder than that of the Sun[6]. Finally, the dust tail hypothesis[7], although explaining the facts in a qualitative way, at least, requires a large and continuous source of dust particles in Earth's neighbourhood.

Before the satellite measures of interplanetary dust concentration became available, there was little reason to suggest such a source of dust in the immediate vicinity of Earth. However, the remarkable fact that Earth is surrounded by a concentrated dust layer[8] seems to be explained in no other way. Whipple has found that the Moon might be the source of Earth's dust layer, for under certain conditions, the velocities of fragments thrown up from the lunar surface as a result of meteorite impact will exceed the lunar escape velocity. Many of these particles will be captured in quasi-stable orbits about Earth, forming just the sort of layer of dust found by the satellite experiments. Smaller particles will be blown out by radiation pressure before they can orbit Earth.

It seems reasonable to suggest that this localized source of dust in the immediate vicinity of Earth is sufficient to maintain a dust tail for Earth acted on probably by radiation pressure analogously with type II comet tails. If such is the case, then Earth has a dust tail as well as a gas tail[9], and we would be in the position of looking down the axis of the tail. To test such a hypothesis it will be necessary to work out theoretically the mechanism of supply of the dust layer and of its depletion. It will also be helpful to have further observations of the gegenschein to confirm its colour, spectrum, location, surface brightness, and variations in these quantities. Especially interesting would be systematic studies of the motions of the gegenschein and any relations these might have with other events. Measures of the parallax of the gegenschein should be attempted from an orbiting vehicle.

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PHYSICS

Mode Characteristics and Coherence in Optical Ruby Masers

AN optical maser, using a good-quality ruby crystal with plane-parallel reflectors, oscillates in modes corresponding to Fabry–Perot resonances. Oscillations are shown to occur at several different wave-lengths corresponding to various numbers of half-wave-lengths between reflecting ends. For each wave-length, there are many angular modes which are coupled so that they oscillate coherently. This implies that a set of angular modes may be considered a single modified mode and that light from the entire set may be focused to a point of minimum size allowed by the wave-length of the light.

There have already been various discussions of the modes in an optical cavity[1–4] and experimental demonstration of a ring structure[5] in light emitted by ruby masers. The photograph shown in Fig. 1 was taken by passing a pulse from a ruby maser through a 410-mm lens, with the photographic plate in the focal plane (far-field pattern). The series of rings observed corresponds within the accuracy of measurement with the pattern of a Fabry–Perot interferometer. Separations between successive sets of rings follow the interferometer equation

$$D_n^2 - D_{n-1}^2 = \frac{4f^2\lambda\mu}{L}$$

where D_n is the diameter of the n^{th} ring on the film, f the focal length of the lens, λ the wave-length, μ the refractive index, and L the geometrical length of the ruby. The value of L obtained from this expression, on substitution of other quantities and measured values of D_n, agrees with the measured length of the crystal to within a few per cent. This was found to be the case for crystal samples of differing lengths, a fact which rules out interpretations of the rings on the basis of diffraction by some unidentified aperture

The ring pattern of Fig. 1 was allowed to fall on two 100μ apertures separated by 2 mm which accepted light from different rings or different portions of the same ring, corresponding to different angular directions of propagation within the ruby interferometer. Interference patterns characteristic of coherent sources resulted (Fig. 2), which reproduce the pattern found at a distance of 2·5 ft with no lens interposed. This implies that various angular modes are of precisely the same frequency and coherent in phase. They must therefore be effectively coupled. Scattering from lattice imperfections is known to be prominent, and can be expected to couple the various modes as a result of transfer of light between them.

One must not, however, assume that only one frequency is present in the oscillation. The photograph in Fig. 3 was taken by interposing between the ruby and camera an external Fabry–Perot interferometer of about 1-cm spacing. The ring structure of Fig. 1 is seen in the background of the various orders of the interferometer. The 'fine structure' components shown were measured and the wave-number difference of adjacent components was about 0·07 cm^{-1} which, for our crystal, is equal within about 10 per cent to the separation of longitudinal modes $\Delta\nu = 1/2\mu L$. Such separations have previously been measured directly as beats in the helium–neon and ruby systems[6,7].

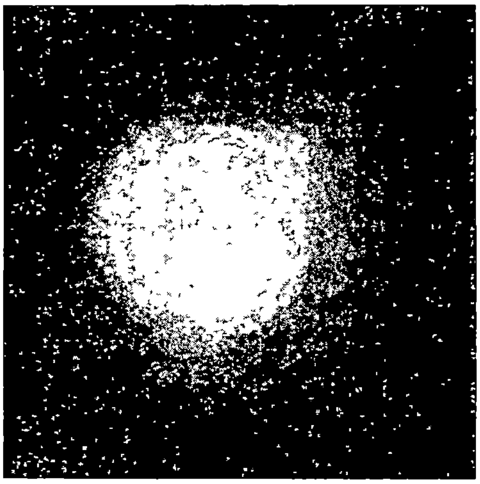

Fig. 1. Ring pattern formed by light beam from ruby maser at the focal plane of a lens. Spacing of rings corresponds to angular modes in ruby rod for a fixed frequency

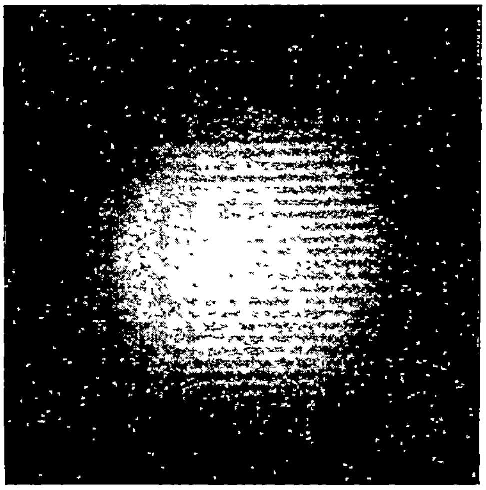

Fig. 2. Interference pattern produced by light from two small apertures transmitting beams from different parts of the ring pattern of Fig. 1

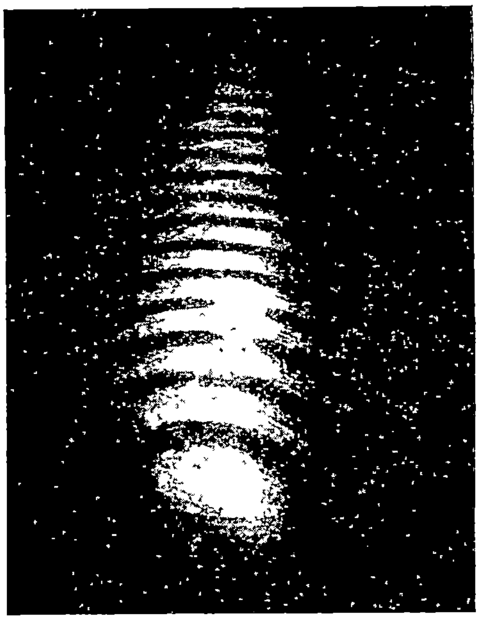

Fig. 3. Rings produced by beam from ruby maser after passage through a Fabry–Perot interferometer, showing resolution of longitudinal modes. The less obvious superimposed series of rings is the same shown in Fig. 1, which occurs without passage through a Fabry–Perot interferometer

The several frequencies shown in the pattern of Fig. 3 are, of course, all within the ruby emission line, and are so close together that their interference patterns can coincide in Fig. 2. Hence the emission of the ruby maser is composed of several different frequencies with separations determined by those of the longitudinal modes. Each of these frequencies is emitted coherently in a series of coupled angular modes. The large number of angular modes spreads the emitted energy into a beam the width of which in solid angle exceeds that of the diffraction pattern of the principal mode by as much as several orders of magnitude[8]. We mean by a principal mode one in which the wave propagation is essentially perpendicular to the reflecting ends, and hence which can be expected to have the lowest losses[1-4]. It should be noted that oscillations under many different conditions always showed a maximum in the direction of the principal modes. Presumably, it is the lower losses of these modes, and their saturation of adjacent frequency regions in the ruby emission during oscillation, which produce just those frequencies that correspond closely to several adjacent principal modes in the cases examined. One must expect under some conditions to find other combinations of frequencies.

It is interesting to note that the coherence observed between the angular modes, and the coupling which must exist between them, implies that the set of angular modes may be regarded as a single modified mode, in which the maser is oscillating. This means, further, that it should be possible to concentrate the energy at a point or in a beam as small as that allowed by coherent oscillations in any other form of single mode. Such a possibility can be of importance in concentrating large amounts of power from solid-state masers into small spots or highly directed beams For example, one may note that the successive rings in the focal plane of the lens may be all made to arrive in phase at any arbitrary point on the axis by interposing a suitable phase-shifting plate at each ring The light would then be focused as by a zone plate, and a suitable following lens could produce a beam of width limited primarily by the aperture of the lens

The phase coherence of the various angular modes implies also the existence of rings in the near field due to interference of the beams from these modes, which may be considered as a set of coherent waves travelling at various angles with respect to the axis Some rings have been observed in the near field by us and by others (Collins, R J , private communication), but they have not been carefully compared with expected theoretical behaviour

This work was supported in part by a contract with the U S Air Force monitored by the Air Force Office of Scientific Research, and in part by a Joint Services contract with the U S Army Signal Corps, the Office of Naval Research, and the Air Force Office of Scientific Research

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de Haas–van Alphen Effect in InBi

THE de Haas–van Alphen effect (oscillatory dependence of magnetic susceptibility on magnetic field strength at low temperatures) has provided one of the most successful methods for studying the Fermi surfaces of pure metals at low temperatures Recently the utility of this technique has been extended to semiconducting compounds with the observation of the de Haas–van Alphen effect in degenerate *p*-type PbTe (ref 1) In this communication we report what we believe to be the first observation of de Haas–van Alphen oscillations in a metallic compound, InBi (ref 2)

The single crystal of InBi used in the present investigations was obtained by mixing stoichiometric quantities of indium and bismuth in a 'Pyrex' glass tube under vacuum The molten compound was then forced under helium pressure into a tapered 'Pyrex' glass capillary which was allowed to cool slowly in a temperature gradient A cylindrical sample of length 13 mm and diameter 0 9 mm was cut from the capillary with a sandblaster, and the glass casing was dissolved with hydrofluoric acid An X-ray analysis showed the direction of the sample axis to be 33° ± 2° from a $<100>$ axis in the basal plane of the tetragonal crystal The pulsed field apparatus used in this work has been described briefly in previous communications[3,4]

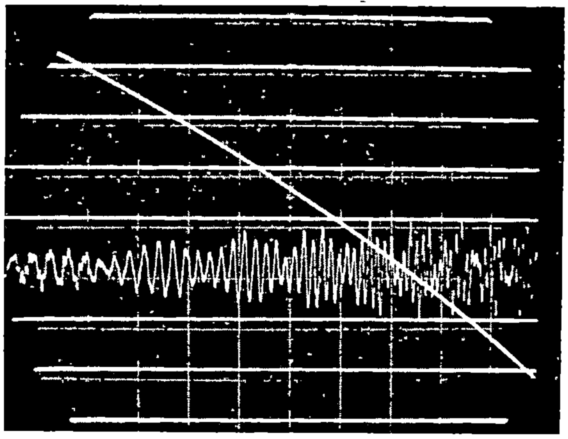

Fig 1 de Haas–van Alphen effect in InBi at 1 11° K The oscillating trace shows the output from a pick-up coil containing the sample The curved trace shows the magnetic field increasing from 80 1 to 130 6 k gauss during a sweep time of 1 8 msec (time increasing from right to left) The beating effect is due to two oscillating terms of different period and amplitude

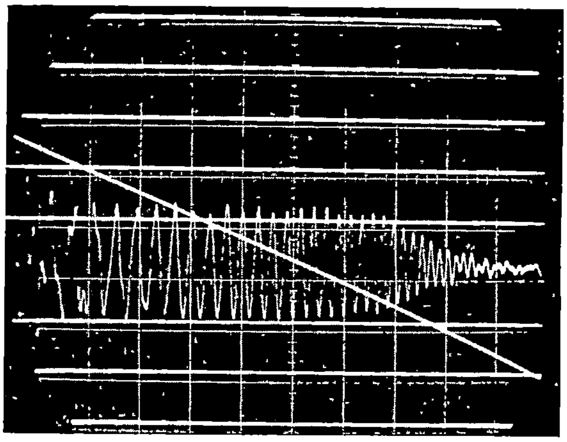

Fig 2 Long period de Haas–van Alphen oscillations in InBi at 1 11° K The diagonal trace shows the field increasing from 36 2 to 70 0 k gauss during a time of sweep of 0 9 msec The change in interval occupied by each successive oscillation is a consequence of the decrease of the time derivative of the magnetic field and the periodicity of the oscillations in H^{-1}

With the sample mounted with its axis parallel to the magnetic field (within ± 2°) we observed three oscillating susceptibility terms which are pictured in Figs 1 and 2 The pertinent de Haas–van Alphen parameters for these three periods are given in Table 1 The extremal cross-sectional areas, A, of the Fermi surface were calculated from the periods, P, by the relation $A = 4\pi^2 e/chP$ The effective mass for each period was determined from the temperature-dependence of the amplitude of the oscillations[5]

It is apparent that the ranges of de Haas–van Alphen parameters in InBi are similar to those characteristics

Table 1 PERIODS, CORRESPONDING FERMI SURFACE EXTREMAL CROSS-SECTIONAL AREAS, AND EFFECTIVE MASSES FOR DE HAAS–VAN ALPHEN OSCILLATIONS IN INBI (ACCURACY OF PERIODS IS APPROXIMATELY 5 PER CENT)

Period (10^{-8} gauss^{-1})	Area (10^{15} cm^{-2})	m^*/m_0
8 43	1 13	0 58 ± 0 1
6 33	1 51	0 64 ± 0 1
25 4	0 38	0 21 ± 0 05

of pure polyvalent metals[5]. The application to InBi of the nearly free electron model[6,7], which has met with some success in understanding pure metals, will be attempted when more data are available. de Haas–van Alphen measurements should be possible on a wide variety of metallic compounds, thus providing insight into the nature of electronic binding and structure in such materials.

This research was supported by the U.S. Atomic Energy Commission.

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A Method of Calibrating Two-Sphere Non-directional Radiometers

When two heated spheres, one black and one bright, but otherwise identical, are kept at the same temperature, they lose an equal amount of heat by conduction and convection[1]. Any difference in heat input is due to the difference in radiational exchange. Neither air temperature nor air velocity enters the calculations for determining the mean radiant temperature of the surroundings. The quantities needed are: the heat input of the two spheres; the temperatures of the two spheres (which should be equal); the surface area; the emissivities of the bright and the black sphere. Nickel[1] or gold[2] is used for plating the bright sphere.

Attempts have been made to predict the accuracy of the two-sphere radiometer from the above quantities. Roose[1] reasoned that, as the heat input can be measured with an accuracy of ± 0·5 per cent, the error in mean radiant temperature would be ± 0·36° F. Sutton and McNall[2] anticipated errors not exceeding ± 2° F.

The method now proposed avoids the need to measure surface areas and emissivities. Advantage is taken of times and circumstances when the readings of a globe thermometer[3] and of air temperature happen to be equal. This is so, for example, out of doors in the evening during the transition from solar heat gain to radiational loss; or it may be so indoors under certain conditions. In such circumstances, it is clear that there is then, for the time being, a state of thermal equilibrium in which globe temperature and air temperature are both equal also to the mean ambient radiant temperature, so that the latter is known. Thus, if the two-sphere radiometer is operated at such a time, the answer which it should give is known, and only the heat inputs to the spheres need be measured, in addition, in order to enable its calibration constant to be found.

Using the above method it was found that the average error when measuring mean radiant temperatures with the Sutton–McNall[2] instrument was 0·4 deg. F.; the highest deviation encountered was 1 deg. F.

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BIOPHYSICS

Montmorillonite as a Caliper for the Size of Protein Molecules

It is known that proteins may be adsorbed from solution by the clay montmorillonite, leading to an increase in $d(001)$ crystallographic spacings[1]. In order to compare these increases with the size of protein molecules one must be certain that a complete mono-molecular layer of protein has been adsorbed, and this can be calculated to require 1·3–2·0 gm. of protein per gm. of clay. As the clay becomes saturated the adsorption isotherm levels off, but the isotherms are initially steeper than curves of the Langmuir type[2]. Assuming that protein molecules are spherical, we have calculated the expected $d(001)$ spacings and compared them with the experimental values found (Table 1). The agreement is moderately good for all but pepsin. Hæmoglobin appears to be adsorbed as a monomer, whereas lactoglobulin enters the clay particles as a dimer.

Table 1. Adsorption of Proteins by Montmorillonite

Protein	Mol. wt.	Adsorption (gm./gm.) Theoretical	Adsorption (gm./gm.) Experimental	Calculated mol. diam., Å	$d(001)$, Å Theoretical	$d(001)$, Å Experimental
Lysozyme						
(native)	14,700	1·3	1·3	32·6	42·2	44
(denatured)	14,700	—	1·3	—	—	46
Chymo-trypsinogen	22,500	1·5	1·4	37·2	46·8	40
Lacto-	35,000	1·7	1·5	43·8	53·4	54
globulin	(17,500)*			(34·8)	(44·4)	—
Pepsin	35,000	1·7	1·0	43·8	53	18
Hæmoglobin	63,000	2·0	1·9	53·2	62·8	53
(human)	(40,000)*			(45·6)	(55·2)	—

* These proteins dissociate under extreme conditions of dilution and/or salt concentration to give the values in parentheses (ref. 3).

Our result with native pepsin agrees with that of Talibudeen[4], namely, that the $d(001)$ spacing is about 18 Å for a wide range of amounts of protein adsorbed; it indicates an unfolding of pepsin molecules into extended polypeptide chains within the clay particles.

It has often been suggested that during heat denaturation of proteins there is a tendency for protein molecules to undergo unfolding or expansion. We have found that the $d(001)$ spacings of montmorillonite–lysozyme complexes are the same for adsorbed, heat-inactivated lysozyme as for native lysozyme, from which it may be concluded that lysozyme does not change drastically in shape on undergoing denaturation in solution.

Tobacco mosaic virus (isoelectric point 3·5) was allowed to react with sodium (Wyoming) bentonite at pH 3·2 (citrate buffer, ionic strength 0·0375). According to spectrophotometric analysis, 1,830 mgm. of virus was adsorbed per gm. of clay. At this value, about 15 per cent of the internal surface area of the clay could be covered with the virus However, X-ray data indicated that internal adsorption had not taken place

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HYDROGRAPHY

Concentration of Chloride in Fresh Waters and Rain Water

A study of analyses of the waters of a number of freshwater lochs in the north-west of Scotland, sampled in 1955, showed that the chloride concentrations (Cl) varied with the distance (D) of the loch from the coast, the most suitable equation relating these parameters being of the form $Cl = K D^{-n}$, where K and n are constants This form of equation was used by Gorham[1] to describe similar data obtained from tarns in the English Lake District A second series of lochs in north-western Scotland, sampled in 1960, and an earlier series of samples from lochs on the island of Rhum, western Scotland, taken in 1958, have also provided data fitting the general equation adequately

For the Scottish lochs, which are situated near a very irregular coastline, the parameter D is estimated in a south-westerly direction (that of prevailing strong winds) to the coast exposed to open sea. Despite the necessarily imprecise measurement, it has been found that the coefficients of correlation r for the regression of chloride concentration on D, after logarithmic transformation, are close to unity Furthermore the various values obtained for the constant n are in close agreement The values of the proportionality constant K differ to some extent, but are nevertheless of a similar order The results are summarized in Table 1, in which other relevant information is included

The samples of loch water in each series were all taken within short periods of one month or less, in areas where chloride derived from terrestrial sources is insignificant All chloride is consequently derived from cyclic salts dissolved in rain water, or directly from sea-spray droplets The analyses of loch water can thus be considered as the integrated values of total precipitation (including sea-spray) draining from the catchment areas of the lochs, the period of accumulation being virtually the same for all lochs in any one group

In work of mine on the rainfall of the island of Rhum it has been shown that the sodium content of the total precipitation at one sampling station (Kinloch) was linearly related to the maximum gust velocity of the wind in the area during the appropriate sampling period, for velocities above a threshold of about 30 knots The chloride/sodium ratio varied little from that of sea-water It seems possible that the proportionality constant K depends at least partly on the wind velocity, the prevalence of high wind velocities in a particular area over a definite period of time giving high values of K

In addition to the four series of data from freshwater lochs, constants have also been calculated from two series of rainfall analyses taken from the references quoted and are included in Table 1 The values of n for these two series are similar to those calculated for the lochs They are derived from mean values of chloride concentrations in precipitation sampled regularly over long periods, and such mean values can be considered as analogous to the loch water analyses

The mean value of the constant n, for the six series of data in Table 1, weighted according to the number of samples, is 0 328, and the equation thus approximates closely to the form $Cl = K/\sqrt[3]{D}$ The correlation coefficient is high in all six series ($P < 0\ 001$), and the relationship appears to be valid for distances ranging from 0 1 to 80 km , and probably much longer It is suggested that the marine influence on fresh water is governed by such a relationship

Other attempts have been made to derive more complex relationships for data of the type discussed, variants of the general equation $Cl = ae^{-pD} + be^{-qD}$ being used (where a, b, p and q are constants) Eriksson[2] applied this equation to the data of Leeflang[3] while Conway[4] used a similar type of equation, with the addition of a further constant, to Jackson's[5] data The four constants in Eriksson's formula differ from the corresponding constants in Conway's formula An attempt to fit the Scottish data to an equation of the Eriksson type yielded constants differing from those of both Eriksson and Conway It is considered that the simpler formula $Cl = K D^{-n}$ has the merit of consistency in that similar values of n have been deduced from data obtained in widely differing regions, while the values of K are of a similar order, and are possibly related to the mean wind velocity in the area and sampling period concerned

No theoretical derivation of the proposed formula has been attempted, but it is probable that deviations can be expected at very short or very long distances. The deduced value of chloride concentration at zero distance would be infinitely large, perhaps represented by salt particles, although in practice this would not be found Topographical features no doubt also have some influence on the salt depositions While the proposed formula is doubtless a simplification of more complex equations which may govern the pheno-

Table 1

Location	No of sampling stations	Range of chloride concentration (Cl) m equiv /l	Range of distance (D) km	Sampling period	r	K	n	Reference
North-west Scotland	21	0 51–1 59	0 8–31	6 days, 1955	−0 887	1 49	0 336	Holden
North-west Scotland	20	0 39–1 07	1 3–14 5	7 days, 1960	−0 890	1 21	0 334	Holden
Rhum	10	0 38–1 39	0 2– 5 8	9 days, 1958	−0 989	0 74	0 346	Holden
Lake District	16	0 19–1 80	0 1–51	1 month, 1957	−0 912	0 67	0 303	Gorham (ref 1)
New Zealand	11	0 09–0 63	0 5–61	2 years rainfall	−0 914	0 46	0 341	Gibbs and Blakemore (ref 6)
Holland	6	0 09–0 46	0 44–86	6 years rainfall	−0 967	0 32	0 287	Leeflang (ref 3)

menon of salt transport, it does suggest that such transport from the oceans is a continuous process extending over considerable distances, rather than the combination of direct transport of spray droplets over short distances near the coast, and the solution of cyclic salts by precipitations alone at all other distances as could be implied from equations of the Eriksson or Conway type

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METALLURGY

A New Reversible Solid-State Transformation in Iron–Nickel Alloys in the Invar Range of Compositions

Unusual physical properties exhibited by alloys of the iron–nickel system in various ranges of composition have led to extensive investigations[1] of this system in the past One group of alloys is that contained in the invar range, namely, 30–45 atom per cent nickel, and is characterized by unusual thermal expansion, magnetic and other physical properties In spite of extensive investigations, the general sluggishness of solid-state transformations in the iron–nickel system has prevented true equilibria from being well established The properties of these alloys, therefore, have remained largely unrelated to their structural and/or crystallographic features

During a systematic re-examination of solid-state equilibria in the iron–nickel system, using new techniques capable of yielding true equilibria within reasonable lengths of time, we have found a new reversible transformation which occurs in these alloys in the temperature-range of ~ 150°–300° C In the present communication, this transformation is described and its possible bearing on the physical properties of the alloys discussed

Equilibrated iron–nickel alloys containing up to 75 atom per cent nickel were prepared in the form of pre-alloyed ultra-fine powders by the thermal decomposition and hydrogen-reduction of iron–nickel mixed-crystal formates[2,3] at various temperatures, including temperatures as low as 200° C Most of these alloys, as-quenched to room temperature from their respective temperatures of preparation, generally showed the equilibrium structures corresponding to the temperature of preparation However, alloys with 30–45 atom per cent nickel showed an exceptionally different behaviour which, on detailed investigation, proved to be due to the occurrence of the new transformation On re-heating, all the as-quenched alloys readily became transformed to yield equilibrium structures corresponding to the temperature of holding

As-quenched alloys were examined by X-ray diffraction at room temperature, using a Philips diffraction camera of 114 7 mm diameter High-temperature X-ray diffraction patterns were next obtained with a Unicam high-temperature diffraction camera which had been previously calibrated against the thermal expansion of gold Using a high-intensity rotating-anode X-ray generator, diffraction patterns could be secured with exposures as short as half an hour Cobalt and iron characteristic radiations were generally used, manganese radiation being used in those cases where high resolution was sought

Fig 1 Room-temperature X-ray diffraction pattern showing (311) back reflexion from a 31 atom per cent nickel iron–nickel alloy, alloy quenched to room temperature from the γ-state (~ 600° C) Manganese K_α radiation

Examination of the diffraction patterns revealed that the transformation occurs in all the face-centred cubic γ-phase alloys which contain approximately 30–45 atom per cent nickel When alloys of this structure and composition are quenched to room-temperature from the γ-phase, their X-ray diffraction lines always appear broad (Fig 1) That this broadening is not to be associated with quenching strains or with effects of fine particle-size became evident from high-temperature X-ray diffraction investigations during thermal cycling of the as-quenched alloys On heating to or above what appeared to be a critical temperature, the broad diffraction lines of the quenched alloys showed a pronounced sharpening Subsequent cooling resulted in loss of sharpness of the lines and the reappearance of their broadening (Fig 2) The effect has been reproduced repeatedly and takes place readily.

Closer examination of the broad lines indicated that the broadening may be interpreted as due to the superposition of two closely spaced reflexions having the same indices but arising from two face-centred cubic phases (designated as γ_1 and γ_2) of slightly different lattice constants The sharpening of the lines on heating may thus be attributed to the coalescence of the two γ-phases into a single γ-phase

In spite of the poor resolution of the X-ray diffraction lines, an attempt was made to measure the lattice parameters of the two γ-phases Typical results are shown in Table 1, from which it may be seen that the difference in the lattice constants is only about 0 008–0 009 Å

Transformation temperature appears to be dependent on the composition of the alloy Exact transformation temperatures for various compositions could not be determined, but they seem to lie in the

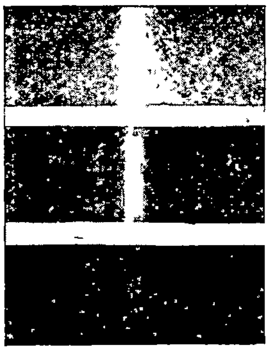

Fig 2 High-temperature X-ray diffraction patterns showing (311) back-reflexion from a 31 atom per cent nickel iron–nickel alloy, alloy as-quenched to room temperature from the γ-state subjected to thermal cycling Iron K_α radiation

Table 1 LATTICE CONSTANTS OF THE TWO γ-PHASES CO-EXISTING AT ROOM TEMPERATURE IN IRON–NICKEL ALLOYS WITH 31 AND 36 ATOM PER CENT NICKEL

Atom per cent nickel in alloy	Lattice constants (Å)	
	γ_1	γ_2
31	3 593	3 585
36	3 593	3 584

approximate range 150–300° C Note that the transformation occurs at relatively low temperatures Considering the general sluggishness of solid-state transformations in most iron–nickel alloys, rendered all the more sluggish at temperatures below 500° C , the reversibility of this transformation is indeed very striking

Observed features of the transformation are consistent with the interpretation that the transformation is of the magnetic kind, more specifically, ferromagnetic ⇄ anti-ferromagnetic Such a transformation may serve as the key to the proper understanding of the anomalies in thermal expansion, magnetic and other physical properties, for which the 30–45 atom per cent nickel iron–nickel alloys are noted The broad X-ray diffraction lines observed may thus be interpreted as due to the co-existence of ferromagnetic and antiferromagnetic modifications of the γ-phase in these alloys at these low temperatures Taking into account exchange interactions between iron–iron, nickel–nickel and iron–nickel atom pairs in iron–nickel alloys, the possible occurrence of such a transformation in precisely the same composition-range as of the alloys of this work has recently been considered by Kondorskii[4]

The validity of our interpretation of data, however, needs further confirmation through more refined experiments using methods other than X-ray diffraction, for example, magnetic analyses as well as neutron diffraction Work on these lines is now in progress

In attempting to correlate the changes of properties, for example, thermal expansion, saturation magnetization, etc , of these alloys with composition and structure, we are led to pose the question Should not magnetic transformations also be viewed as regular phase changes and therefore be given a fitting place in phase diagrams ? This question has far-reaching implications For example, if magnetic transformations are considered as regular phase changes, such concepts as 'solid phase' and 'crystal symmetry' will have to be modified to recognize the magnetic state of matter and the atomic symmetries associated with this state

Results discussed here together with unreported results covering compositions up to 75 atom per cent nickel, indicate that the iron–nickel equilibrium diagram is much more complex than the most recent diagram[5] drawn for this system We have constructed a new iron–nickel phase diagram which will be reported in the near future

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CHEMISTRY

Relaxation Enhancement by Paramagnetic Ion Binding in Deoxyribonucleic Acid Solutions

THE effect of paramagnetic ions on proton relaxation times depends not only on their concentration but also on the binding of the ion to other molecules which may be present in the same solution By measuring longitudinal relaxation times (T_1) by nuclear magnetic resonance we have compared the proton relaxation-rates at constant ion concentration with and without deoxyribonucleic acid (DNA) present In this way it was possible to detect binding and to distinguish between interior and exterior DNA binding sites for different transition metal ions

In the present experiment T_1 was measured by the Carr–Purcell method[1] with a conventional spin-echo apparatus operating at 50 Mc /sec , for a wide range of magnetic ion concentrations in water and in calf-thymus DNA solutions

The Solomon[2]–Bloembergen[3] expression for T_1 in the presence of paramagnetic ions is

$$\frac{1}{NT_1} = \frac{4}{30}\,\frac{S(S+1)g^2\beta^2\gamma^2{}_I p}{r^6}\left[3\tau_c + \frac{7\tau_c}{1+\omega_s{}^2\tau_c{}^2}\right] + \frac{2}{3}\,\frac{S(S+1)A^2p}{\hbar^2}\left[\frac{\tau_e}{1+\omega_s{}^2\tau_e{}^2}\right]$$

in which N is the molar concentration of paramagnetic ions, γ_I the proton gyromagnetic ratio, p the probability that a proton is in the hydration sphere of the paramagnetic ion in a $1M$ solution of the ions, τ_c the correlation time for dipolar interaction, ω_s the angular electron precessional frequency, A the isotropic indirect hyperfine interaction, τ_e the correlation time for the isotropic hyperfine interaction (electron spin relaxation time, τ_s, or correlation time for proton chemical exchange in the hydration sphere, τ_h, whichever is shorter) and r is the ion-proton internuclear distance

At high fields, such as were used in the present experiments, the second term is negligible The correlation time τ_c is given by $1/\tau_c = 1/\tau_r + 1/\tau_h + 1/\tau_s$, where τ_r is the rotation correlation time

Consider how $1/NT_1$ is affected by paramagnetic ions originally in solution being bound to a molecule If the ion is bound to a single point (that is, not chelated) on the outside of the molecule, two degrees of rotational freedom are eliminated and the third may be reduced by the 'friction' of the hydration sphere against the molecule If the ion is chelated, rotation will in general be stopped completely In any event $\tau_r{}^*/\tau_r > 1$, where the asterisk denotes values for the bound ions If τ_s and τ_h are greater than τ_r and do not change appreciably when the ion is bound as is often the case, then $\tau_c{}^*/\tau_c > 1$ The number of water molecules in the hydration sphere of the bound ion is reduced slightly so that $p^*/p \approx 5/6$ In this case of large values of τ_s, therefore, the rate of proton relaxation in the presence of externally bound ions is greater than when the same concentration of ions is in solution If we define a relaxation enhancement factor $\varepsilon = T_1/T_1{}^*$ then $\varepsilon > 1$ This case is illustrated in Fig 1 for aqueous solutions of Mn^{++} ions and DNA

The second case is $\varepsilon < 1$, implying that p/r^6 and/or τ_c are reduced when the ion is bound, this has been used in several recent experiments[4]. The first alternative clearly indicates that the ion site is an interior one and not accessible to hydration water The latter can only arise from a large decrease in

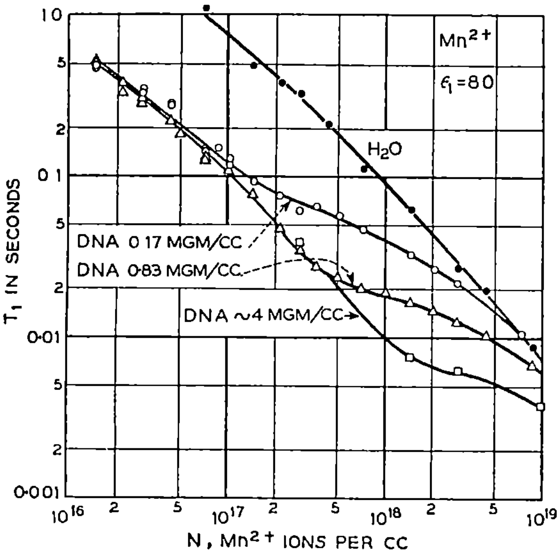

Fig 1 The curves show the proton T_1 as a function of Mn^{2+} ion concentration in pure water and DNA solutions of various concentrations The relaxation enhancement factor ε is approximately 8 0 for Mn^{2+} concentrations below the saturation value of 7 × 10^{17} Mn^{2+}/mgm DNA, which agrees with conductiometric measurements (ref 5)

electronic relaxation time on binding so that $\tau_s{}^*$ dominates $\tau_c{}^*$ (that is, $\tau_s{}^* < \tau_c$). In many cases even a rough knowledge of τ_s and $\tau_s{}^*$ makes the distinction between exterior and interior sites unambiguous

Our experimental results for aqueous DNA solutions may be summarized as follows

(1) For Cu^{2+}, Mn^{2+} and Cr^{3+} the measured values of ε are 10, 8 and 4 7, respectively We conclude that these three ions are bound to exterior DNA sites, probably to the phosphate groups

(2) For Fe^{3+} ε is 0 3 and if, as can be shown to be the case for the previously mentioned ions, $\tau_s \approx \tau_s{}^*$, we can conclude that Fe^{3+} is bound to an interior site

(3) Ni^{2+}, Fe^{2+} and Co^{2+} show $\varepsilon = 1$ Since these ions are known to have a very short τ_s, we can only conclude that they are either not bound to DNA or bound to an exterior site with $\tau_s{}' = \tau_s$ Recent experiments indicate that for Ni^{2+} and Co^{2+} the latter is the case[6,7] The number of available metal ion sites was found to be approximately 7 × 10^{17}/mgm of DNA for Mn^{2+}, Cr^{3+} and Fe^{3+} (~ 0 6 phosphate equivalent)

A detailed account of these experiments, which includes a discussion of the effect of the ionicity of the solution on the metal-DNA system, is being prepared

The experimental method described here should be generally applicable to other problems of paramagnetic ion bonding to large molecules An expression for $1/NT_2$ analogous to $1/NT_1$ leads to T_2 enhancement, which proves useful in the investigation of some ions

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Detection by Ionization of Gases in Helium used in Gas Chromatography

THE recent communication of Karmen *et al*[1] on analysis by gas chromatography illustrates an interesting mode of operating a Lovelock ionization detector. The results given, however, do not indicate that the behaviour of the cell is in any way different from that used in the conventional manner The cell used by me[2] has a similar electrode configuration and the results, where comparable with Karmen's, are practically identical (see Table 1)

Table 1

	Concentration (volumes per million) for maximum conductivity		Relative responses for equal volumes		
	Karmen	Wiseman	Karmen	Wiseman (ref 2)	(*a*)
Hydrogen	—	~1,000	—	1	1
Nitrogen	520	750	4	3	~5
Argon	960	>170	3	5	5
Oxygen	280	250	11	10	10
Methane	—	⩾100	—	20	~10
Krypton	—	—	—	—	~18
Xenon	—	—	—	—	20
Carbon dioxide	80	170	5	70	—

(*a*) Previously unpublished results (Wiseman) using microargon detector

Some allowance must be made for the greater impurity of their helium, particularly in the case of carbon dioxide It is therefore not possible to accept their contention that the design of cell is more important than the purity of the helium

There are several points of considerable importance raised by their communication First, their implication that a cell specially designed for use with helium is likely to work better than one based on work with argon is almost certainly correct Secondly, to obtain the best results, a leak-tight detector is essential, but the short outlet illustrated in their diagram would appear to be inadequate to prevent back-diffusion once helium of really high purity was used. Thirdly, there are some serious implications overlooked in their acceptance of helium of commercial grade without further purification It is well known that bottles of commercial gases vary substantially in trace composition and in particular in water content Water appears to have an even higher response in these detectors than carbon dioxide Further, it is clear from Table 1 that were carbon dioxide the major constituent of the 100 v p m. impurity-level of the commercial helium, the helium would not give increases of conductivity with any gases Hence a mere specification of 99 99 per cent would be meaningless without some data on the nature of the impurities It is apparent from published data[1,2] that the tolerance of gases like argon and hydrogen is about 10 times that of carbon dioxide and probably water Karmen *et al* do not state whether they dry their helium. If they did, then possibly their specification is much better than indicated Even one molecular sieve trap in liquid nitrogen is a very effective purifier

Table 2

	Tolerable impurity-level (v p m)
Neon	10 (?)
Argon	10
Hydrogen	10
Nitrogen	5
Oxygen	3
Carbon dioxide	1
Water	<1
Hydrocarbons	1
Carbon monoxide	1
Total impurities	≯20

These figures are based on 1 per cent of the figure for maximum conductivity

Further disadvantages of using helium of relatively low purity are the reduction of the linear range—possibly even its complete elimination and the irregular calibration curve likely to result This could be S-shaped in character, and in any event as the conductivity/concentration curve reaches a maximum, the sensitivity of the device to changes of concentration reaches a minimum

In general, therefore, users should be recommended to obtain the purest helium possible compatible with reasonable trouble in purification A suggested specification for commercially available pure helium is given in Table 2

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Quantitative Estimation of Peak Areas in Gas-Liquid Chromatography

Recently, Carroll[1] advocated the use of the product of retention time (t_R) and peak height (h) for the quantitative estimation of relative peak areas This method[2], based on the assumption of a proportionality of peak width (w) and retention time (t_R) or volume (V_R), gives satisfactory results with respect to the reproducibility. In fact, we have found that the reproducibility (under equal operating conditions) of this method is better than that of any other 'manual' estimation of peak areas However, the method is not commonly used in our Laboratory, as the results obtained with it are erratic This is due to the fact that in most cases the proportionality between w and V_R is only approximate Differences between the relative areas, as found by the triangulation method and from the product of h and V_R, can amount to several per cent relative In principle the non-linearity of w and V_R can be taken into account when calibrating the detector However, changing the column or altering the carrier gas velocity will change the calibration factors

Equation (17) in ref 3 can be written as

$$C_v = \frac{n^{1/2}}{V_R\sqrt{2\pi}} \exp\left(-n(V - V_R)^2/2V_R^2\right) a \qquad (1)$$

where n is the number of theoretical plates, V_R is the retention volume, V is the volume of carrier gas passed through the column since the introduction of the sample, C_v is the concentration of the component in the carrier gas, a is the amount of the component in the sample

From this equation it follows that (for a Gaussian peak)

$$h \times V_R \simeq \frac{n^{1/2}}{\sqrt{2\pi}} a \qquad (2)$$

Since n depends *inter alia* on the distribution coefficient[4], and thus on the retention time of the component, the relation between the product of h and V_R and the amount a will, in general, not be the same for every component. Therefore the method can only be expected to yield good results when the differences in retention volumes are very small, or when the C-term in the van Deemter equation is relatively unimportant, that is, when the linear gas velocity and the amount of stationary phase are small (influence of diffusion coefficients on n being neglected) In Carroll's measurements this is certainly not the case as the relative areas of the early peaks are larger when the triangulation method is used, whereas the relative areas of the late peaks are larger (with one exception) when the estimation is made from the product $h \times t_R$. Most likely in these experiments the distribution coefficient of the most volatile component (methyl palmitate) was larger than the ratio of the volumes of mobile and stationary phases in the column In consequence the number of theoretical plates must be expected to increase with the retention time This will explain the discrepancies between the results obtained by the different methods The same trend can be observed from the results summarized in Table 1

Table 1 AVERAGE RELATIVE AREAS OF SEVEN PEAKS IN A CHROMATOGRAM OF A HYDROCARBON MIXTURE

Distribution coefficient of component	Column length	2 m		2 m		4 m		4 m	
	Sample volume	2 5 µl		5 µl		2 5 µl		5 µl	
	Method	$h\,w$*	$h\,V_R$	$h\,w$*	$h\,V_R$	$h\,w$*	$h\,V_R$	$h\,w$*	$h\,V_R$
55		2 7	2 6	2 7	2 6	2 8	2 5	2 8	2 5
72		13 8	13 8	13 6	13 7	13 7	13 5	13 7	13 6
148		12 6	11 8	12 6	11 7	12 3	11 4	12 5	11 4
172		7 6	7 2	7 7	7 3	7 2	7 1	7 3	7 2
206		46 0	46 7	45 8	46 6	46 2	47 2	46 3	47 1
267		6 3	6 3	6 4	6 4	6 4	6 3	6 2	6 3
380		11 1	11 7	11 2	11 8	11 5	12 0	11 3	11 9

* w, width at half-height

Column, squalane on sterchamol (20 100), temperature, 50° C , ratio mobile to stationary phase, 6 (approximately)

The Perkin–Elmer *ABC* test mixture (hydrocarbons) was run on a 2-m and on a 4-m squalane column using 2 5 and 5 µl samples The relative areas of seven peaks of this mixture, given in Table 1, are the averages of the values obtained from five chromatograms

It appears that for accurate quantitative analysis the method advocated by Carroll is not suitable

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In proposing the method referred to by Dijkstra, I tried to stress its convenience rather than its extreme accuracy and the examples which I gave were chosen to show that some lack of agreement might be expected in comparing areas determined by this method with areas determined by other methods However, as Dijkstra points out, results obtained with other methods are less reproducible Slight differences in the way in which triangles are drawn and small inaccuracies in cutting out curves can give rise to appreciable differences in estimated area It is also more difficult to measure peak widths at half height accurately than it is to measure retention times Furthermore, in the case of overlapping peaks it is frequently impossible to get a good estimate of the individual areas by other methods whereas the retention times and peak heights can still be measured and appropriate corrections for peak height applied if necessary

A convenient and rapid method with somewhat limited accuracy can still be useful in work where extreme accuracy is not required or where accuracy cannot be obtained in any event because of errors introduced by other steps in the analytical procedure However, it is always desirable to improve the accuracy of a convenient method, and this can sometimes be done when sources of inaccuracy are known For this reason I welcome Dijkstra's critical comment on the method for estimation of peak areas

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Behaviour of 5*A* Molecular Sieve in Subtractive Gas Chromatography

5*A* molecular sieve can be used to remove selectively normal paraffins from hydrocarbon samples in the gas phase at the inlet of a gas liquid chromatographic column[1,2] This subtraction technique has formed the basis of analytical procedures for the determination of the normal paraffins in complex petroleum fractions and has been applied in conjunction with both packed and capillary columns[3]

It has also been shown that at high temperatures (*c* 300° C) normal paraffins could be removed in a similar manner from waxes but in this molecular weight-range only 2-methyl eicosane and the highly branched paraffin squalane were available to test the selectivity of the molecular sieve Neither of these compounds was sorbed by the sieve at a temperature of 320° C

Recently, Murray *et al* [4] have reported that unspecified single methyl branched paraffins in the wax range are completely removed from the gas phase by 5*A* sieve. This affinity for the singly branched paraffins was destroyed by repeated passage of these compounds through the sieve until no further sorption occurred However, the sieve was still capable of retaining the normal paraffins

We have now studied the behaviour of the single methyl branched hydrocarbons in greater detail using in particular a sample of 2-methyl dotriacontane synthesized in our laboratories[5] In these experiments a pre-column 6 cm long and 6 mm internal diameter was packed with activated 30–60 B S mesh 5*A* molecular sieve This pre-column was attached to the inlet of an 86-cm gas-liquid chromatography column, also of 6 mm diameter, containing 26 per cent wt of silicone grease *E* 301 on 52–60 B S mesh 'Sil-O-Cel' firebrick The pre-column was maintained at 320° C and the gas-liquid chromatography column at 296° C These conditions had been used previously for wax separations and were known to be satisfactory for such mixtures[3] 10-μl samples of pure paraffin hydrocarbons were introduced into the pre-column and their behaviour is shown in Table 1

At C_{33} the 2-methyl paraffin is completely sorbed whereas at C_{24} and below this type of compound is not sorbed by the sieve Also, when the branching occurs in the middle of the molecule the C_{44}-branched paraffin is retained, but the similar C_{26} compound is not sorbed At some critical chain-length between the limits indicated, single methyl branched paraffins start to be sorbed by the 5*A* sieve

Table 1 Behaviour of Paraffin Hydrocarbons in a Gas-Liquid Chromatography Pre-column containing 5*A* Molecular Sieve

Completely sorbed from the gas phase		Not sorbed from the gas phase	
Docosane	C_{22}	2-Methyl eicosane	C_{21}
Tetracosane	C_{24}	2-Methyl tricosane	C_{24}
Hexacosane	C_{26}	13-Methyl pentacosane	C_{26}
Octacosane	C_{28}		
Dotriacontane	C_{32}		
2-Methyl dotriacontane	C_{33}		
22-Methyl tritetracontane*	C_{44}		

* Gas-liquid chromatography column temperature, 350° C

Further work on the selectivity of the sieve will be carried out as more reference hydrocarbons in this range become available

We wish to thank Mrs M Colwell for the synthesis of some of the paraffins used in the work

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Improved Gas Chromatography of Unesterified Fatty Acids

It has been demonstrated by Bottcher *et al* [1] and by Hunter *et al* [2] that unesterified fatty acids can be separated, by gas chromatography, by the use of 'liquid' phases of the polyester type Metcalfe[3] has pointed out the necessity to have a highly acidic 'liquid' phase in order to suppress the association of the carboxyl group of the fatty acids, and has shown that the separation of C_4–C_{22} fatty acids is very much improved by the incorporation of phosphoric acid in the polyester 'liquid' phase Using a column packing of 'Celite 545' previously coated with 25 per cent of diethylene glycol adipate polyester and 2 per cent of phosphoric acid, mixtures of fatty acids were separated at temperatures of 220–235° C , yielding well-defined symmetrical peaks and separating saturated and unsaturated acids from each other

The development of the argon ionization detector has resulted in higher sensitivities of detection allowing a reduction of sample size This reduction of sample size itself improves column efficiency and, in addition, allows the use of columns with lower content of 'liquid' phase, which in turn leads to improved resolution and a faster analysis A faster analysis is particularly important with the separation of fatty acids which are more thermo-labile than their corresponding methyl esters However, the choice of solid support is important when low ratios of 'liquid' phase to solid support are considered The use of a solid support having a large surface area per unit weight, such as 'Celite', is not particularly suitable in some respects, since treatment with the 'liquid' phase could leave much of the surface area uncoated, allowing the solid support to act as an absorbent and thus to interfere with the distribution of the analysis sample between the 'carrier' gas and the liquid phase

Bohemen and Purnell[4] have discussed the requirements of an efficient solid support These authors have shown that, as predicted by the van Deemter, Zuiderweg and Klinkenberg rate equation[5], high column efficiencies are attained when the columns have the following characteristics (*a*) narrow range of particle sizes, (*b*) spherical shape of particle, (*c*) small quantity of 'liquid' phase

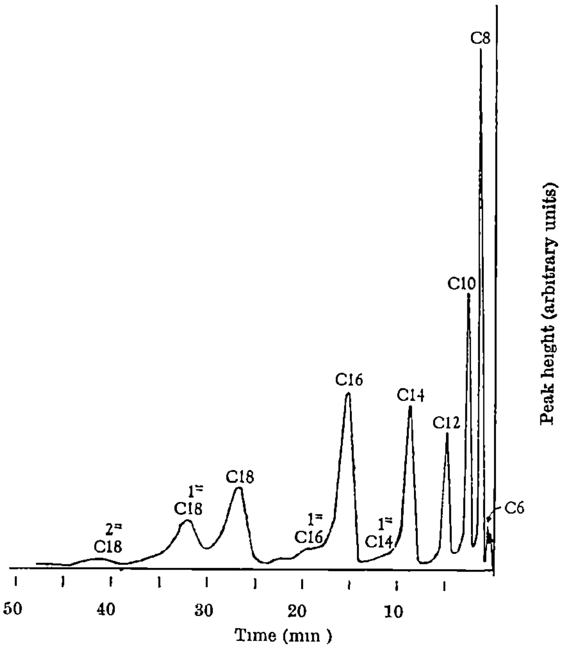

Fig 1 Chromatographic separation of a mixture of C_6—C_{18} fatty acids Sample size, 0 1 µl of a 50 per cent solution in cyclohexane Column temperature was 180° C , column size 1 2 m × 4 mm , argon pressure was 12 p s i at column inlet, atmospheric pressure at outlet

All these requirements can be fulfilled by using small glass beads of uniform size, which can retain up to 3 per cent of 'liquid' phase by surface tension

We have demonstrated that mixtures of unesterified C_6–C_{18} fatty acids can be analysed at temperatures ranging from 140° C to 200° C , by the use of a glass-bead support coated with a mixture of 1 per cent of poly-ethylene glycol succinate and 0 4 per cent of phosphoric acid

Poly-ethylene glycol succinate was prepared by heating a mixture of 1 0 mole of ethylene glycol and 1 05 moles of succinic acid at 150° C for 3 hr The resultant viscous liquid solidifies at room temperature and has a melting point of 80–82° C The polyester was used as 'liquid' phase without further treatment The polyester (0 5 gm) was dissolved in chloroform (30 ml) and to the solution was added, with constant stirring, 50 gm of glass beads (Ballotini, No 13 diam = 0 075 – 0 1 mm , manufactured by the English Glass Co , Ltd , Leicester), followed by 0 2 gm of phosphoric acid (85 per cent H_3PO_4) The stirring was continued while the chloroform was allowed to evaporate at room temperature When the packing was almost dry, it was finally rendered solvent-free by heating for 0 5 hr in an oven maintained at 100° C The coated beads were then sieved, and only the portion passing a 85–100 mesh sieve was used to pack the column The 1 2 m × 4 mm glass column was vibration-packed with the coated beads in the normal way The packed column was purged for 72 hr with a stream of argon, while the column was maintained at a temperature of 200° C For fatty acid analysis, the Pye gas chromatography apparatus was used, which utilizes argon as carrier gas and strontium-90 as the radioactive source for the ionization detector The mixtures of fatty acids analysed were dissolved in cyclohexane (50 50 w/v) and 0 1 µl of the solution was used for each experiment Normally, the detector voltage was 1,000 and the sensitivity control was set at × 10

Table 1 RETENTION TIMES (MIN) OF C_6—C_{18} SATURATED FATTY ACIDS AT VARIOUS COLUMN TEMPERATURES

Saturated fatty acids	Column temperature (° C) 140	150	160	170	180	190	200
C6	3 0	1 6	1 2	0 6	—	—	—
C8	6 4	4 0	2 8	1 6	1 4	1 0	—
C10	13 2	8 0	5 4	3 4	2 8	1 8	1 0
C12	27 0	16 0	10 6	6 6	5 0	3 0	2 0
C14	54 0	31 8	20 2	12 4	9 0	5 6	3 8
C16	107 0	62 0	37 2	22 4	15 2	9 8	6 2
C18	214 0	122 0	69 2	40 4	26 2	16 2	10 4

The separation of a mixture of C_6–C_{18} fatty acids, at 180° C , is shown in Fig 1 The approximate retention times of each of the component acids of this mixture, at various column temperatures, are given in Table 1 This illustrates that long-chain fatty acids can be separated rapidly at comparatively low temperatures

We are grateful to the Directors of Armour Hess Chemicals, Ltd , for permission to publish this communication

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BIOCHEMISTRY

Oxidized Cytochrome and Chlorophyll C_2^+ in Photosynthesis

CHANCE *et al* [1a] reported the presence of trapped oxidized cytochrome in bacteria at –200° C , and in a previous communication[1b] we supplied evidence for the presence of trapped oxidized cytochrome during photosynthesis of green plants at –150° C We have now refined and extended the measurements over the whole range of the visible spectrum The difference spectrum of the trapped products in fresh spinach chloroplasts is shown in Fig 1*a* It shows in addition to the oxidized cytochrome Cyt$^+$ (solid line) the trapping of what is probably oxidized chlorophyll C^+ (dotted line) Both substances are products of one and the same light reaction $h\nu_1$ It has been shown that in green plants photosynthesis is triggered by two light reactions $h\nu_1$ and $h\nu_2$, one being sensitized by a chlorophyll pigment already sensitive at 710 mµ and the other sensitized by a chlorophyll pigment (*P*) sensitive at shorter wave-lengths (see refs 2 and 3) Exciting only $h\nu_1$ (at +20° C and 710 mµ) gives almost the same difference spectrum as presented in Fig 1*a* Two light-reactions in photosynthesis are discussed in the literature[2,5,6,8]

Information about the difference spectrum of C/C^+ in the range of 400–440 mµ (in Fig 1*a* masked by the absorption changes of cytochrome) and about the chemical nature of C/C^+ is given by experiments with aged chloroplasts of spinach Vernon, Kamen and Zaugg[4] have shown that aged inactive chloroplasts can again reduce triphosphopyridine nucleotide (TPN) in light after addition of reduced substances (dichlorophenol-indophenol with ascorbic acid) We observed that after such additions (reduced phenazine methosulphate, with ascorbic acid in excess) the aged

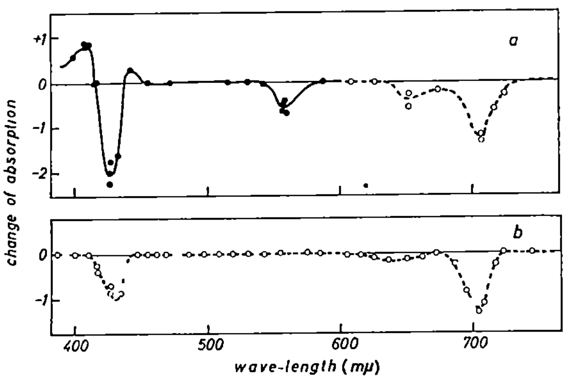

Fig 1 *a* Difference spectrum of the trapped products of photosynthesis in spinach chloroplasts at −150° C *b* Difference spectrum caused by illumination of aged chloroplasts with reduced phenazine methosulphate (PMS) at +20° C

chloroplasts showed a difference spectrum depicted in Fig 1*b* The changes of absorption at 430 and 703 mμ are both caused by one and the same substance *C* This follows from the identical behaviour of the changes at 430 and 703 mμ at different physical and chemical parameters For example. with increased concentration of reduced phenazine methosulphate (red PMS) the decay-time of the absorption changes at 430 and 703 mμ decreases from approximately 10^{-2} sec at 10^{-6} *M* to 10^{-4} sec at 10^{-4} *M* This means in addition that *C* is oxidized by light: $C \xrightarrow{h\nu_1} C^+$, and that C^+ is reduced by red PMS in a dark reaction $C^+ \xrightarrow[\text{PMS}]{\text{red}} C$ With the addition of reduced dichlorophenol-indophenol (red In) the decay-time at 430 and 703 mμ also decreases but only to a limited value of 10^{-2} to 10^{-3} sec depending on the condition of the chloroplasts This means that red In , contrary to red PMS, does not react directly with C^+ but via an intermediate reaction (with cytochrome, see later) with a duration of 10^{-2} to 10^{-3} sec By addition of different mixtures of ferro/ferricyanide it was possible to change the steady-state concentration of C/C^+ This can be seen by the dependency of the size of the light-induced absorption changes at 430 and 703 mμ on the ratio of ferro/ferricyanide From such measurements we have estimated the redox potential of C/C^+ as $+ 0{\cdot}45 \mp 0\ 01$ V at *p*H 6 and 8 Kok[5] has discovered and already reported in detail about the changes at 703 mμ and +20° C. He found that the unknown substance causing these changes has a redox potential of +0 46 V. and can be oxidized by light at 710 mμ and reduced by shorter wave-lengths Our measurements at 703 mμ and + 20° C confirm his results Our additional observation that in chloroplasts capable of reducing TPN the oxidation of *C* causes, apart from the changes at 703 mμ, a pronounced change at 430 mμ (see also ref 3) leads us to suggest that the difference spectrum shown in Fig 1*b* represents the oxidation of a chlorophyll-*a* Besides type 0 and type 1 (see ref 2) this is the third chlorophyll reaction observable in plants

By exciting *Chlorella* with 'flashing light' at +20° C it follows from the time of the changes of absorption (estimated at 703 mμ) that $C \longrightarrow C^+$ reacts within $< 10^{-5}$ sec The decay time of the changes, that is, the duration of the back reaction $C^+ \longrightarrow C$ in *Chlorella*, has been estimated at 430 mμ as approx 10^{-3} sec Contrary to the strong temperature-dependent life-time ($\tau \approx 10^{-2}$ sec) of the absorption changes at 475 and 515 mμ (see ref 2)—caused by an enzymic back-reaction (*E*) of a reduced substance $X^- \longrightarrow X$—the back-reaction of $C^+ \longrightarrow C$ is virtually independent of temperature (0–40° C)

Previous work[2] showed the time of cytochrome oxidation $\text{Cyt} \longrightarrow \text{Cyt}^+$ at 20° C and 420 mμ to be 10^{-5} sec This value is falsified by the absorption changes of $C \longrightarrow C^+$, which, as now can be seen, also take place at 420 mμ New measurements of the time of increase of $\text{Cyt} \longrightarrow \text{Cyt}^+$ at 405 mμ give a temperature-independent value of about 10^{-3} sec This value corresponds to the decay-time of $C^+ \longrightarrow C$.

The rapid increase of $C \longrightarrow C^+$ (less than 10^{-5} sec), the trapping of C^+ and Cyt^+ at −150° C , the production of C^+ and Cyt^+ in one and the same light reaction $h\nu_1$, the identification of *C* as chlorophyll-*a* and the fact that the decay-time of C^+ corresponds to the time of increase of Cyt^+ gave us the idea that the cycle of C/C^+ is the chlorophyll reaction operating between cycles 1 and 2 in Fig 2 of ref 2 This led to an extended reaction pattern shown in Fig 2 The trapping of both C^+ and Cyt^+ at −150° C is probably caused by a charge compensation at −150° C between C^+ and Cyt $C^+ + \text{Cyt} \rightleftarrows C + \text{Cyt}^+$ This is further evidence for the direct connexion of these substances

The energy difference between the cycles provides evidence that phosphorylation is generated by the energy released between cycles 3 and 4 (see ref 2) Losada *et al* [6] suggest phosphorylation between chlorophyll and cytochrome

As aged chloroplasts with red PMS show only changes of absorption at 430 mμ and 703 mμ (Fig 1*b*) it follows from Fig 2 that this system only operates with cycles 1 and 2 and is the most simple and stable photo-active unit in plants (C^+ reacts only with PMS in aged chloroplasts, in fresh chloroplasts C^+ is shielded from the attack of PMS) Addition of *p*-chlorophenyl-dimethylurea (CMU) or phenanthroline (strong poisons for oxygen production) have no influence on the absorption changes at 430 and 703 mμ in this simple system, this is in accordance with our scheme Aged chloroplasts with small amounts of red In show absorption changes at 430 and 703 mμ and the difference spectrum of cytochrome (Fig 1*a*) From Fig 2 it follows, therefore, that in this case cycles 1, 2, and 3 are operating (Cyt^+ is reduced by red In) In fresh chloroplasts with CMU or with phenanthroline, oxygen production is stopped, and

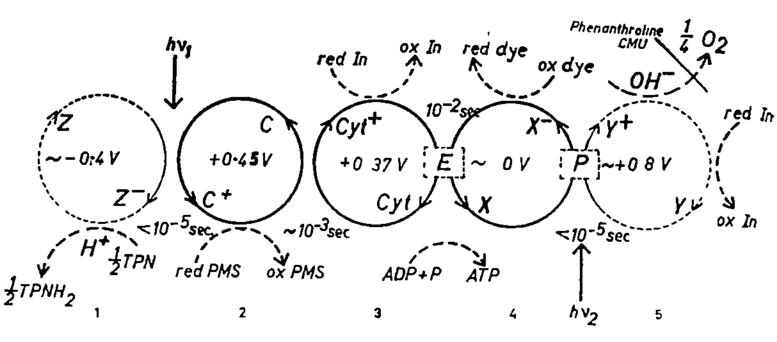

Fig 2 Reaction pattern of photosynthesis

no change of absorption can be observed By addition of red In , however, all changes of absorption, as well as those caused by X/X^-, reappear Therefore, in this case, cycles 1, 2, 3, 4 and 5 are operating and Y^+ must be reduced by red In instead of OH^- (Fig 2)

Losada *et al.*[6] have assumed that in fresh chloroplasts with CMU and red In one light reaction was separated by blocking the other one with CMU This cannot have been the case, since from the results reported here it follows that addition of CMU (blocking the production of oxygen by poisoning the reaction of Y^+ and OH^-) has together with red In no effect on either light reaction, because Y^+ can react with red.In instead of water (OH^-)

Note added in proof. Recently Kok[7] has reported changes in absorption at 432 mμ in inactive chloroplasts extracted with acetone He tends to ascribe these changes to those at 703 mμ and assumes tentatively that both changes are caused by a chlorophyll-*a*

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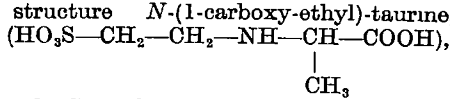
New Ninhydrin-Reactive Substance from Red Algae

During an investigation on the free amino-acids in marine algae, I isolated a new ninhydrin-reactive substance from three species of red algae *Chondrus ocellatus*, *Neodelsea yendoana* and *Iridaea cornucopiae* by the use of anionic ion-exchange resin 'Amberlite *IR*–45' and 'Dowex 2'. This substance gave an empirical formula $C_5H_{11}O_5NS$ and melted at 258° C Optical rotation gave a value $[\alpha]_D^{13} = -1\ 15$ ($c = 5$, *M* sodium hydroxide solution) It was not very sensitive to ninhydrin, but gave a bright brown colour with Plattner's reagent. Titration with 0 1 *N* sodium hydroxide, in alcoholic solution, gave a value 63 80 per cent (calculated as C_4H_7N—SO_3H—COOH, 66 16 per cent) By the method of Kuhn and Roth, at least one radical, C—CH_3, was estimated The oxidation products obtained, after treatment with potassium permanganate solution, were taurine, alanine and propionic acid From these results, the structure *N*-(1-carboxy-ethyl)-taurine, that is  $(HO_3S{-}CH_2{-}CH_2{-}NH{-}\underset{\displaystyle CH_3}{\underset{|}{CH}}{-}COOH)$, was assumed Crystals obtained from α-brompropionic acid and taurine by chemical synthesis corresponded well with the isolated substance not only in melting point but also in R_F values on various paper chromatograms

Further investigations on the non-absorbable fraction of algal extracts on strong cationic ion-exchange resin revealed the wide distribution of this substance with taurine in red algae The only exception was in the case of *Chondria crassicaulis*, from which a sulphur-containing amino-acid, 1,4-thiazane-3-carboxylic acid *S*-oxide had previously been isolated[1] Not a trace of this substance could be detected here Furthermore, they have not been detected so far in brown or green algae

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Sex Hormones as a Possible Factor controlling Biosynthesis of Arylesterase

Blood plasma arylesterase in pigs is controlled genetically by a set of alleles, each determining a plasma arylesterase activity of 0, 25, 50, 75 and 100[1] The distinguishable phenotypes are the manifestation of an additive effect of a pair of these alleles The plasma of new-born piglets has no arylesterase activity, and differentiation into groups of various esterase activities begins on the second to fourth day of life, adult values being reached at the age of about 50 days There are no sex differences for the biosynthesis of this enzyme, which, however, is influenced by sex hormones, as will be briefly reported here

In the genetical work, the male piglets were castrated at the age of three to four weeks. The adult arylesterase activity values of these boars were fairly constant However, certain male piglets were selected for use in later cross-tests With these uncastrated boars, it was observed that arylesterase activity after maturity was much lower than before sexual maturity[2]. For example, it was demonstrated that with a boar of esterase activity (phenotype) 150 before maturity, the activity gradually decreased with increasing age to a value of about 30, and that this low phenotypic activity was then fairly constant This phenomenon was found to be common to all mature boars, the low phenotypic esterase activity of which had no influence on expected segregation in the progeny. Evidence has now been presented that this decrease in arylesterase activity is due to the influence of male sex hormones on the biosynthesis of active protein. When a mature boar with a low phenotypic activity of 30 was castrated (Fig. 1*B*) the arylesterase activity increased gradually and had reached after 3 weeks a value (120–140) close to that found before maturity (150). Moreover, this high activity could again be reduced to the low value when 100–250 mgm of testosterone propionate was administered daily intramuscularly The low value was reached about three weeks after hormone treatment began Testosterone had no effect on the enzyme *in vitro*.

This observation has been confirmed with boars of various genotypes Testosterone also enhanced the decrease in arylesterase activity when administered shortly after maturity (Fig 1*A*) Female sex hormones (for example, œstradiol) had no effect on the biosynthesis of this esterase It is possible, however, that in addition to testosterone other agents (for example, the adrenocortical hormones) are also engaged in the regulation of plasma arylesterase activity in boars.

In contrast to mature boars, sows show small normal variations in plasma arylesterase activity

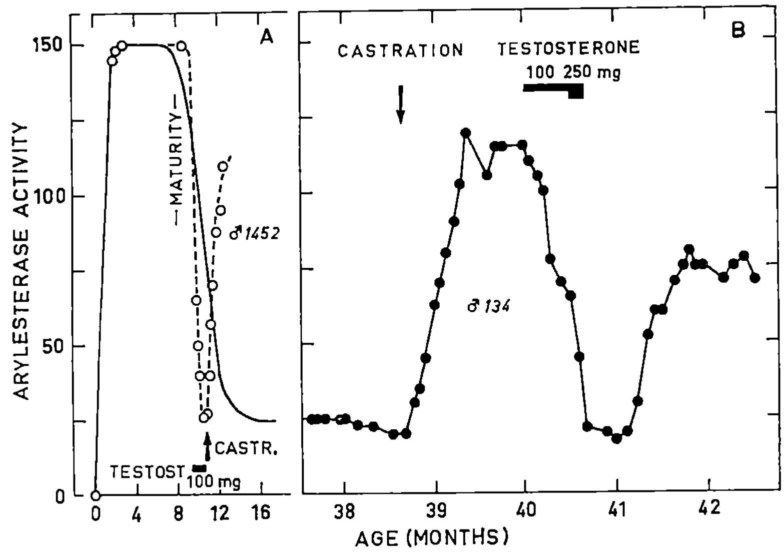

Fig 1 Effect of castration and testosterone propionate on plasma arylesterase activity of boars *in vivo*, the hormone was administered intramuscularly daily as indicated Esterase activity expressed in b_{30}/0 02 ml plasma with phenyl acetate as substrate (Warburg technique) *A*, solid line, variation in plasma arylesterase activity of boars from birth to adult age (generalization), broken line and open circles, effect of testosterone shortly after maturity and before castration of boar 1452 (phenotype 150, weight 140 kgm) *B*, effect of testosterone after castration of boar 134 (phenotype 150, weight 250 kgm)

In some cases, sows had higher esterase activity when sexually matured than two to three months before maturity It was observed that the activity was slightly higher shortly before partus, decreased below the normal value at the time of delivery, increased during 2–3 weeks after delivery and then again decreased to normal It is possible that these variations in activity are partly due to changes in volume of blood during pregnancy Œstradiol had no effect on the esterase activity in mature sows Testosterone had an opposite effect on this activity in sows compared with boars, that is, increase in esterase activity which, however, was not so marked as the decrease in boars

This work was supported by grants from the Swedish Medical Research Council

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A New Unsaturated Uronide isolated from Alginase Hydrolysate

Alginase, which brings about the enzymic hydrolysis of alginic acid, has been found in the organs of a few of the lower animals[1,2] and in some kinds of bacteria[3,4]

According to Kooiman[5], and others[3,4], the enzyme of the bacteria can split alginic acid into smaller units and mannuronic acid monomers Recently, Eller and Payne[6] isolated a bacterium, *Alginomonas alginica*, which can oxidize mannuronic acid, but experiments concerning the isolation of the intermediates have not been reported During work on alginase hydrolysates we found a new type of mannuronide containing an unsaturated sugar acid and a mannuronic acid, the compound is the main component of the breakdown products of alginic acid The isolation and properties of this new compound are described here

One part of homogenized liver of abalone (*Haliotis discus hannai*) was mixed with 4 parts of distilled water at 4° C After 2 hr the mixture was centrifuged, and the supernatant filtered through pulp The clear water extract which was obtained was used as the enzyme solution 7 5 litres of 1 per cent sodium alginate solution were incubated with 750 ml of the enzyme solution at 30° C The *p*H value of the reaction mixture was adjusted to 7 0 with dilute sodium hydroxide during the course of the reaction Progress of the enzyme reaction was followed by noting the increase in reducing power, which was measured by the iodometric method of Somogyi[7] After 20 hr the reaction was stopped by the addition of sulphuric acid The precipitate was filtered off, and the filtrate neutralized with barium carbonate, re-filtered, and evaporated under reduced pressure below 40° C About 300 ml of the concentrate were passed through 'Amberlite *IR*-120' The neutralization with barium carbonate, filtration, and resin treatment were repeated, and the eluates and washing water combined. About 500 ml of the solution were put on a carbon–'Celite' column, 11 × 9 cm, and eluted with water, 10 per cent ethanol, and then 30 per cent ethanol The eluates were collected in 500-ml amounts at a flow-rate of 4 ml /min

The course of the separation on the column was checked by submitting the concentrate taken from each fraction to ascending paper chromatography in the systems *n*-butanol/acetic acid/water (5 2 3) and pyridine / ethyl acetate / acetic acid / water (5 5 1 3) According to the results of the chromatograms, individual fractions obtained from the column were pooled As the water fractions between 3 5 and 25 1 were observed to contain one main product of hydrolysis, they were combined, evaporated under reduced pressure below 40° C, and lyophilized Colourless needle-like crystals were isolated, with an approximate yield of 7 5 gm The crude product was recrystallized from water and had a melting point of 135 5–136·5° C (decomposed) (Calculated for $C_{12}H_{16}O_{12}\,H_2O$, C, 38 92, H, 4 86, found, C, 38 98, H, 5 22 per cent) An infra-red spectrum of this compound in potassium bromide showed two carboxylic groups The molecular weight was 400 ± 40 (Barger's method) This compound showed a strong absorption peak in the ultra-violet at 232 mμ (in water) with $E^{1\%}_{1\,\mathrm{cm}}$ of 143 0, which disappeared on bromination or palladium hydrogenation, indicating the presence of an α,β-unsaturated acid[8]. On paper chromatograms, significant differences between this and the brominated or palladium-hydrogenated compound were observed On hydrolysis with sulphuric acid at 100° C for 1 hr, the spots on chromatograms of the hydrolysates showed the characteristics of mannuronic acid and of mannuronic acid lactone, from which the occurrence of the mannuronic acid moiety in the compound can be inferred The colour reactions of this compound are shown in Table 1

Table 1 COLOUR REACTIONS OF THE UNSATURATED URONIDE

Reagent	Maximum absorption (mμ) or colour tone	Remarks
Carbazole*	530	Same as hexuronic acid
Phenol-H_2SO_4†	480	On bromination shows λ_{max} 480 and 560 mμ
Thiobarbituric acid‡	550	Same as pectin trans-eliminase hydrolysate§
Aniline hydrogen phthalate	brownish red	Same as hexuronic acid
o-Aminophenol	Yellow ⟶ brown	Differs from hexuronic acid which shows blue

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From these results it can be seen that the structure of this new type of uronide may be a diuronide containing $\Delta^{4,5}$-mannuronic acid and the mechanism of the enzyme action seems to involve the splitting of the glycosidic bond by an elimination reaction, thereby introducing unsaturation into the liberated molecules, as already seen in the splitting mechanisms of hyaluronic acid[8] and pectin[9]

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Determination of the Amino-Acid Composition of Bovine Growth Hormone

THE amino-acid composition of bovine growth hormone has been determined[1] by Levy's method[2] This procedure which involves paper chromatography of dinitrophenyl derivatives of amino-acids is subject to certain limitations such as variable recoveries of tyrosine and lack of resolution of the leucine derivatives The development of automated ion-exchange chromatography of amino-acids[3] has prompted a re-investigation of the amino-acid composition of this protein

All the samples of bovine growth hormone employed in this work were furnished by Dr C H Li and were prepared by a previously described method[4] Determination of the nitrogen content of the anhydrous, ash-free protein by the Dumas method has given a value of 16 7 per cent in contrast to that of 15 8 per cent reported elsewhere[1] This value was confirmed by the amino-acid composition reported here and also by an independent analysis performed by the Elek Micro Analytical Laboratories, Los Angeles, California

Hydrolyses were carried out for 24 or 72 hr at 110° C in a sealed evacuated tube Approximately 4 mgm of protein was transferred to the tube in a weighing tube followed by 10 ml of constant-boiling hydrochloric acid redistilled in an all-glass system After hydrolysis, acid was removed by repeated evaporation, the hydrolysate was then diluted to 10 ml with 0·2 *N* citrate buffer, *p*H 2 2 Chromatograms of each hydrolysate were run in triplicate on a Spinco model *MS* amino-acid analyser

Table 1 gives the detailed amino-acid analysis of one bovine growth hormone sample, preparation III Appropriate corrections have been made for hydrolytic destruction Including the tryptophan and amide values of Li and Chung[1], it will be seen that the analysis will account for the weight of the protein and the nitrogen content A summary of the analyses for the three preparations is also shown in Table 1 Li and Chung's results[1] have been included for purposes of comparison Preparation I is from the sample of growth hormone used for the original analysis of Li and Chung It will be seen that the agreement is generally good There are two discrepancies worthy of note The total number of leucine and isoleucine residues obtained in the present analysis ranges from 62 to 68, the higher figure being found in two of the three preparations The older figure of 76 residues would appear to be subject to question In this work significantly higher values for serine have been obtained It is possible that this reflects a shortcoming in the Levy procedure The hydrolysis conditions might also be different to the extent that

Table 1 AMINO-ACID ANALYSES OF BOVINE GROWTH HORMONE

	Preparation III				Summary (residues)		
	Gm amino-acid residues/ 100 gm protein	Deviation*	N as percentage of total N	Residues 45,652 m w	Preparation I	Preparation II	Li and Chung (ref 1)
Lys	6 20	0 5	8 02	22 1	23	23	23
His	2 03	1 4	3 68	6 8	7	7	7
Arg	8 20	1 1	17 39	24 0	26	26	26
Asp	9 06	0 6	6 52	35 9	33	34	35
Thr	4 70	0 8	4 67	25 7	24	25	26
Ser	5 26	1 2	5 01	27 6	25	27	22
Glu	14 03	1 3	9 01	49 6	51	52	50
Pro	3 21	4 3	2 74	15 1	13	14	14
Gly	2 84	0 7	4 12	22 7	22	22	20
Ala	4 61	0 7	5 38	29 6	29	30	31
CyS/2	1 80	5 0	1 35	8 1	8	8	8
Val	3 02	2 4	2 53	13 9	14	14	14
Met	2 41	2 1	1 52	8 4	7	8	7
Ileu	3 27	0 9	2 40	13 2	14	14	76 §
Leu	12 27	0 6	8 99	49 4	54	54	
Tyr	4 26	1 4	2 16	11 9	12	13	11 †
Phe	8 15	0 7	4 59	25 3	25	26	27
Tryp	1 25‡		1 13	3 1			3
NH_2			6 50‡				
	97 57		97 71	392 4	387	397	400

* Mean deviation as ± per cent of mean
† Léonis and Li (ref 6) report 12 residues
‡ Taken from Li and Chung (ref 1)
§ Sum of isoleucine and leucine residues

different destruction factors have been obtained Other differences may be indications of 'microheterogeneity'[5] The tyrosine values are in good agreement with the spectrophotometric data of Léonis and Li[6] for bovine growth hormone The chromatograms did not reveal the presence of any new or unusual amino-acids

I wish to thank Dr C H Li, of the University of California, for the gift of the growth hormone samples Dr S. H Eppstein has contributed valuable advice during the course of this work Microanalyses were performed by the Physical and Analytical Laboratory of the Upjohn Co and the Elek Micro Analytical Laboratories, Los Angeles, California

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Starch Gel Electrophoresis of Hen Egg White, Oviduct White, Yolk, Ova and Serum Proteins

THE Smithies[1] starch gel technique has recently been successfully applied by Lush[2] to demonstrate genetic polymorphism in proteins of the white of a hen egg

Lush[2] referred to two electrophoretically distinct components of crystalline hen ovalbumin described by me[3]. Three—and not two—components were reported in this work, which was later briefly reported[4] The electrophoretic technique used in an examination of hen serum and egg proteins is now described in more detail Suitable starch gels containing 15–17 per cent w/v starch were prepared from B D.H soluble starch and buffered as recommended by Smithies The 'Perspex' troughs containing the starch gels were placed on a metal sheet in contact with an ice block in a cold room at 0° The run was continued for 8 hr , at 400 V and 10 m amp

The components of the 'thin', 'inner thin and 'thick' layers[5] of hen egg white proteins were compared with those of the viscous fluid obtained from the magnum region of the oviducts of laying hens The protein composition appeared to be identical by comparison of pairs of these fluids on a single gel in all four cases Sixteen well-defined bands were observed (Fig 1*a*) The fast anodic components 16 15 . 14 were shown to be identical with three components of a sample of five-times crystallized ovalbumin, A_1 A_2 A_3 respectively, as described by Cann[6].

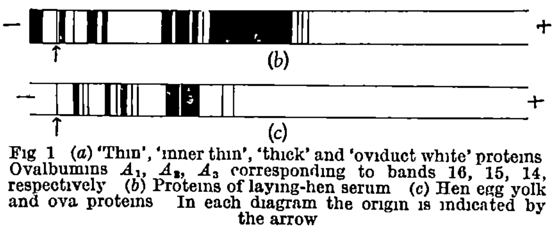

Fig 1 (*a*) 'Thin', 'inner thin', 'thick' and 'oviduct white' proteins Ovalbumins A_1, A_2, A_3 corresponding to bands 16, 15, 14, respectively (*b*) Proteins of laying-hen serum (*c*) Hen egg yolk and ova proteins In each diagram the origin is indicated by the arrow

For comparative purposes the protein patterns of hen serum (Fig 1*b*), egg yolk and ova isolated from the ovary (Fig 1*c*) are given The actual position of the bands in the figures cannot be directly compared since these patterns were obtained from different gels It can be seen that the high resolving power of starch electrophoresis is well suited to the study of species differences in complex protein and lipoprotein mixtures

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Electrophoretic Separation of Conjugated Bilirubin on Paper

IN previous experiments[1] the 0 02 *M* phosphate buffer of *p*H 6 8 was used for electrophoresis of bile pigments on paper Under these conditions (in the absence of proteins) the conjugated bilirubin migrating anodically separates from the unconjugated bilirubin which remains on the starting line The presence of bile salts clearly influences this separation Purified preparations[2] of conjugated bilirubin which are devoid of bile salts migrate in the form of a tail beginning just from the starting line If bile salts are present a sharply defined zone of conjugated bilirubin separates, migrating in front of the bile salts The velocity of migration of this zone in the presence of bile salts is considerably higher than the rate of migration of pure preparations of the pigment.

For example, the purified preparation, containing about 80 per cent of pigment II (as determined according to Billing[3]) after paper electrophoresis (1 m.amp /1 cm of Whatman No 1 paper, potential gradient about 12·5 V /cm , 2 hr 15 min) in 0 02 *M* phosphate of *p*H 6 8, yielded a diffuse zone beginning at the starting line and tailing to 9 cm The same preparation in the presence of about 10-fold excess (on the weight basis) sodium taurocholate (and taurodeoxycholate) migrated simultaneously as a relatively narrow zone between 12 and 13 cm from the start The separation of both fractions of conjugated bilirubin (pigment I and pigment II[4]) could not be observed Analogous results were obtained with the use of sodium acetate–acetic acid buffer of *p*H 5·2, only the rate of migration was slightly lower Barbital buffer of *p*H 8 6 cannot be recommended owing to the instability of conjugated pigments in the alkaline range. The migration of unpurified preparations in this buffer proceeds in the same way as in phosphate buffers

For the purpose of electrophoretic separation of purified preparations of conjugated bilirubin (that is, those which were devoid of excess bile salts) the systems containing pyridine were tested Such systems were described by Grassmann *et al* [5] (*p*H 3 9) and by Runeckles and Krotkov[6] (*p*H 6 0) Both yielded better results than mere aqueous solutions, systems containing more pyridine caused less tailing The system (*p*H 6 1) was selected containing 5 ml of glacial acetic acid, 70 ml of pyridine in 1 litre Under

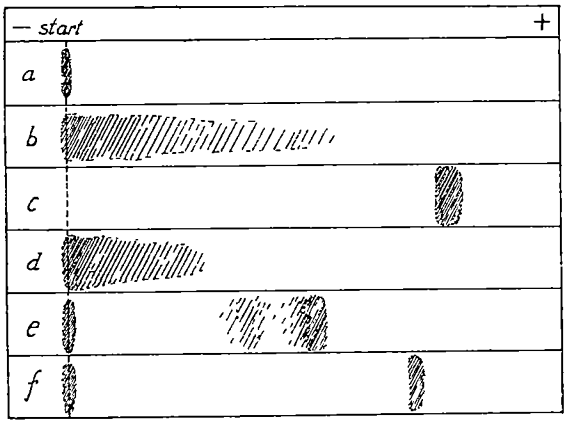

Fig. 1. Electrophoresis of bile pigments on filter paper. *a*, Unconjugated bilirubin in 0.02 *M* phosphate, *p*H 6.8; *b*, conjugated bilirubin (about 80 per cent of pigment II) in 0.02 *M* phosphate, *p*H 6.8; *c*, same in the presence of excess bile salts; *d*, same preparation as in *b* but in 0.08 *M* sodium acetate, *p*H 5.2; *e*, same preparation as in *b* with free bilirubin added, in pyridine acetate; *f*, same as in *e* in the presence of excess of bile salts

these conditions the separation of conjugated from unconjugated bilirubin was complete and both fractions of conjugated bilirubin were visible on the electrophoretograms if an appropriate amount of pigment was applied (corresponding to about 100 µgm of bilirubin on 2–3 cm width of paper). The presence of an excess of bile salts precludes the possibility of distinguishing between the two conjugated forms.

The use of buffers containing pyridine acetate enables the electrophoretic separation even of preparations devoid of bile salts, which was not possible in mere aqueous buffer solutions.

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Enzymatic Hydrolysis of Biliary Conjugates of 3,4-Benzpyrene on Filter Paper

METABOLITES of numerous biologically active endogenous and exogenous organic compounds are excreted in bile and urine as glucuronic acid conjugates. In the course of studying the mechanism of action of carcinogenic polycyclic hydrocarbons, we wished to identify the metabolites of certain of these agents. For this purpose, ^{14}C-3,4-benzpyrene was injected into rats, and bile was collected by an indwelling cannula, utilizing a technique already described[1]. The bile fractions were collected in an automatic fraction collector, and enzymatic hydrolysis with β-glucuronidase was undertaken. Characterization of all the metabolites by this technique proved to be impossible because of the extreme instability of some of the hydrolysis products. We therefore attempted to determine whether the extent of oxidation and the class of conjugate formed could be established by direct analysis of the conjugates.

Two methods utilizing paper chromatography with subsequent enzymatic hydrolysis on paper were developed. Williams and Bevenue[2] have reported the use of a technique for the identification of raffinose and sucrose by enzymatic hydrolysis on paper chromatograms. In our work, migration was initially accomplished along one axis using a solvent system consisting of *n*-butanol/pyridine/water (3 : 2 : 2) for the separation of fluorescent compounds. If satisfactory separation resulted, the enzyme was applied to the individual spots, which were then incubated for 90 min. A second chromatographic separation of compounds in an axis perpendicular to the first was then done, using the same solvent system. Under these conditions, unhydrolysed conjugates were found lying on the diagonal across the paper while those undergoing hydrolysis were usually detected in greater proximity to the solvent front of the second migration.

When the number of metabolites was large or when separation in one dimension was unsatisfactory for further analysis, a two-dimensional chromatogram was prepared prior to application of the enzyme. The papers were then scanned for radioactivity in a two-dimensional scanner (Packard Instrument Co.), following which fluorescence spectroscopic analysis was used to characterize the metabolites. (In certain experiments, non-isotopic 3,4-benzpyrene and pyrene were used and qualitative results were then obtained exclusively by fluorescence analysis.)

Following two-dimensional chromatography, using *n*-butanol/pyridine/water (3 : 2 : 2) as the first solvent system and *n*-butanol / acetic acid / water (4 : 1 : 5) as the second, the individual spots were identified by fluorescence, following which they were incubated for 90 min with a solution containing 100 units of the enzyme. The spots were then cut out of the chromatogram and attached to a new paper strip for a third chromatographic separation for which either the original or the second solvent system was used. Excellent results were obtained by cutting two semi-circles in the fresh paper strip and slipping the cut-out spots into these slits in a manner which permitted satisfactory contact between the two papers. Following development of the chromatogram, the strip was analysed for fluorescent spectra and/or the presence of radioactive foci. By this technique data on the original compound, its conjugation, rate of hydrolysis, and stability and nature of the free parent substance were obtained. Wherever technically feasible, ultra-violet absorption spectroscopy was used in addition to fluorescence spectroscopy.

Basic information concerning the optimal conditions for enzymatic action on filter paper was obtained by studying the hydrolysis of phenolphthalein glucuronides (California Foundation for Biochemical Research) as suggested by Talalay *et al.*[3]. All work was carried out on Whatman No. 1 filter paper using 0.1 molar acetate buffer at *p*H 4.5. Time studies were performed in which a substrate containing 25 µgm phenolphthalein as the glucuronide and 100 units of the enzyme at 37° C were used. These were placed on a screen inside a still Dubnov incubator. These results are presented in Fig. 1, where they are further compared with results obtained following (*a*) substitution of a test-tube for the filter paper; (*b*) changing of the incubation temperature to 25° C;

(c) changing the concentration of phenolphthalein in the substrate to 5 μgm. It will be noted that, in the presence of 100 units of enzyme, 90 per cent hydrolysis occurred after 10 min. incubation at a concentration of 5 μgm. of substrate. In contrast, 60 per cent hydrolysis occurred at the five times greater concentration. As anticipated, a greater yield resulted from hydrolysis in a test-tube, and a lower yield was obtained when the temperature was reduced to 25° C.

Progressively increasing the enzyme concentration above 50 units per spot resulted in diminishing the rate of increase of hydrolysis, suggesting that 50 units of enzyme would provide an excess for all concentration ranges likely to be encountered (Fig. 2). The constant use of 100 units of enzyme in the presence of altered concentrations of the substrate resulted in a hydrolysis-rate of 100 per cent down to 60 per cent with the decreasing yield paralleling the increase in concentration of substrate (Fig. 3). At the concentration-range encountered in work on bile, that is, between 1 and 2 μgm. of metabolite, a yield of almost 100 per cent may properly be anticipated.

Our work has further established that hydrolysis does not occur if the enzyme is denatured, while little hydrolysis is noted when the strips are not perfectly free of solvent after chromatography. Hydrolysis was uniformly decreased in the presence of bile, and for this reason enzymatic hydrolysis was carried out only after chromatography in all cases. This emphasizes the need for enzymatic hydrolysis subsequent to chromatography. Hydrolysis of conjugates of oxidation products of benzpyrene does not occur as rapidly as the hydrolysis of phenolphthalein glucuronide, and therefore the hydrolysis time was increased to 90 min. in contrast to the 10 min. used for phenolphthalein. Detailed analysis of the biliary metabolites of polycyclic aromatic hydrocarbons will be presented in a subsequent publication.

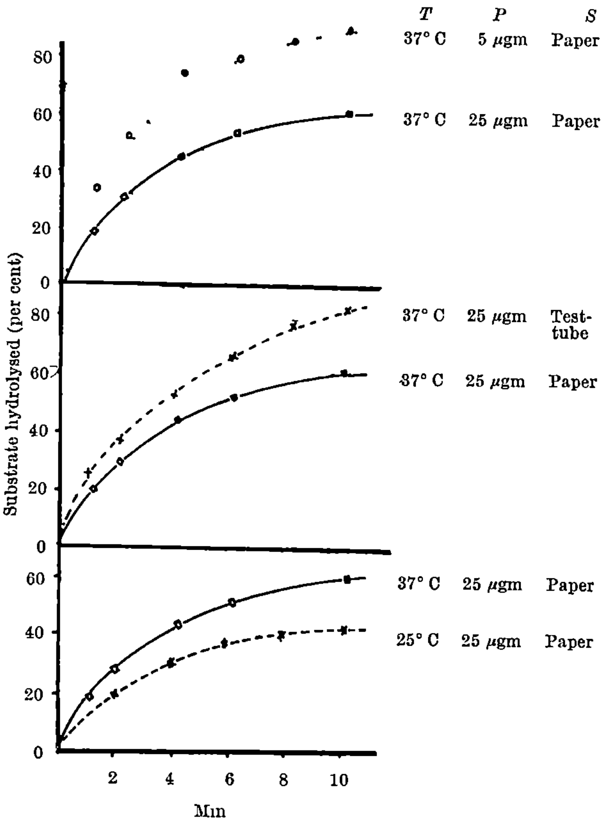

Fig. 1. Hydrolysis of phenolphthalein glucuronide on filter paper by β-glucuronidase. Effect of temperature (T), phenolphthalein glucuronide concentration (P) and site (S)

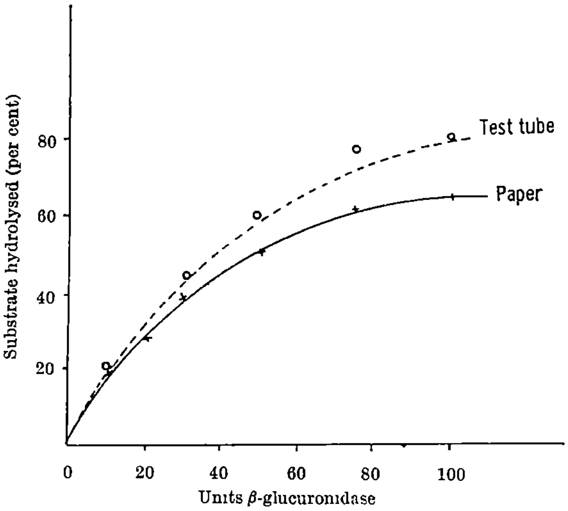

Fig. 2. Effect of enzyme concentration

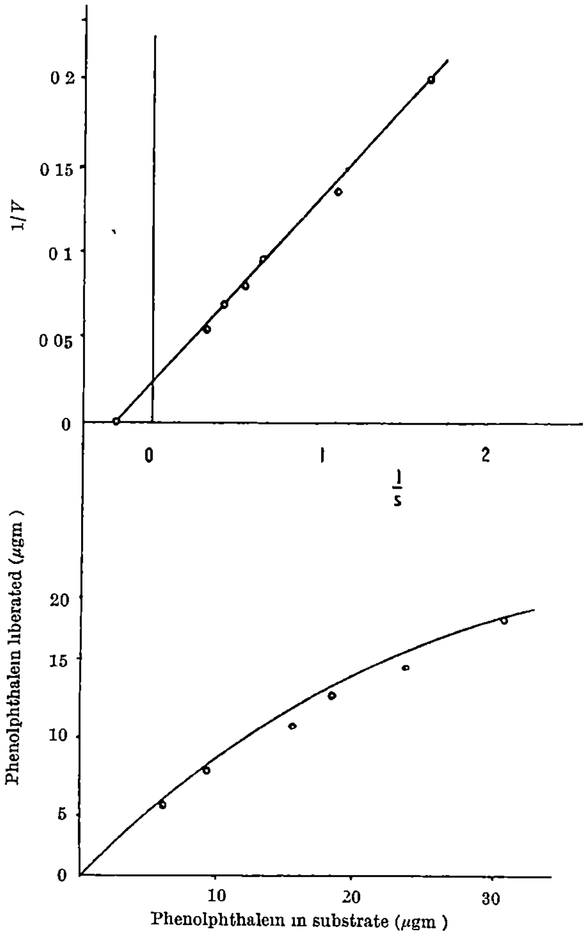

Fig. 3. Effect of substrate concentration

This investigation has been supported by grant CS-9156 from the Field Investigations and Demonstrations Branch of the National Cancer Institute and grant *E*–215 from the American Cancer Society

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PHYSIOLOGY

Respiratory Chain Phosphorylation in Sub-Cellular Particles isolated from the Hepatopancreas of *Carcinus maenas*

IN previous publications[1,2] I have shown that the respiratory chain present in respiring particles isolated from the hepatopancreas of *Carcinus maenas* (the common shore crab) contains pyridine nucleotides, flavoproteins and cytochromes c_1+c, b, a and a_3 in the proportions 19 3 1 1 1 1 All these respiratory pigments were shown to undergo oxidation and reduction during the oxidation of succinate, but the pyridine nucleotides reacted too slowly to be considered as a member of the respiratory chain associated with the oxidation of succinate This communication presents evidence which shows that the oxidations catalysed by this respiratory chain are associated with the production of adenosine triphosphate

The respiring particles used in these experiments were isolated by differential centrifugation of homogenates of hepatopancreas in 0 25 *M* sucrose[1] The P/O (phosphorus to oxygen) ratios associated with the oxidation of succinate, fumarate and α-oxoglutarate were measured using Warburg respirometers and an inorganic phosphate uptake method. The respiring particles were suspended in a reaction mixture containing 5 m*M* substrate, 5 m*M* magnesium chloride, 1 m*M* adenosine triphosphate, 5 m*M* phosphate buffer, *p*H 7·4 (10 m*M* for α-oxoglutarate), and malonate in the concentrations shown in Table 1 Sucrose was used to adjust the tonicity to 0 25 osmolar 0 9-ml samples of the suspension of respiring particles were placed in the main compartment of a 6-ml respirometer flask, 0 1 ml. of a 20 per cent potassium hydroxide solution was placed in the centre well and 0 1 ml of a 0 25 *M* glucose solution containing 4 mgm of crystalline hexokinase was in the side-arm of the flask After a 7-min equilibration period at 25° C the hexokinase–glucose solution was mixed with the contents of the main compartment and the oxygen consumption measured The reaction was stopped by the addition of 0 1 ml of a 40 per cent trichloroacetic acid solution The uptake of orthophosphate was determined as the difference between the orthophosphate contents of the experimental flasks and flasks to which the hexokinase was added after the trichloroacetic acid. Sumner's method[3] was used to assay orthophosphate. The results of these experiments are given in Table 1

Table 1 P/O RATIOS ASSOCIATED WITH THE OXIDATION OF VARIOUS SUBSTRATES BY THE RESPIRING HEPATOPANCREAS PARTICLES

Substrate	P/O ratio*	Qo_2 (μl /hr /mgm protein)*
Succinate	2 04 ± 0 32 (11)	29 9 ± 4 8 (10)
Fumarate	2 06 ± 0 46 (6)	18 7 ± 4 5 (6)
α-Oxoglutarate	2 72 ± 0 23 (7)	29 0 ± 4 8 (7)
+ 10 m*M* malonate	2 35 and 2 55	14 8 and 14 7
+ 30 m*M* malonate	2 07 and 1 89	12 0 and 11 9

* Data given as mean ± standard deviation (number of determinations)

The mean value of the P/O ratios allied to the oxidation of 4 5 m*M* succinate is 2 04 This value lies between those reported for rat heart sarcosomes, P/O ratio = 0 95, and for rat liver mitochondria, P/O ratio = 2 17 (ref 4) It should be noted that the P/O ratio associated with the oxidation of succinate by rat liver mitochondria is inflated by the phosphorylation associated with the further oxidation of the fumarate produced by the action of succinic acid dehydrogenase This would not appear to be the case in the respiring hepatopancreas particles, since the mean value of the P/O ratio associated with the oxidation of fumarate, 2 06, is only fractionally higher than that for the oxidation of succinate The P/O ratios for the oxidation of α-oxoglutarate by the respiring hepatopancreas particles is just lower than the value given by rat heart sarcosomes, P/O ratio = 2 80 ± 0 20 (10), in experiments where a similar method of assay to that described above was used (Beechey and Alcock, unpublished results) In experiments where the further oxidation of succinate was prevented by the addition of malonic acid, the P/O ratio associated with the oxidation of α-oxoglutarate was actually lowered It was 2 45 in the presence of 10 m*M* malonic acid and 1 92 in the presence of 30 m*M* malonic acid These results suggest that the malonic acid is acting as an uncoupling agent in this system

It was Loomis and Lipmann[5] who first demonstrated the uncoupling of respiratory chain phosphorylation from oxygen consumption by dinitrophenol The effect of dinitrophenol on the coupled oxidation succinate and fumarate by the hepatopancreas is shown in Fig 1 It can be seen that the complete uncoupling of phosphorylation from respiration occurs at concentrations of dinitrophenol greater than 60 μ*M* Thus the sensitivity of the phosphorylating mechanisms in these hepatopancreas particles to dinitrophenol is of the same order as that of the corresponding systems in mammalian liver mitochondria[6] and heart sarcosomes[7]

The results presented here show that the oxidation of succinate, fumarate and α-oxoglutarate by these respiring hepatopancreas particles is associated with the production of adenosine triphosphate and also

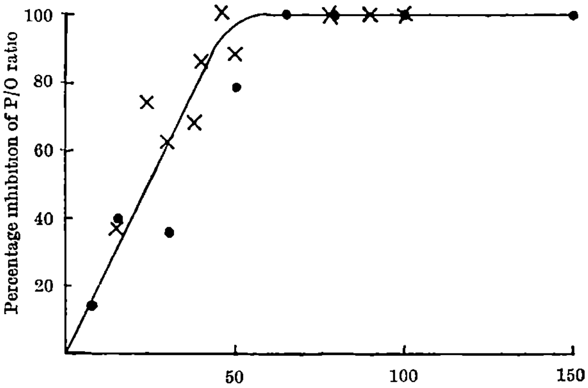

Fig 1 Effect of dinitrophenol on the P/O ratio associated with the oxidation of succinate (●) and fumarate (×) by the respiring hepatopancreas particles

that the phosphorylating mechanism is uncoupled by dinitrophenol. The P/O ratios measured are quite similar to those given by mammalian mitochondria These facts, together with the knowledge that these particles contain an active respiratory chain[1,2], suggest that respiratory chain phosphorylation occurs on these sub-cellular particles isolated from the hepatopancreas of *Carcinus maenas*

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Growth Hormone Activity of the Anterior Pituitary Lobe of the Male Rat at Various Ages

WITHIN the past few years several reports have dealt with the relationship between the age of an animal and the pituitary content of growth hormone. Pigs[1,2], ox[3], and the rat[4,5] have been examined In general, it has been found that although the total amount of growth hormone in the pituitary gland increases greatly with age, the concentration of the hormone in the gland tends to remain fairly constant. The present work on the male rat supports this concept, but also indicates that relative to body-weight there may be a decreased amount of total growth hormone in the anterior lobe as the animal advances in age. This latter relationship has likewise been found in the larger species which have been examined, but previous evidence[5] on the rat has indicated a lack of correlation between the total pituitary growth hormone/body-weight ratio and age

Male rats of the Long-Evans strain which were raised in the Bucknell University colony were used predominantly as the source of the pituitaries which were assayed At ages of 10, 30, 60, 90, 180, 360 and 600 days the animals were killed by decapitation, the pituitary rapidly exposed, the posterior lobe removed and discarded, and the anterior lobe removed, weighed and placed in acetone. After 24 hr. in acetone the glands were air dried and placed in a vacuum desiccator until time for use At the time of assay the desiccated anterior lobes were weighed, homogenized in saline, and injected intraperitoneally into young hypophysectomized rats prepared as indicated below The homogenate, freshly prepared each day, was administered once daily for 4 days With the exception of two of the age periods, the injections were given to assay animals at three dosage-levels, namely, 80, 280 and 720 μgm of dry anterior pituitary a day Parallel injections of standard growth hormone were given to another three groups of hypophysectomized animals at dosage-levels of one-tenth the amounts of homogenate used Another group of animals received saline The 10- and 30-day-old pituitaries were injected at two levels only 80 and 280 μgm. per day for the 10-day-group, and 32 and 392 μgm daily for the 30-day pituitaries. For each of these two ages the growth hormone standard was likewise given in amounts equal to one-tenth the homogenate used Six to eight hypophysectomized rats were used at each dosage-level for all groups

The recipient animals used for each assay were albino male rats obtained from a commercial source at 22–24 days of age, and hypophysectomized when they were 28 days old At 40–42 days of age they were randomized into a number of groups and the four-day period of injection begun[6] Rats which had gained more than 8 gm or lost more than 3 gm post-operatively were eliminated from the assay The operated animals were maintained on calf meal, fresh bread, and 10 per cent glucose in their drinking water

Twenty-four hours after the last injection the rats were killed by ether anæsthesia and the tibias removed and prepared for epiphyseal measurement according to the procedure of Evans, Greenspan *et al* [7,8] Statistics were calculated according to methods given by Emmens[9]

It can be seen in Table 1 that the concentration of growth hormone activity in the anterior pituitary gland showed little change with age, the ranges of the estimated potencies being essentially the same for all groups In Table 1 are also given the anterior pituitary- and body-weights at the various ages examined, it will be noted that the ratio of dry (or wet) anterior pituitary-weight to body-weight exhibited a decrease with age The ratio was highest at 10 days and then dropped to a somewhat constant value for the ages up to 3 months A further decrease was observed at 6 months, and the still lower ratio seen at 12 months was maintained at 20 months of age Thus, with a rather similar concentration of growth hormone in the pituitary at all the ages investigated, these decreasing pituitary-weight/body-weight ratios would indicate that in the anterior pituitary gland there is less total growth hormone per gram of body-weight as the male rat ages

Solomon and Greep[5], using female rats, found that the growth hormone concentration in the whole

Table 1 THE RELATION OF AGE TO THE GROWTH HORMONE POTENCY OF THE ANTERIOR PITUITARY LOBE AND THE ANTERIOR PITUITARY-WEIGHT/BODY-WEIGHT RATIO IN THE MALE RAT

Age (days)	Potency and range* (per cent)	N†	Anterior pituitary average weights		Average body-weights (gm)	Ratio of dry pituitary-weight to body-weight mgm of pituitary per gm rat ($\times 10^{-3}$)
			Wet (mgm)	Dry (mgm)‡		
10	11 3, 8 2–15 6	162	1 20 ± 0 03§	0 19	21 ± 0 3§	9 05
30	15 8, 7 1–35 3	26	3 76 ± 0 04¶	0 60	84 ± 1 8¶	7 14
60	19 8, 9 0–44 0	31	7 5 ± 0 1¶	1 42	191 ± 3¶	7 43
90	13 3, 9 4–19 0	24	9 2 ± 0 1¶	1 74	247 ± 4¶	7 04
180	14 0, 8 1–24 1	26	9 4 ± 0 3	1 85	332 ± 8¶	5 57
360	20 0, 10 5–36 5	25	10 0 ± 0 3	1 96	386 ± 8¶	5 08
600	17 2, 11 3–26 3	18	11 0 ± 0 5	2 04	401 ± 15	5 09

* Growth hormone potency of pituitary preparation and range of potency at 95 per cent significance level
† Number of pituitaries and number of animals weighed
‡ Desiccated pituitaries of a given age were weighed collectively, thus standard errors are not available
§ Standard error of the mean
¶ $P < 0\ 001$ that the difference between this value and that of any younger age group in this column is due to chance

pituitary likewise fluctuated within only a small range as the animals aged They observed in addition, however, that the weight of the whole pituitary continued to increase in an approximate proportion to the body-weight increments of the animals Therefore the total amount of pituitary growth hormone relative to body-weight was calculated to change very little, and showed neither a regular decrease nor increase throughout the age range studied Whether the apparent discrepancy between this latter observation and that of the present communication, in this regard, is due to the sex of the animal investigated or to anterior lobe and whole pituitary differences is not clear In support of the present results, it has been reported in both male and female pigs[1,2] and in the cow[3] that the total amount of growth hormone in the anterior lobe shows a decline in relation to body-weight as these animals age

It is recognized that the amount of hormone present in a gland may well not indicate the amount or rate of secretion, and information concerning blood-levels of, and tissue sensitivities to, growth hormone at various age periods would be of interest

The bovine growth hormone used as the standard was a gift of the Endocrinology Study Section of the National Institutes of Health This work was supported by National Science Foundation grant *G*–5069

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Permeability of the Mammary Gland to Sodium-24, Potassium-42, Chlorine-36 and Iodine-131

Phosphorus-32 and calcium-45 appear in the blood of goats a few minutes after their administration through the teat[1,2] The regulation of milk electrolyte content is little understood, and it seems necessary to examine the problem of udder permeability further in order to understand these problems better

In the present investigation the permeability of the mammary gland to sodium-24, potassium-42, chlorine-36 and iodine-131 has been studied, using goats in the first half of lactation Polyethylene glycol (mean mol wt 4,000), which does not penetrate the udder epithelium[3], was used as a reference substance The disturbances in udder function, which often follow the injection of fluid through the teat, have been avoided so far as possible by reducing the injected amount of fluid The injected solutes are rapidly and fairly evenly distributed throughout the whole milk volume of the injected udder half[3].

Half of an udder was cannulated and about 2 ml physiological saline containing tracer (10–40 μc. of sodium-24, potassium-42 or iodine-131, 1–2 μc of chlorine-36) and polyethylene glycol was slowly injected, usually 4 hr after milking

In the experiments with iodine-131, 5-ml samples of milk were drawn at regular intervals, and the disappearance of iodide was examined by comparing the expansion of the iodide and polyethylene glycol spaces

In the other experiments the udder was cannulated some time after the injection and the milk collected in fractions When the milk ceased to flow spontaneously an additional amount of milk was obtained with oxytocin The volume of milk still left in the udder was calculated from the amount of polyethylene glycol retained in the udder.

The radioactive isotopes were counted in a Friesecke and Hoepfner *FH* 49, using an ordinary Geiger–Muller-tube for sodium and iodide and a proportional counter for potassium and chloride Polyethylene glycol was determined turbidimetrically[4]

The results from one experiment with iodine-131 are summarized in Table 1 Between the 4th and the 19th hr of the experiment the volume of water in the milk, measured as the distribution volume of polyethylene glycol, expands from 95 to 145 ml while the iodide space increases to nearly 5 litres during the same time

Table 1

Time after injection (hr)		4 5	6 3	8	9 8	11 3	19
Distribution volume (ml)	Polyethylene glycol	95	98	102	120	126	145*
	Iodine-131	265	346	447	875	1,192	4,606

* The goat used in this experiment was in an advanced stage of lactation, which explains the low production of milk

After the first 4 5 hr 40 per cent of the dose was still present in the udder This agrees well with the results from other experiments with iodide

The results of the experiments with sodium-24, potassium-42 and chlorine-36 are given in Table 2

Table 2

	No of experiments	Time between injection and sampling (hr)	Activity remaining in the milk (per cent)
Sodium-24	2	1	13
Sodium-24	2	4	1
Potassium-42	3	4	36
Chlorine-36	6	4	37
Sodium-24*	1	2	10
Chlorine-36*			70

* Examined simultaneously in the same udder half

It is evident that the sodium ions disappear far more rapidly than the others The difference between sodium and chloride is especially well demonstrated in the experiment where they were examined simultaneously

The ratio of the concentrations of the isotope and polyethylene glycol is usually lower in the last fractions of milk. Table 3 gives the results of one typical experiment with each isotope

Table 3

Fraction No	1	2	3	4	5	6	7	8
Sodium/PEG	38	33	30	27	29	7	7	
Potassium/PEG	21	20	19	18	16	16		
Chlorine/PEG	38	35	32	24	26	25	26	27

PEG, polyethylene glycol

Clearly the epithelium of the udder is fairly permeable to the ions examined, and Table 3 tends to show that this applies at least to the epithelium of the 'proximal' parts of the gland, that is, the finer ducts and the alveoli. More information is obviously needed before definite conclusions can be drawn with regard to the processes involved in the permeation of these solutes. In view of the low sodium content of the milk the apparently high permeability of the udder with regard to sodium ions may, however, indicate participation of both diffusion and active transport of this ion.

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Particle-bound and Free Catecholamines in Dog Hearts and the Uptake of Injected Norepinephrine

Submicroscopic structures containing catecholamines have been found by high-speed centrifugation of homogenates or pressed juice of adrenal medulla[1-3], splenic nerves[4-6] and brain[7]. A granular fraction containing catecholamines has been isolated from the pressed juice of cyclostome hearts[8]. It was the purpose of this investigation to see whether also in mammalian hearts the catecholamines are stored in a particle-bound state; the question was also examined whether injected norepinephrine is taken up by submicroscopic cell elements. This is of interest since Raab and Gigee[9] reported a specific avidity of the heart muscle to absorb and store catecholamines.

Six puppy hearts, which have a high catecholamine content (Wegmann *et al.*, unpublished data), were submitted to successive low- and high-speed centrifugation after homogenization. The catecholamine content in the different fractions was analysed fluorimetrically. In another series of 7 animals an identical procedure was applied 20 min. after the intraperitoneal administration of 10 mgm. norepinephrine/kgm. The hearts were dissected into very small pieces in ice-cold physiological sodium chloride solution. After washing three times and re-suspending in ice-cold saline solution the hearts were homogenized in a Potter–Elvehjem glass homogenizer with a loose-fitting pestle. The homogenates were centrifuged at 600–1,000*g* for 10 min. The homogeneous supernatant was again centrifuged at 50,000–100,000*g* for 60 min. The sediment from this centrifugation was taken up in a few ml. of *N* hydrochloric acid. Both sediment and supernatant were then deproteinized by trichloracetic acid for fluorimetric estimation according to Euler and Lishajko[10] after adsorption on alumina and elution with acetic acid. Only a set of two filters (excitation at 412 mμ, emission at 508 mμ) was used; thus norepinephrine and epinephrine were determined together.

The catecholamine content in the different fractions of the normal hearts is shown in Table 1. The supernatant layer obtained by low-speed separation did not contain microscopically visible cell structures. After high-speed centrifugation 67–85 per cent (average 74.5 per cent) of the catecholamines were located in the small sediment.

The catecholamine content in the different fractions 20 min. after injection of 10 mgm./kgm. norepinephrine is shown in Table 2. In these experiments only 9–31 per cent (average 19.6 per cent) of the catecholamines were found in the sediment after high-speed centrifugation, the greater part being recovered in the supernatant. The difference between the results in the two series is highly significant ($P < 0.001$).

Table 1. Distribution of Catecholamines in Heart Homogenates of Puppies

Weight of heart (gm.)	Total homogenate (μgm./gm.)	Supernatant (800*g*) (μgm./gm.)	Supernatant (800*g*) Total homogenate (per cent)	Sediment (75,000*g*) (μgm./gm.)	Sediment (75,000*g*) Supernatant (per cent)
13	1.00	0.54	54	0.36	67
14	0.64	0.34	50	0.26	76
16	1.20	0.56	47	0.38	68
10.5	1.10	0.41	37	0.35	85
12.5	1.04	0.58	56	0.41	71
13.5	0.71	0.34	48	0.27	80
Mean	0.95	0.46	49.0	0.34	74.5

Table 2. Distribution of Catecholamines in Heart Homogenates of Puppies 20 min. after Intraperitoneal Injection of 10 mgm. Norepinephrine/kgm.

Weight of heart (gm.)	Total homogenate (μgm./gm.)	Supernatant (800*g*) (μgm./gm.)	Supernatant (800*g*) Total homogenate (per cent)	Sediment (75,000*g*) (μgm./gm.)	Sediment (75,000*g*) Supernatant (per cent)
12	2.15	1.16	54	0.36	31
11.5	12.2	10.33	85	0.93	9
10	4.00	2.64	66	0.78	30
7	3.76	1.83	49	0.39	21
11	2.75	1.44	52	0.41	29
12	3.90	2.59	67	0.34	13
18	13.3	10.92	82	0.42	4
Mean	6.01	4.42	65.0	0.52	19.6

This investigation demonstrates the presence of particle-bound catecholamines in dog hearts. More than 95 per cent of injected norepinephrine found in the supernatant after low-speed centrifugation were not particle-bound. Whether or not these large amounts recovered in the soluble fraction were intra- or extra-cellular cannot be stated at the present time.

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Response of the Reticulo-Endothelial System to Stimulation with Œstrogens

We have previously shown that diethylstilbœstrol and tri-*p*-anisylchloroethylene are strong stimulants of the reticulo-endothelial system[1]. We have also found that when the reticulo-endothelial system is stimulated by diethylstilbœstrol the body defence is raised, as indicated by increased phagocytic activity, elevation of the serum γ-globulin to about three times the normal level, increased antibody formation, and the protection of mice and guinea pigs against lethal doses of virulent bacteria. In previous experiments it was found that the minimum dose of diethylstilbœstrol required to stimulate the reticulo-endothelial phagocytes is about a hundred times greater than that necessary to induce œstrus[2] and that after treatment with 0.5 mgm. diethylstilbœstrol daily for 6 days the phagocytic activity returns to normal about 8 days later[3]

The present work was carried out to ascertain (1) the speed of action of diethylstilbœstrol and tri-*p*-anisylchloroethylene in stimulating the reticulo-endothelial system, (2) whether this stimulation is maintained during repeated treatment with diethylstilbœstrol

In the following experiments the phagocytic activity was taken as the measure of stimulation of the reticulo-endothelial system, it was assessed by the rate of disappearance of a known amount of specially prepared carbon from the circulating blood, the method being a modification of that of Biozzi *et al*[4] The procedure used has been described in previous communications[5] The total body phagocytic activity or phagocytic index is denoted by the symbol *K*

*Speed of action of diethylstilbœstrol and tri-*p*-anisylchloroethylene* Fifty-five male white mice (*T O* Swiss strain) of 20–25 gm body-weight were used in this experiment Fifteen animals each received 0 1 mgm diethylstilbœstrol in 0 1 ml arachis oil subcutaneously once daily, fifteen received 0 5 mgm diethylstilbœstrol in 0 1 ml arachis oil subcutaneously once daily, and a further fifteen received 1 mgm tri-*p*-anisylchloroethylene in 0 2 ml olive oil orally once daily On the first. second and third day after the commencement of treatment with each compound the phagocytic activity was measured in groups of 5 animals by the carbon method referred to above Ten animals were used as controls and received no treatment

The results are shown in Table 1 The control animals showed a phagocytic index of 15 ± 2 Compared with the control values it can be seen that on the first day after the commencement of treatment with each of the compounds there is slight stimulation of the reticulo-endothelial system, by the second day there is moderate stimulation, and by the third day the system has become strongly stimulated

Effect of repeated treatment with diethylstilbœstrol Forty male white mice (*T O* Swiss strain) of 20–25 gm body-weight were used in this experiment Ten of the animals were used as controls and received no treatment The remaining 30 animals were divided into three groups of 10 and were treated as follows Group *A*, 10 animals each received 0 5 mgm, diethylstilbœstrol in 0 1 ml arachis oil subcutaneously once daily for 6 days On the eighth day after the commencement of treatment the phagocytic activity was measured in each animal by the carbon method, and the animals were then killed Group *B*, 10 animals were given diethlystilbœstrol for 6 days, as described above They were then left for 3 weeks, then given a second course of diethylstilbœstrol for 6 days, on the following day the phagocytic activity was measured in each animal, then the animals were killed Group *C*, 10 animals were given a similar course of diethylstilbœstrol for 6 days They were left for 3 weeks then given a second course of diethylstilbœstrol for 6 days, they were now left for a further 3 weeks then given a third course of diethylstilbœstrol for 6 days On the following day the phagocytic activity was measured in each animal and then the animals were killed

Table 1 SPEED OF ACTION OF DIETHYLSTILBŒSTROL AND TRI-*p*-ANISYLCHLOROETHYLENE IN STIMULATING THE RETICULO-ENDOTHELIAL SYSTEM

Compound used	Daily dose	Day after treatment commenced	Mean phagocytic index (*K* value) ± *S E*
Diethylstilbœstrol	0 1 mgm in 0 1 ml arachis oil subcutaneously	First	18 ± 1
		Second	37 ± 4
		Third	40 ± 1
Diethylstilbœstrol	0 5 mgm in 0 1 ml arachis oil subcutaneously	First	19 ± 1
		Second	33 ± 7
		Third	72 ± 4
Tri-*p*-anisylchloroethylene	1 0 mgm in 0 2 ml olive oil orally	First	25 ± 1
		Second	37 ± 2
		Third	60 ± 4
Controls			15 ± 2

Table 2 EFFECT OF REPEATED TREATMENT WITH DIETHYLSTILBŒSTROL ON PHAGOCYTIC ACTIVITY OF RETICULO-ENDOTHELIAL SYSTEM

Animal group	Mean phagocytic index (*K* value) ± *S E*
Group *A*	66 ± 3
Group *B*	58 ± 1
Group *C*	77 ± 1
Controls	16 ± 1

The results are shown in Table 2 The control animals showed a phagocytic index (*K* value) of 16 ± 1 Compared with the controls it can be seen that the total phagocytic activity or phagocytic index *K* remains high in all three experimental groups *A*, *B* and *C* and that the phagocytes continue to respond to stimulation during repeated treatment with diethylstilbœstrol

The above results show that œstrogen treatment produces strong stimulation of reticulo-endothelial activity by the third day after the administration of the drug is commenced When the treatment is stopped the stimulative effect lasts for about 1 week, during which it gradually diminishes, and by the eighth or ninth day the phagocytic activity has returned to normal[3] These findings may be of clinical interest since they indicate the short period during which œstrogens need be used to raise the body defence in acute illness The results also indicate that the reticulo-endothelial phagocytes continue to respond to stimulation during repeated courses of treatment with diethylstilbœstrol over a period of at least 9 weeks

We gratefully acknowledge financial assistance from the Medical Research Council and the Tobacco Manufacturers' Standing Committee

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Production of Aortic Occlusion resembling Acute Aortic Embolism Syndrome in Cats

A SYNDROME of acute aortic embolism occurring in cats resembles the condition found in man An embolus, usually fragmented from an intracardiac thrombus, lodges at the iliac bifurcation, disrupting blood supply to the rear limbs The symptoms include paraplegia, loss of femoral pulse, cold rear extremities, initial muscular tenseness of the rear limbs followed by atrophy, and pain In an effort to reproduce the signs of aortic embolism or 'saddle embolus', the posterior aortas of thirteen cats were occluded by various methods The work here suggests that the blood-clot occluding the aorta is responsible for the hind-limb paralysis, accomplishing this by some means other than mechanical circulatory blockage.

Experimental aortic ligations have been performed frequently on laboratory animals, many being concerned with occlusion of the aorta above the renal arteries[1,2]. According to Adams[3], suprarenal aortic occlusion causes paralysis by ischæmic damage to the spinal cord, whereas infrarenal aortic occlusion accomplishes this by peripheral nerve damage. Giuseffi[4] states that infrarenal aortic occlusion of more than 4 hr. is apt to produce paraplegia in the dog. In his work on dogs, Brooks[5] reports that there is a constant marked disturbance in function of the posterior extremities following ligation of the aorta between the inferior mesenteric and external iliac arteries.

Long-term infrarenal aortic ligation work on the cat is not readily found in the literature. Because clots were found at the iliac bifurcation in naturally occurring cases, these experiments were designed to look into the effects of low aortic ligation on symptomatology referable to the rear limbs.

Cats between the ages of 7 months and 5 years were divided into 3 groups according to the surgical procedure used on each. A pre-surgical aortogram was performed on every cat to determine patency of the aorta. Braided stainless steel wire or heavy silk was used as the ligature material.

Group 1 consisted of five cats in which a single ligature was placed around the aorta at the level of the iliac bifurcation. Complete occlusion of the aorta was determined by aortography immediately after surgery. From the day following ligation until they were killed one month later, these cats showed no symptoms of paraplegia. Subsequent aortograms demonstrated a progressive increase in collateral circulation at the ligation site. Although no femoral pulse could be palpated post-surgically, the rear legs remained warm.

Group 2 consisted of five cats which were ligated as above with a second ligature placed approximately 1·5 cm. proximal to the first ligature. In most cases the circumflex iliac arteries were also ligated since they were the only vessels emerging from the aorta between the two aortic ligatures. As in Group 1, these cats demonstrated no symptoms of paraplegia. The purpose of the double ligation was to determine whether or not a 1·5 cm. 'dead space' in the aorta would cause a slower build-up of collateral circulation than a single ligature. Results did not differ from Group 1.

Group 3, in which a clot was produced in the posterior aorta of three cats, was done in order to simulate the naturally occurring syndrome of aortic embolism. A permanent ligature was placed around the aorta at the iliac bifurcation as in Group 1. The circumflex iliac arteries were obliterated as in Group 2. A length of umbilical tape was then placed around the aorta 1·5 cm. above the first ligature to occlude the aorta temporarily. When the tape was drawn snugly, blood was trapped between the ligatures. 10–20 units of bovine thrombin (about 0·2 c.c. of fluid) was injected through a 27-gauge needle into the lumen of the aorta between the ligatures. After allowing 5 min. for a clot to form, the temporary tape ligature was removed. On the day after surgery all three cats were paralysed in the rear limbs. Sensory and motor functions were lacking. There was no femoral pulse and the rear limbs were cold to the touch. Muscle atrophy of the rear limbs was extensive at the end of one week. Aortography showed no increase in collateral circulation. After ten days of unabated paraplegia, all three cats were killed. In contradistinction to Groups 1 and 2, necropsy showed a small, ragged clot extending approximately 1 cm. above the ligature at the iliac bifurcation. The muscles of the rear limbs were pale in colour. There were no gross lesions of the spinal cord or peripheral nerves to the rear limbs.

The following conclusions are drawn from this preliminary study: (1) ligation of the aorta of the cat at the iliac bifurcation has no demonstrable effect on locomotion in spite of the diminution of femoral pulse, and (2) since the methods used in Groups 1 and 2 produced no paretic symptoms but the Group 3 method did, the clot apparently played some part in causing the paraplegia. Collateral circulation was evidently impeded in some way by the presence of the clot. It is doubtful whether this happened by mechanical blockage of collateral channels from the aorta since the clot extended up the aorta from the iliac bifurcation for only a short distance. In fact, the clot did not extend as far up the aorta as the proximal ligature in Group 2. The similarity of the symptoms of naturally occurring and experimentally produced aortic embolism is striking and leads us to believe that the mechanism involved in producing paraplegia is the same in both instances.

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RADIOBIOLOGY

Mast Cell Turn-over in Adult Mice

Mast cells are generally thought to be derived from undifferentiated mesenchymal cells and perhaps heteroplastically from lymphocytes, plasma cells or histiocytes[1]. Various conditions can cause mast cell granules to be discharged in a manner that has led some investigators to believe the cell is destroyed in the process. Yet Padawer[2] reported never having seen a mast cell in mitosis during his extensive investigation of these cells with colchicine and related substances. This does not preclude the possibility of mast cells being continually replaced by transformations from a population of proliferating precursor cells. Extensive mast cell degranulation occurs after administration of compound 48/80 and is followed within 4 days by the re-appearance of granulated mast cells[3]. This sequence of events was investigated radioautographically with sulphur-35 by Watson and Kennedy[4]. They observed sulphur-35 uptake within 24 hr. by 'ghost cells' which had a tissue distribution equivalent to that of the original granulated mast cells.

The question of mast cell turn-over has been investigated in the present experiment by using radioautography after repeated injections of tritiated thymidine because this method will detect cells synthesizing deoxyribonucleic acid (DNA) even if they represent only a very small fraction of the population, and because transformations to mast cells from a proliferating population of precursor cells would be detected through the gradual accumulation of labelled nuclei in the mast cell population.

Six adult, female, strain *A/Jax* mice were given 6 injections of 100 µc tritiated thymidine at 4-hr intervals and killed at 1, 2, 3, 4, 8, or 21 days after the initial injection Tissues were fixed in Carnoy's fluid and processed for radioautography using a fluid emulsion method described elsewhere[5] with the modification that a 'Celloidin' coating over the sections was omitted The radioautographs were developed after exposing for a month and then stained with aldehyde-fuchsin[6] or toluidin blue (0 01 per cent aqueous solution followed by air drying and immersion in 95 per cent alcohol) or hæmatoxylin and eosin Mast cells and radioactive connective tissue cells were counted in one aldehyde–fuchsin-stained section from each time-interval for the three areas of connective tissue

Table 1 FREQUENCY OF RADIOACTIVE AND NON-RADIOACTIVE MAST CELLS AND OF RADIOACTIVE CELLS FROM OTHER CONNECTIVE TISSUE CELL POPULATIONS IN ALDEHYDE–FUCHSIN-STAINED RADIOAUTOGRAPHS

Tissue	Days after injection	Mast cells Radio-active	Mast cells Non-radioactive	Other cells radio-active	Area (sq mm.)
Skin	1	0	20	84	7 05
	2	0	21	38	3 37
	3	0	57	140	8 66
	4	0	77	112	7 92
	8	0	85	27	10 08
	21	0	26	23	2 44
Foot-pad	1	0	6	16	0 88
	2	0	10	54	2 70
	3	0	122	33	15 30
	4	0	8	7	0 83
	8	0	9	11	0 59
	21	1	110	11	5 50
Tongue	1	0	10	7	1 64
	2	1	64	6	5 23
	3	1	58	6	3 74
	4	0	8	12	5 51
	21	0	33	3	4 22
Totals		3	724	590	85 66

The use of aldehyde-fuchsin, which left the nuclei unstained, made it easy to identify silver grains over a nucleus, and facilitated the locating of mast cells by providing a specific stain for their cytoplasm Slides stained with toluidin blue or hæmatoxylin and eosin were scanned as a further check on the mast cell population and to identify the various radioactive cells in the connective tissue Counts of non-radioactive and radioactive mast cells and radioactive connective tissue cells in aldehyde–fuchsin-stained sections are given in Table 1 Radioactive mast cells were rare in these tissues, regardless of the time-interval Radioactive cells were plentiful in other cell populations of the connective tissue, many radioactive fibrocytes, macrophages, neutrophils, lymphocytes and plasma cells were seen

Considering the frequency of radioactive nuclei among proliferating cell populations seen in radioautographs of adult mice given single[7] or multiple[8] injections of tritiated thymidine, the mast-cell population sampled in this work was obviously very stable in terms of cell turn-over. If the multiple injections labelled all cells dividing during the 24-hr period, then it would take 9–18 months to replace completely a population in which only three cells out of 727 were found labelled after the 24-hr injection period The three radioactive mast cells listed in Table 1 may represent transformations rather than DNA synthesis by mature mast cells Radioactive cells which might serve as mast cell precursors were abundant in the areas where mast cells were found (Table 1) Yet, formation of mast cells could not have been the principal objective of mitotic activity in any of these cell populations (including their stem cells) since the number of radioactive fibrocytes, macrophages, lymphocytes and plasma cells was greatly in excess of the number of radioactive mast cells The frequency of radioactive leucocytes varied with time as would be expected[8], and decreased greatly at the later time intervals

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Detection of Inclusion Bodies containing Deoxyribonucleic Acid by Means of Autoradiography

WE have been examining extensively the *in vitro* multiplication of oil-induced, rabbit peritoneal histiocytes by means of autoradiography The cells were incubated on flying coverslips in Leighton-tubes, containing 40 per cent homologous serum in Tyrode solution in the presence of 1 µc tritiated thymidine/ml In general, little incorporation occurred within the first 48 hr, but then an increasing number of cells became labelled

In our first experiments all the label was localized in the nucleus, the cytoplasm remaining free (even in autoradiographs exposed for 6 months) However, in several rabbits which were bought from the same breeder we noticed the presence of a cytoplasmic inclusion body, a finding later closely paralleled in time with the occurrence of a non-bacterial peritonitis The shape of the inclusion body was that of a star (Fig. 1), and it was quite regular

This cytoplasmic label appeared irrespective of that of the nucleus The percentage of histiocytes containing these 'inclusion bodies' increased with time in cell culture The number of cells containing 2 or 3 bodies in the same cell was also increased

Table 1 presents quite typical data where several hundred cells were scored for each time period

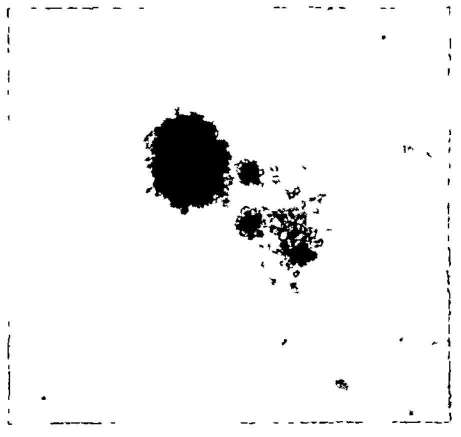

Fig 1. Cytoplasmic inclusion bodies labelled with tritiated thymidine in rabbit histiocyte

Table 1 INCREASE WITH AGE OF TRITIATED CYTOPLASMIC INCLUSION BODIES IN RABBIT HISTIOCYTES

Time (hr)	Percentage of histiocytes labelled
3	0
24	0
48	4
72	9
96	12

It is suggested that autoradiography could be a useful and sensitive method for detection of cytoplasmic parasites containing deoxyribonucleic acid which are in the latent stage but in which turn-over of deoxyribonucleic acid is occurring

This work was supported by the National Science Foundation

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HÆMATOLOGY

Fœtal Hæmoglobin in Fanconi Type Anæmia

THE persistence of fœtal hæmoglobin formation after infancy is usually taken to indicate an anæmic state existing from early life and is most commonly found in association with hereditary hæmoglobinopathies Despite an extensive search among other types of anæmia only very slight increases have been found, with the exception of quite large amounts present in acute leukæmia in children[1] and in adolescents[2]

A further example of an anæmia developing long after infancy which is also associated with an increased amount of hæmoglobin *F* has been found in a 9 yr old boy suffering from Fanconi-type anæmia This child had been under observation from the age of six months because of his poor physical development and the fact that he had congenital deformities of both thumbs Before the age of 9 yr the hæmoglobin was estimated to be 10 4–11 gm /100 ml on three occasions, but at this age a severe anæmia developed with a hæmoglobin of 5 3 gm /100 ml which was associated with a fœtal hæmoglobin-level of 14 per cent The hæmoglobin-level was raised by transfusions and, as a result of treatment with methyl testosterone and prednisone as described in ref 3 this level was maintained for six months, during which time the level of hæmoglobin *F* fell to 6 4 per cent At this point the testosterone was discontinued because of its adverse side-effects and this has resulted in a progressive fall in the level of hæmoglobin to 7 4 gm /100 ml over the last eight months associated with a rise in the level of hæmoglobin *F* to 10 7 per cent

This apparent variation in levels of hæmoglobin *F* with the total amount of hæmoglobin present suggests that in certain circumstances children, as well as neonates, react to anæmia by the production of hæmoglobin *F*, particularly as it would appear that no significant degree of anæmia was present in this case during the first nine years of life

There is, however, an alternative possibility that, as production of hæmoglobin *A* is under different genetic control from hæmoglobin *F*, its presence here is due to a genetic abnormality associated with the other genetic abnormalities occurring in this condition It is interesting to note in regard to the earlier finding of hæmoglobin *F* in acute leukæmias that there are on record three instances of Fanconi's anæmia and leukæmia occurring among different members of the same family[4], a remarkable association in view of the rarity of Fanconi's anæmia.

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Agglutination and the Electrical Surface Potential of Red Blood Cells

THE reduction of the electrical surface potential of erythrocytes has been considered for a long time as a possible cause of cell agglutination

Certain colloids coagulate only after decrease of their surface potential below a certain critical value The same was found for bacteria, the cause of both clumping and electrical discharge being either antibody or acidification[1,2] Similarly, erythrocytes have been observed repeatedly to show a reduced surface potential after treatment with antibody or with other agglutinating agents

This communication reports measurements of the surface potential of erythrocytes after treatment with one of the following agglutinating substances iso-antibodies, immune antibodies, agglomerins[3] (specific proteins in plasma of blood with high sedimentation-rate), certain salts and hæmagglutinating viruses The effects of non-agglutinating (incomplete) antibodies on the surface potential have also been investigated

A modified Abramson cell electrophoresis apparatus which was adapted for small volumes of test solution (2 0 ml) was used This modification permits investigation of suspensions in either salt solutions or protein solutions

After applying a current of 10 0 m amp , the velocities of single cells were observed with the microscope in one of the stationary planes of the electrophoresis cuvette and measured by means of a stop-watch and an ocular micrometer calibrated in microns 10 determinations were made of each sample and the arithmetic mean calculated The velocities were corrected for field strength (V /cm) and viscosity The mobility values were taken as a measure of the electrical surface potential of the cells

The buffer for diluting most agglutinating materials was a 0 9 per cent sodium chloride solution which contains 5 volumes per cent of $M/15$ phosphate buffer The final *p*H was 7 2 The (anodic) mobility of human red blood cells in this buffer was 1 080 ± 0 008 μ/sec /V /cm

All measurements were carried out directly in the appropriate test solutions, adequate time and temperature for incubation being allowed By this method, the effect on agglutination and surface potential of the medium itself was taken into account The erythrocyte concentration was 0 1 per cent

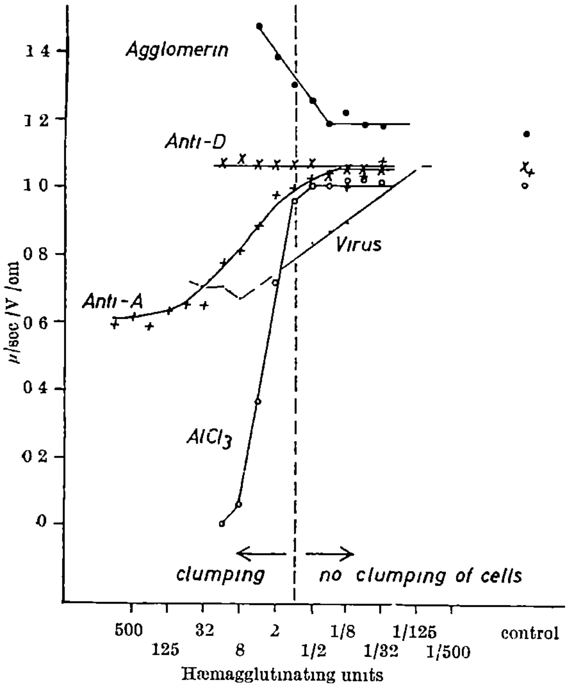

Fig. 1. Electrophoretic mobility of human erythrocytes after treatment with agglutinating agents. Dilutions in geometric progression in phosphate-buffered saline (anti-*A*, anti-*D*, virus), non-buffered saline (aluminium chloride) or compatible normal plasma (agglomeration of blood with high sedimentation-rate)

Following the measurements, the suspensions were recovered and tested for agglutination after 2 min. of centrifugation at 150*g*. In addition, the specific electrical resistance and the viscosity of the supernatant liquids were determined. All measurements including cell electrophoresis were carried out at 23.0° C. Dilutions of all materials in geometric progression in standard buffer were prepared. The highest dilution which gave a macroscopically detectable agglutination was taken as the titre of the solution and was assigned a value of one hæmagglutinating unit.

Typical results are shown in Fig. 1, which indicates that anti-*A* isoagglutinin caused a marked decrease of the electrophoretic velocity of the erythrocytes. However, the effect was small at the concentration at which agglutination began. This behaviour differed from that of hæmagglutinating virus (fowl plague, inactivated by sodium periodate), where a much higher reduction of cell mobility still did not alter the stability in suspension of the blood cells. Anti-*D* had no effect and the electrophoretic mobility remained unchanged even after addition of 32 hæmagglutinating units. Complete and incomplete anti-*D* were electrophoretically similar. The agglomeration of the red blood cells by plasma from a patient with a high sedimentation-rate was accompanied by increase in electrophoretic mobility of the cells. In this case the dilution was carried out with compatible normal plasma. A hæmagglutinating salt (such as aluminium chloride) had a profound effect on cell mobility. No electrophoretic effects were observed on erythrocytes with compatible blood groups. The results will be published later in greater detail.

In summary, the present work indicates several possible relationships between agglutination and surface charge: (1) both agglutination and reduction of the electrophoretic mobility of erythrocytes (like virus); (2) agglutination and no reduction (anti-*D* in saline acting), (3) no agglutination in saline but reduction (special anti-erythrocyte-rabbit sera), (4) no agglutination in saline and no reduction (anti-*D* in albumin acting), (5) increase of mobility in combination with cell agglomeration (plasma of high sedimentation-rate).

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Serum β-Globulin Types in Red Deer and Other Species and their Stability in the Presence of Bacteria

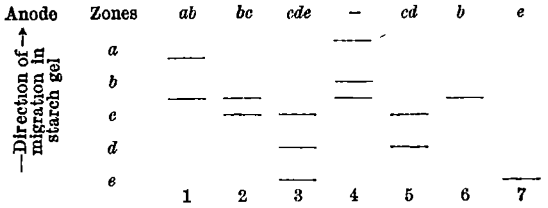
INDIVIDUAL variations in the serum β-globulin patterns obtained on starch-gel electrophoresis have been shown to be genetically determined in a number of species[1-5] and in some the proteins concerned have been shown to be transferrins[7,8]. These variations have been ascribed to a number of alleles[5,6] which have been used in the genetic characterization of animal populations[2,3,7]. This communication reports the serum β-globulin patterns found in red deer (*Cervus elaphus*) with some observations and experiments on their stability, shows that they are due to transferrins and makes some comparisons with the β-globulin patterns in other species.

Serum samples of *C. elaphus* were obtained through several culling seasons from around Braemar, by courtesy of the Mar Estate, and from Rhum, a national nature reserve. A few samples of fallow deer (*Dama dama*) and Sika deer (*C. nipon nipon*) were obtained from parks in Kent. The blood was collected at death and the serum separated before posting to the Rowett Research Institute, where it was stored at + 1° C for examination. Starch-gel electrophoresis was carried out in phosphate buffer *p*H 7.6 (ref. 9) using hydrolysed starch (Connaught) and paper electrophoresis in 0.05 *M* barbiturate buffer *p*H 8.6 (ref. 5). Transferrins were detected by radioautographs[7] of both starch gel and paper electropherograms of sera to which iron-59 had been added.

The β-globulin zones in *C. elaphus* were found to occupy five possible positions showing a regular decrease in mobility, on starch-gel electrophoresis. Calling these zones *a*, *b*, *c*, *d* and *e*, 254 samples showed patterns of types *ab*, *abc* or *bc*. Six samples showed slower-moving zones of type *cd* or *cde* and two samples showed atypical patterns in which three zones occurred in the region of zones *ab*. Twelve samples of *Dama dama* and five of *C. nipon nipon* were all type *bc*: the zone *b* corresponded to the single β-globulin zone (transferrin) of a human serum. Some examples of the different types of sera are shown schematically in Table 1 and their distribution in *C. elaphus* is given in Table 2.

The samples of *C. elaphus* for the first two seasons were re-examined after storage for several months at + 1° C., when it was found that seventeen of them had changed their type, showing slower-moving zones; this change was also detectable on paper electrophoresis. As an example, five samples of type *ab* become type *cd*. In the subsequent season, care was taken to use a preservative ('Thiomersal', British Drug Houses, Ltd.) in the sample bottles and no

Table 1



Serum β-globulin types, *C elaphus*, common types, 1 and 2, slow-moving type 3, atypical pattern, 4 Pattern produced from serum type *ab* 5, *Dama dama* and *C nipon nipon* also 2 Human, normal sample for comparison 6, pattern produced from same sample 7.

Table 2. DISTRIBUTION OF SERUM β-GLOBULIN TYPES IN *C elaphus*

Zones	*ab*	*abc*	*bc*	Slow	Others	Total
Samples from Braemar						
Seasons 1958–61	33	14	2	—	—	49
Samples from Rhum						
Seasons 1958–60	41	36	8	6	1	92
Season 1960–1 (stags)	35	23	2	—	—	60
(hinds)	32	25	3	—	1	61
All seasons	108	84	13	6	2	213

slow-moving types were found Comparison of the two lots of sera showed that the stored samples had often supported a considerable growth of Gram-positive and negative streptococci and bacilli, accompanied by a slightly more acid reaction

An attempt was made to produce changes in the β-globulin patterns experimentally by inoculating fresh sera with stored sera from the previous season Incubation for 24 hr. at 37° C produced bacterial growth but no changes in pattern, however, on subsequent standing at + 1° C. for four weeks, slower-moving zones appeared and this did not occur in uninoculated control sera Examples of the changes that can be produced in *C elaphus* and in human sera are included in Table 1.

Experiments with iron-59 were carried out on various sera to see if the β-globulin patterns being studied were due to transferrins The radioautographs showed that the zones *a*, *b* and *c* in *C elaphus* and *b* and *c* in *Dama dama* and in *C. nipon nipon* and the slow-moving zones *c*, *d*, and *e* in *C elaphus* samples altered on storage were the only ones in the sera combining with iron This was also found to be true of the strong zones characterizing eight sheep, six cattle and three goat-type sera that have been described[2-4] The radioautographs of the paper electropherograms confirmed that the proteins studied were β-globulins in all the species mentioned

It would seem that the various β-globulin types that have been reported are due to transferrins and that these proteins can readily show a stepwise decrease in mobility, comparable to the differences observed between individual types and without affecting the ability of the protein to combine with iron As the decreased mobility is detectable on paper electrophoresis, the changes are probably due to a decreased negative charge rather than to a gel filtration effect on a system of polymers A possible explanation of these changes is suggested by the report of Schultze[10] that the bacterial enzyme neuraminidase reduced the electrophoretic mobility of a number of human serum proteins. It would appear advisable, therefore, to accept with caution, as genetic types, rare slow-moving zones, in samples obtained under primitive conditions, unless such changes can be excluded and the type confirmed by a fresh sample from the same individual.

We are grateful to G Macnaughton, M Douglas, P Macrae, L Stewart and G Mackay, of the Nature Conservancy, and J. Bonner-Williams, of Tonbridge, for help in obtaining samples of deer sera, and to Dr G. C Ashton for the sheep and cattle type sera

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Amino-Acid Composition of the Polypeptide Chains of Gorilla Hæmoglobin

COMPARISON of tryptic peptide patterns has demonstrated the great overall similarity of the primary structure of hæmoglobins from normal adult man and anthropoid apes[1]. Work is now in progress to define this similarity more precisely. We report here the determination of the amino-acid composition of the polypeptide chains of gorilla hæmoglobin

Recent investigation in these laboratories has shown that gorilla hæmoglobin contains two pairs of polypeptide chains These have the same *N*-terminal sequence by the dinitrophenyl method as the polypeptide chains of human hæmoglobin[2,3] We designate them, therefore, the α- and β-chains

The α- and β-chains of gorilla hæmoglobin were separated by chromatography according to the method of Wilson and Smith[4] Individual chains were hydrolysed in 6 *N* hydrochloric acid at 110° C for 22 and 70 hr The amino-acid composition was determined by means of a Spinco automatic amino-acid analyser

The results are given in Table 1

On the basis of the amino-acid composition, the α- and β-chains of gorilla hæmoglobin are almost indistinguishable from the corresponding chains of human hæmoglobin The α-chain of gorilla hæmoglobin appears to have one more residue of aspartic acid and one less residue each of glutamic acid and serine Amino-acid analysis of tryptic peptides from the α-chain has revealed the substitution of an aspartyl for a glutamyl residue in a peptide equivalent to spot No 23 (ref 8) or peptide αT-4 (ref 9) of the α-chain of human hæmoglobin Assuming an essentially identical sequence in the two types of α-chains, this substitution probably is in the 23rd residue from the *N*-terminus Whether the α-chain of gorilla hæmoglobin actually contains one less seryl residue is uncertain because of the destruction of serine during hydrolysis The value given, however, was obtained from each of two independent determinations of the destruction of serine as a function of the time of hydrolysis

The β-chain of gorilla hæmoglobin clearly contains one less arginyl residue than that of human hæmoglobin and may contain an additional lysyl residue

Table 1 Number of Residues per α- and β-Chain of Gorilla and Human Hæmoglobins

Amino-acid	α-chain Human*	α-chain Gorilla	β-chain Human*	β-chain Gorilla
ala	21	21	15	15
arg	3	3	3	2
asp	12	13	13	13
cys/2	1	1	2	2
glu	5	4	11	11
gly	7	7	13	13
his	10	10	9	9
ileu	0	0	0	0
leu	18	18	18	18
lys	11	11	11	11 or 12
met	2	2	1	1
phe	7	7	8	8
pro	7	7	7	7
ser	11	10	5	5
thr	9	9	7	7
try	1	1†	2	2†
tyr	3	3	3	3
val	13	13	18	18
Total	141	140	146	145 or 146

* Based on refs 5 and 6 for the α-chain, on ref 7 for the β-chain, and on a personal communication from G Braunitzer

† Estimated from the tryptic peptide pattern and from the ultraviolet spectrum.

In the hydrolysates of the β-chain, homocitrulline was found Presumably, this was formed by reaction of lysine with cyanate[10] in the urea which was used in the separation of the chains although its formation would not have been anticipated because of the acidity of the solutions The value for lysine in the β-chain thus is reported as the sum of lysine and homocitrulline

These amino-acid compositions thus yield further evidence of the similarity of gorilla and human hæmoglobins The substitution of an aspartyl for a glutamyl residue in the α-chain and the possible substitution of a lysyl for an arginyl residue in the β-chain are compensatory in charge in each chain. As a result, the electrophoretic behaviours should be alike. Indeed, migration-rates of the two hæmoglobins on paper electrophoresis at *p*H 8 6 are indistinguishable (Paper electrophoretic migrations were kindly determined by Dr W. R. Bergren) The chromatographic behaviours on 'Amberlite *IRC*-50' are detectably, but very slightly, different[11]. Whether the above are the sole differences in composition is, of course, to some extent uncertain because of the usual inaccuracies of amino-acid analysis; for this reason, one cannot be sure that the serine content of the α-chains is different The amino-acid composition in no way can provide information about changes in sequence without changes in composition

While these restrictions must be kept in mind, the α-chains of gorilla and human hæmoglobin may well differ by only two residues and the β-chains by one residue. The differences, then, appear to be of the order observed between normal and abnormal human hæmoglobins Indeed, abnormal human hæmoglobins such as hæmoglobin *S* or hæmoglobin *I* differ from normal human hæmoglobin apparently only by one amino-acid substitution in one or the other chain[12,13] One might say that the β-chain especially has the characteristics of an abnormal human β-chain although a human β-chain corresponding to the gorilla β-chain has not yet been found. The results suggest that the hæmoglobin genes of man and gorilla constitute a single population

We wish to express our appreciation of valuable discussions with Prof Linus Pauling. Mrs Joan Balog Shelton and Mr Johnson Cua have given competent technical assistance.

This work was supported in part by a grant (*H*-2558) from the National Institutes of Health, U S Public Health Service One of us (E Z) is on leave from the Centre National de la Recherche Scientifique, Paris

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IMMUNOLOGY

A 'Microspot' Test for Antigens and Antibodies

The immunologist is often confronted with the problem of determining the presence of low concentrations of antigens in solution, or of establishing the antigenic specificity of a substance available only in minute quantities On the contrary, he is also frequently concerned with the conservation of small quantities of hard-won antisera One answer to both these problems is a technique worked out in these Laboratories

The principle of the technique encompasses the coating of microscope cover-glasses with a thin layer of antiserum-agar, on which microspots of the solutions of the antigens are deposited from fine capillary tubes The cover-glasses are kept in a moist atmosphere for a suitable period of time and then examined for the presence of spots of antigen-antibody precipitate at the sites of antigen application In a strong antigen–antibody system precipitation spots will be visible in 1–2 hr After 24 hr most positive reactions will be detectable; but it is advisable to keep the cover-glasses for 3 days for the possible appearance of further very weak reactions.

Reading of the moist preparation will generally suffice, and photographs may be made for record In addition, the preparations may be soaked for periods successively in saline and distilled water and dried at 37° C The agar film adheres firmly to the cover-glass and a permanent mount is produced At this stage viewing the surface of the agar by reflected light against a black background may reveal faint spots not apparent in the moist state The precipitation spots on the dried preparation are readily stained with a suitable dye, such as ponceau *S*, with virtually no background staining Such stained preparations can be mounted between lantern-slide cover-glasses for projection

In practice, we have used 5–15 per cent serum-agar for the coating of the cover-glasses, that is, 0·025–0·075 ml antiserum plus 0·475–0 425 ml

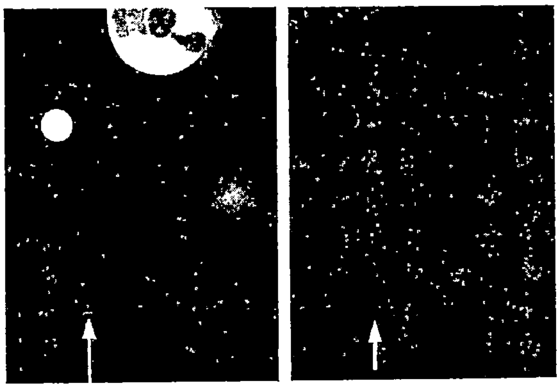

Fig 1 Comparison of microsp detection of duck ovalbumin in wet preparation (left) and after drying and staining (right) Antigen concentrations, from top 10,000, 1,000, 100, 10 μgm /ml 1 per cent specific antiserum in agar gel (× c 3 5)

1 per cent agar respectively, mixing the two at about 57° C It is advantageous to use specially purified agar[1] One-half ml of agar will readily coat 5–6 one-in circular cover-glasses, which may be stored in a moist atmosphere until required No 3 thickness cover-glasses are preferred for their lower fragility

The amount of antigen which can be detected depends on the antigen–antibody system involved We have succeeded in detecting as little as 10 μgm / ml of duck ovalbumin and the γ-globulin in a 1 100 dilution of human serum We have not yet determined the minimum amount of antibody required in the antiserum to produce a positive test

We have already applied the technique to the detection of antigen in eluate fractions from chromatographic columns This, together with the detailed methodology of the technique and alternative use of serum-impregnated papers and membranes, will be reported elsewhere

I am indebted to Alan W Wheeler for technical assistance and to Douglas F Lawson for the photographic recordings

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Acrolein and Mengo-Semliki Virus Immunity

A PREVIOUS report[1] and unpublished qualitative immunochemical findings indicated that acrolein combines with various serum proteins without altering species characteristics During this work the antiseptic qualities of acrolein were evident by the absence of growth of microscopic organisms, no growth was demonstrable at any time in any of the sealed acroleinized preparations that had been stored at room temperature during a ten-year period This is of special interest since strict sterile precautions were not exercised during collection of any of the animal sera These observations, and the known toxic effects of acrolein, suggested application in the preparation of vaccines

This initial report concerns animal experimental work with acroleinized Mengo and Semliki viruses Mengo and Semliki Forest viruses were chosen for this initial acute experiment because of their known lethal virulence for mice Paralysis and death following infection occur in unprotected mice over a period of 1–8 days, depending on the age of the mice, route of administration and the number of lethal doses of live virus injected

Stock virus was obtained from the Department of Clinical Virology, Sloan-Kettering Institute Mengo virus stock 4, dated November 10, 1958 (unknown number of brain passages), stored frozen as 20 per cent mouse brain supernatant with 5 per cent human serum-saline, intracranial titre, 10^{-8} in 5–6-week-old mice, Semliki Forest virus stock 4, dated December 3, 1959 (8th brain passage), stored frozen as 20 per cent mouse brain supernatant in 5 per cent mouse serum-saline, intracranial titre, 10^{-7} in 5–6-week-old mice Swiss Webster mice, approximately 5 weeks old, weight 16–18 gm , were used Acrolein (bulk acrolein stabilized with hydroquinone, maximum hydroquinone 0 1–0 2 per cent, supplied by Dr T J Hall, Union Carbide Chemical Co , New York, N Y) 0 2 per cent in 0 85 per cent chilled sterile saline was freshly distilled and used immediately in each experiment

Stock Mengo virus (20 per cent) was diluted tenfold with sterile distilled water to 2×10^{-3} Stock Semliki virus (20 per cent) was diluted tenfold to 2×10^{-2} This was injected intracranially in two groups of mice in 0 3-ml doses per mouse Brains from only the living moribund as well as the clinically well Mengo- and Semliki-infected mice were collected rapidly and aseptically and pooled approximately 24 hr and 44 hr respectively following injection This was not titred The brain tissue was immediately macerated with scissors and homogenized in a 'Tri-*R* Teflon tissue homogenizer with a small quantity of 0 2 per cent acrolein necessary to maintain a liquid state Following homogenization, sufficient chilled 0 2 per cent acrolein was added to make a final 20 per cent brain suspension The suspensions were put in brown sterile rubber-stoppered vaccine bottles, exposed to room temperature for 24 hr and stored in a refrigerator at 1–5° C As control for the detection of live virus, Mengo and Semliki vaccines 12 days and 28 hr respectively after preparation were serially diluted tenfold with sterile distilled water and injected intracerebrally in 0 03-ml doses in groups of mice approximately five weeks old There was no evidence of illness or death over an observation period of 15 days, at which time the animals were killed

In a similar test experiment, live stock Mengo virus, diluted 1 10 with 0 2 per cent acrolein was shown to be inactivated after exposure for 30 min at room temperature (Incidentally, 0 2 per cent acrolein inactivates Rous sarcoma, Friend's leukæmia and Egypt 101 viruses after 24 hr exposure at room temperature) Tissue cultures for the detection of live virus were not carried out

For immunization tests, mice of the same delivery tag-date age were divided into three separate groups One group was vaccinated with Mengo vaccine (age 3 days) subcutaneously, one group peritoneally, and the third group was unvaccinated to be used as controls Vaccination consisted of a 0 1-ml injection of acroleinized 20 per cent suspension of mouse brain initially, repeated every seven days for a total of four injections Ten days following the last injection, the vaccinated and unvaccinated controls were challenged intraperitoneally with live stock Mengo virus With the Semliki vaccine (age, 1 day), mice of the same tag-date age were divided into four groups Two groups were vaccinated intraperitoneally, receiving 0 1 ml of vaccine initially, repeated every seven days for a

Table 1. MORTALITY FOLLOWING INOCULATION WITH LIVE VIRUS (DEAD/TOTAL)

Live virus inoculated	Group and route of challenge	Virus dilution 2×10^{-1}	$^{-2}$	$^{-3}$	$^{-4}$	$^{-5}$	$^{-6}$	$^{-7}$	$^{-8}$
Mengo	Unvaccinated controls (*IP*)		5/5	5/5	5/5	5/5	5/5	4/5	1/5
	Intraperitoneal vaccinated (*IP*)*		0/5	0/5	0/5	0/5	0/5	0/5	0/5
	Subcutaneous vaccinated (*IP*)		1/3	1/3	1/3	1/3	1/3	1/3	0/3
Semliki	Unvaccinated controls (*IC*)	3/3	5/5	5/5	2/4				
	Intraperitoneal vaccinated (*IC*)*	0/3	0/5	0/6	0/3				
	Unvaccinated controls (*IP*)		3/5	1/5	0/5	0/5	0/5	0/5	0/5
	Intraperitoneal vaccinated (*IP*)*		0/5	0/5	0/5	0/5	0/5	0/5	0/5

* No deaths over a 15-day period of observation

Intraperitoneal (*IP*), intracranial (*IC*) route of administration of live virus

total of four injections. Two groups remained unvaccinated for use as controls. Ten days following the last injection, the four groups were challenged with live stock Semliki Forest virus.

Results are summarized in Table 1. (1) The group vaccinated intraperitoneally was protected against approximately one million (LD_{50}) lethal doses of live Mengo virus, while the group vaccinated subcutaneously showed only irregular partial protection. (2) The vaccinated group challenged intracerebrally was protected against approximately 2 000 (LD_{50}) lethal doses of Semliki Forest virus. It is interesting to note that three mice from each of these groups received undiluted 20 per cent stock virus (2×10^{-1}). The results of challenge via the intraperitoneal route in the other two groups shows a one log protection in the vaccinated mice.

Space does not permit a detailed discussion; however, it appears that acrolein stabilizes without altering the basic compound molecular pattern of the viruses studied. While the molecular configuration was probably altered to some extent by acrolein, there was no noticeable loss in the capacity of the acroleinized virus to act as an antigen when injected into mice. The immune response was determined by the ability of the vaccinated mice to survive challenge against overwhelming lethal doses of live virus injected, and not by the test-tube type determination, based on the amount of reactive humoral substances present in serum.

I am indebted to Dr. Emerson Day for his co-operation and help in initiating this work and to Dr. Chester Southam for his criticism and giving me facilities for this investigation.

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PATHOLOGY

Absorption and Excretion of Penicillin injected into the Muscles of Patients with Diabetes Mellitus

THE observation that two patients with sub-acute bacterial endocarditis due to alpha-streptococci highly sensitive to penicillin *in vitro* failed to respond to administration of six million units of the drug a day led to investigations which revealed low peak blood-levels and abnormal urinary excretion of the antibiotic in both. When the dose of penicillin was increased to a quantity greatly in excess of that suggested by the test-tube sensitivity of the organisms, favourable clinical response followed promptly. Other than the endocarditis, the only finding common to both individuals was the presence of diabetes mellitus. Since a review of published information concerning the pharmacology of penicillin[1-4] revealed no data indicating any abnormalities related to the diabetic state, it was decided to investigate this problem.

Two types of subject were investigated: (*a*) patients with established diabetes mellitus controlled by diet alone or with insulin, (*b*) individuals with no clinical or laboratory evidence of diabetes who were approximately the same age as the diabetics. They were selected on this basis in order to eliminate, so far as possible, the effect of age on the efficiency of renal tubular excretion of penicillin. A single dose of 10,000 units (6 mgm.) of crystalline benzyl penicillin *G* per kgm. of body-weight was injected into the gluteus. Blood and urine specimens were obtained before treatment and 1/2, 1, 2, 4, 6, 8, 24, and 48 hr. after antibiotic administration. Concentration of penicillin in the blood was determined by the tube-dilution and agar-cup methods. The quantity of drug in the urine was estimated by the tube-dilution technique. The test organism used was *Staph. aureus* (var. *pyogenes*) *FDA* 209*P*.

Thirty-one diabetic subjects and twenty-nine normal 'controls' were investigated. There was some variation in the rates of absorption and excretion of penicillin, some individuals with diabetes being indistinguishable in this regard from those without this disease. However, as illustrated in Fig. 1, calculated averages revealed striking differences. Peak blood-levels were reached earlier (1 hr.) and were higher (8 units/ml.) in the controls than in the diabetic patients (2 hr. and 5·4 units/ml. respectively). Disappearance of penicillin from the serum appeared

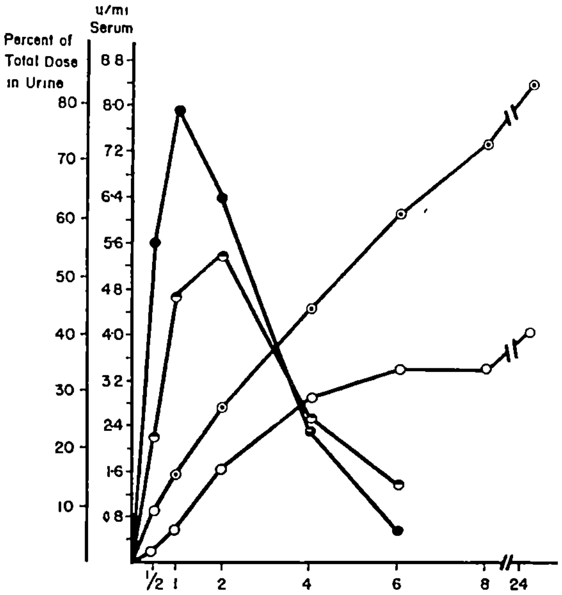

Fig. 1. Serum penicillin content determined by cup-plate assay and cumulative urinary excretion assayed by a two-fold serial dilution method. ● Penicillin-level in serum non-diabetic, ⊙, non-diabetic urine concentration. ⊖, serum-level in diabetic, O, diabetic urine concentration

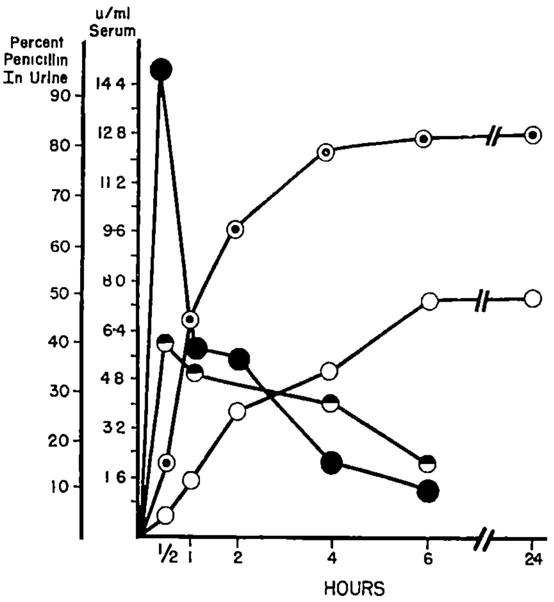

Fig 2 Penicillin content of serum and urine in one diabetic subject after both intravenous and intramuscular administration ○, Cumulative penicillin excretion in urine of diabetic after intravenous dose 10,000 units/kgm , ○, penicillin excretion after intramuscular dose of 10,000 units/kgm , ●, serum content after intravenous dose, ◓, serum content after intramuscular dose

to proceed at the same rate in both groups The half-life of the antibiotic was found to be almost identical, 0 79 hr in the non-diabetics and 0 83 hr in those with diabetes

The urinary excretion of penicillin by diabetic patients, although somewhat variable, was, on the average, very different from that of the controls (Fig 1) The normal subjects cleared approximately 50 per cent of the injected dose of drug via the kidney in 2 hr , 80 per cent after 8 hr , and 95 per cent after 24–48 hr The average urine output of antibiotic in diabetics was found to be only 25 per cent after 2 hr and 50 per cent after 24 hr Specimens of urine obtained from both groups after 36 hr contained too little penicillin to be detected by the method used, since concentrations less than 10 units/ml. were not measured

Since the data obtained indicated that the absorption and excretion of penicillin were abnormal in diabetes mellitus, investigations were carried out to determine the responsible mechanism. The possibility that variation in degree of protein-binding of the antimicrobial agent might be involved was eliminated by failure to demonstrate significant changes in antimicrobial activity when penicillin, in varying quantities, was incubated *in vitro* with serum, plasma, whole blood, and urine of diabetic and normal subjects The fact that the half-life of the antibiotic was found to be essentially the same in both groups suggested that (*a*) derangements in kidney function associated with the diabetic state, (*b*) destruction of the drug, or (*c*) a metabolic pathway other than that available in normal individuals was probably not responsible for the reduced serum-levels and renal clearance in diabetes An investigation was undertaken, therefore, to determine the role of the site of injection of penicillin on its absorption and excretion in normal and diabetic patients

One patient with diabetes mellitus controlled by diet and insulin has thus far been investigated. He was first given the standard dose of penicillin intramuscularly and blood and urine concentrations were determined 1/2, 1, 2, 4, 6, and 24 hr after treatment Concentrations of drug in the blood were determined using the agar-cup method and in the urine by the tube-dilution technique Several days later, he was given the same dose of penicillin intravenously and blood and urine collected at the same intervals The results of this work are presented in Fig 2 The curves of serum and urine concentrations when penicillin was given intramuscularly approximate closely those presented in Fig 1 as an average for thirty-one diabetic subjects Quite distinct are the blood-levels and cumulative urinary excretion following intravenous administration of the antibiotic, these resemble closely those presented in Fig 1 as average for twenty-nine 'controls' The total quantity of penicillin excreted in the urine was considerably larger when it was given intravenously than when it was injected into a muscle These results suggest that the low peak blood-levels and decreased urinary excretion of antibiotic noted in patients with diabetes mellitus are probably due to defective absorption from an intramuscular depot and not to altered renal function, increased rapidity of destruction, excessive protein binding, or metabolism via an unusual pathway

The observations which have been presented appear to be significant not only because they indicate that absorption of penicillin from intramuscular sites is abnormal in diabetes mellitus, but also because they suggest the possibility that other substances may also be absorbed relatively poorly and that this represents a basic absorptive defect in this disease

This work was carried out under a grant from Lilly Laboratories, Indianapolis, Indiana

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Establishment of Amœbic Hepatitis as a Distinct Clinico-Pathological Entity

THERE is much controversy as to whether or not amœbic hepatitis occurs as an entity, as distinct from the well-recognized amœbic liver abscess Recently, we reported a case of long-standing amœbiasis with hepatomegaly which, on liver biopsy, showed histologically the presence of cells morphologically similar to amœbæ, diffusely infiltrating the liver without abscess formation[1]

Further support to our concept of amœbic hepatitis is given by two patients on whom we now report These patients, both with amœbiasis and hepatomegaly without liver abscess, were found to have cells, morphologically similar to amœbæ, diffusely infiltrating the liver on biopsy examination

On laparotomy, the liver of both patients had a fine granular appearance and histologically showed inflammatory infiltration and some portal fibrosis Liver tissue from the biopsies was injected into the liver of hamsters, guinea pigs and rabbits In the hamsters and the rabbits, liver abscesses were produced which showed the presence of cells morphologically similar to amœbæ, such cells had also diffusely infiltrated the liver

The passage of the disease from the human to the experimental animals, we believe, gives further support to the view that diffuse amœbic hepatitis exists as a distinct clinico-pathological entity

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ANATOMY

Third Molar Polymorphism and the Timing of Tooth Formation

AGENESIS (congenital absence) of the third molar tooth is a familiar polymorphism in man, with a population frequency of at least 9 per cent in Europeans[1]. Hitherto, congenital absence of this tooth has been considered as an independent trait, or at most associated with reduction in size when agenesis is incomplete However, there is evidence connecting the presence or absence of the third molar tooth with the timing of formation of the premolar and molar teeth as well

In a series of 172 white children born in Ohio (excluding monozygotic pairs), agenesis of the lower left third molar was radiographically confirmed in 22 subjects aged fourteen years or older The time of formation of the remaining lower left premolar and molar teeth was determined from serial, longitudinal oblique-jaw radiographs[2] and expressed as sex-specific normalized T-scores[3] so that data on boys and girls could be combined As shown in Table 1, children lacking the mandibular left third molar were consistently later in premolar and molar tooth formation than 126 'controls' from unaffected sibships

Table 1 CALCIFICATION TIMING OF THE POSTERIOR TEETH IN CHILDREN LACKING $\overline{M}_3$, THEIR SIBLINGS, AND IN CHILDREN FROM UNAFFECTED SIBSHIPS

	Calcification timing									
	$\overline{P}_1$		$\overline{P}_2$		$\overline{M}_1$		$\overline{M}_2$		$\overline{M}_3$	
Group	N	T*	N	T*	N	T*	N	T*	N	T*
Affected children	21	53	20	54	18	52	21	59	—	—
Siblings of above	22	56	20	53	17	52	24	52	24	57
Unaffected children	111	48	118	49	81	48	126	48	125	49

* Normalized sex-specific T-scores (ref 3) for beginning tooth formation, primarily beginning cusp calcification (ref 4)

Furthermore, 24 unaffected siblings of children with third molar agenesis were compared with the unaffected children from unaffected sibships These unaffected siblings of the propositi also proved late in tooth formation, especially for mandibular third molar formation which averaged 8 T-scores behind the controls (Table 1) For all 150 unaffected children, earliness or lateness of $\overline{M}_3$ formation was significantly associated with the type of sibship from which they came ($\chi^2 = 6 4$) Thus, the tendency for delayed tooth formation when the third molar is missing is characteristic of affected lineages and not restricted to affected individuals alone

Since absence of the mandibular third molar was associated with delayed formation of the molar teeth, it was not surprising to find differences in the sequence of tooth formation[5] between the 22 children with third molar agenesis and the 126 normal controls Thus, 60 per cent of children in the present work who lacked the mandibular third molar were of the P_2M_2 sequence of cusp calcification, while this sequence or order was far less common (22 per cent) in the 126 controls Third molar polymorphism was unquestionably associated with the P_2M_2/M_2P_2 sequence polymorphism ($\chi^2 = 25 01$)

Third molar agenesis, therefore, may be viewed as the extreme degree of expression of factors delaying tooth formation over a long developmental period ranging from the first month of life (for M_1) to the eighth year or beyond (for M_3) Moreover, this common polymorphism is related to the previously described familial P_2M_2/M_2P_2 calcification sequence polymorphism[6], indicating a common determinant for the two

The work reported in this paper was supported in part by grant D–1294 from the National Institutes of Health and the United States Public Health Service

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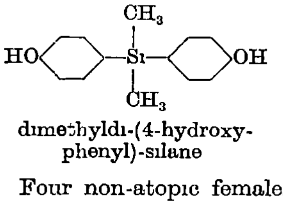
HISTOCHEMISTRY

Allergy to a Carbon-Functional Organic Silicon Compound, Dimethyldi-(4-hydroxyphenyl)-silane

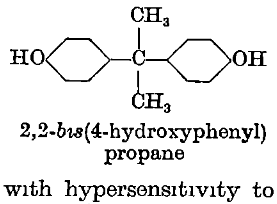
THE noxious effects of certain inorganic silicon compounds are well known[1] Polymeric organic silicon compounds, on the contrary, are chemically very inert and show a low toxicity[2] Some organic silicon compounds may, however, produce toxic effects[3]

Inorganic or organic silicon compounds—the latter not present in Nature[4]—are so far not known as allergens

As a close relationship exists between silicon and carbon atoms, it was assumed that cross-sensitization could occur between silicon and carbon compounds Therefore the allergenic nature of dimethyldi-(4-hydroxyphenyl)-silane[5] was investigated since the carbon compound, 2,2-*bis*(4-hydroxyphenyl)propane ('Bisphenol *A*'), has been demonstrated as an allergen[6]



dimethyldi-(4-hydroxyphenyl)-silane



2,2-*bis*(4-hydroxyphenyl) propane

Four non-atopic females with hypersensitivity to 2,2-*bis*(4-hydroxyphenyl)propane, demonstrated by positive patch test (1/100 dilution in ethyl alcohol), showed positive reactions to dimethyldi-(4-hydroxyphenyl)-silane (1/100 dilution in acetone) The positive tests were characterized by the presence of erythema, infiltration and papules/vesicles

This is the first report on allergy to an organic silicon compound Absence of earlier contact with the silane is evidence of cross-sensitization.

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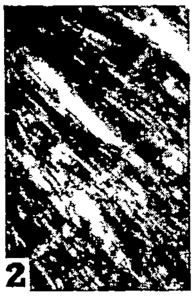
Use of Tetrazolium Compounds in Oxidative Enzyme Histo- and Cyto-Chemistry

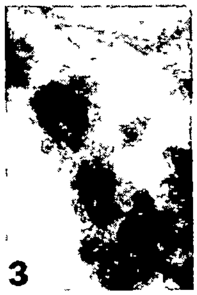
A NEW tetrazolium salt previously synthesized has been evaluated for its ability to overcome the technical and theoretical barriers of either the MTT-Co (ref 1) or nitro-BT methods[2,3] This compound is TNBT or 2,2′,5-5′-tetra-*p*-nitrophenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride (Fig 1), the formazan of which is purplish black[4] The TNBT used in the following investigations produced a brownish-black precipitate in tissue sections Though quite low in water solubility, minimal concentrations of TNBT are required for the effective demonstration of the succinic dehydrogenase system in fresh, frozen sections of tissue of mammalian tissues or human vaginal smear preparations The following were investigated in mouse myocardium and kidney (1) effect of concentration of dye on the products of TNBT formazan in slices of these tissues; (2) effect of *p*H on the reduction of TNBT by the succinic dehydrogenase system in tissue sections in the presence or absence of appropriate substrate, (3) solubility of TNBT formazan in aqueous, alcoholic and other organic solvents necessary for the effective resin-mounting of sections of tissue containing precipitates of formazan.

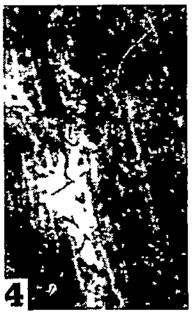
Results indicate that

(1) Varying the concentration of TNBT in the incubating medium from 0·001 mgm /ml to 1·25 mgm /ml through the use of an appropriate solvent has considerable effect on activity of the succinic dehydrogenase system in sections of heart and kidney. The best concentrations of this tetrazolium appear to be in the range 1 25 mgm /ml –0 25 mgm /ml. (Figs 2 and 3)

(2) Maximal deposition of TNBT formazan occurs in succinic dehydrogenase-stained tissue sections over a wide range of *p*H. Tissues incubated in TNBT solutions containing succinate and phosphate buffer with *p*H values of 6 8–8 4 show heavy staining reactions with little variation in intensity as a result of the concentration of hydrogen ions In the absence of succinate, extremely pale non-granular tints appeared in the tissue sections, especially at *p*H 8 4 The very faint staining, when visible in such specimens, was distinctly different from the TNBT formazan granulations seen in localizations of the succinic-dehydrogenase system







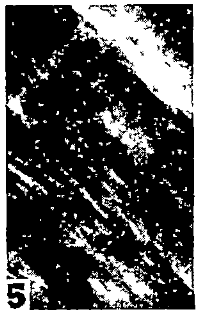


Fig 2 Mouse myocardium stained for the succinic dehydrogenase system using TNBT The muscle cells are well stained and cross-striations are evident in many regions of the myofibrils Note the absence of staining in nuclei and intercalated disks (upper right, lower left, centre) 5-μ section (× 300)

Fig 3 Mouse kidney stained for succinic dehydrogenase system using TNBT Heavy deposition of the diformazan is seen in the proximal tubules while no staining is apparent in the glomerulus (left) Note faint staining of smooth muscle in the tunica media of the artery (top) 10-μ section (× 60)

Fig 4. Mouse myocardium stained for the succinic dehydrogenase system nitro-BT Coarse granular precipitate marks the site of enzyme while occasional crystals can be seen (middle) in extracellular regions 5-μ section (× 600)

Fig 5 Mouse myocardium stained for the succinic dehydrogenase system using TNBT The fineness of the diformazan precipitate sharply delineates the mitochondria within the muscle cells In addition, note the clearly defined inactive nucleus (centre) under which is situated a portion of a stained myofibril Unstained intercalated disk (upper left) 5-μ section (× 600)

(3) TNBT formazan, enzymatically produced in tissue sections or in non-enzymatic systems using dithionite, is as insoluble as nitro-BT formazan in any of the ordinary organic solvents used in the resin-mounting of tissue sections.

(4) Crystallization phenomena such as those observed in nitro-BT formazan specimens (Fig 4) have never occurred in TNBT-stained tissue sections (Fig. 5).

(5) The granular precipitate of TNBT formazan can be of such a fine dimension, suggesting a more correct staining picture rather than that of an

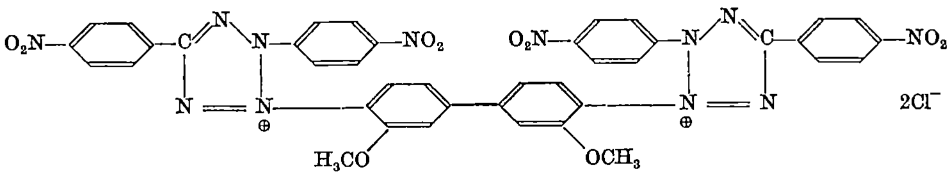


Fig. 1. Structural formula of 2,2′,5,5′-tetra-*p*-nitrophenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride (TNBT)

accumulated precipitate over intracellular organelles This localization is under investigation at present using electron histochemical techniques similar to the procedures of Sedar and Rosa[5]

(6) Cyanide inclusion into incubating media shows little effect in highly active tissues such as heart and kidney Its use for enhancement of staining in tissues of the female reproductive tract appears to be warranted, especially in regions where low succinic dehydrogenase activity prevails

In conclusion, TNBT appears to possess qualities suitable for the histo- and cyto-chemical localization of oxidative enzymes in tissue sections of mouse heart and kidney These properties strongly suggest that TNBT may be a more efficient reagent for the cytochemical demonstration of enzyme systems such as succinic dehydrogenase and diphosphopyridine nucleotide diaphorase

This work was supported in part by United States Public Health research grants *RG*-6008(*C*-3) and *C*-2560.

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BIOLOGY

Anaerobiosis and Sodium Accumulation

Accumulation of most cations by higher plants is considered to be an aerobic process However, we now find that sodium is in part an exception to this generalization. Uptake of sodium by 6-day old plants of *Hordeum vulgare*, variety Atlas 46, occurs under both aerobic and anaerobic conditions Accumulation in the roots under anaerobic conditions is restricted to a period of about 2 hr (Fig 1), during which time transport to the shoots is almost nil (Fig. 2) The amount of sodium accumulated by plants the roots of which are in either aerobic or anaerobic solutions is inhibited by substances such as iodoacetate and methylene blue, indicative of the initial uptake being coupled to metabolic reactions in both cases rather than to passive processes Non-steady-state uptake by the anaerobic plants, which decreases with time as if some process is attenuating, suggests that the reactions involving sodium uptake are several steps removed from the respiratory chain.

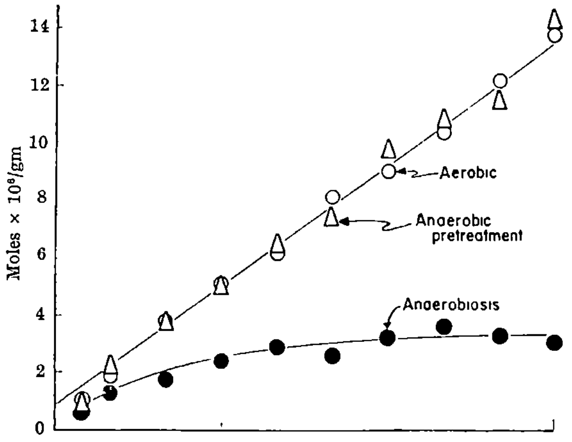

Fig 1 Accumulation of sodium ions by roots from a 10^{-3} *M* solution at *p*H 5 0

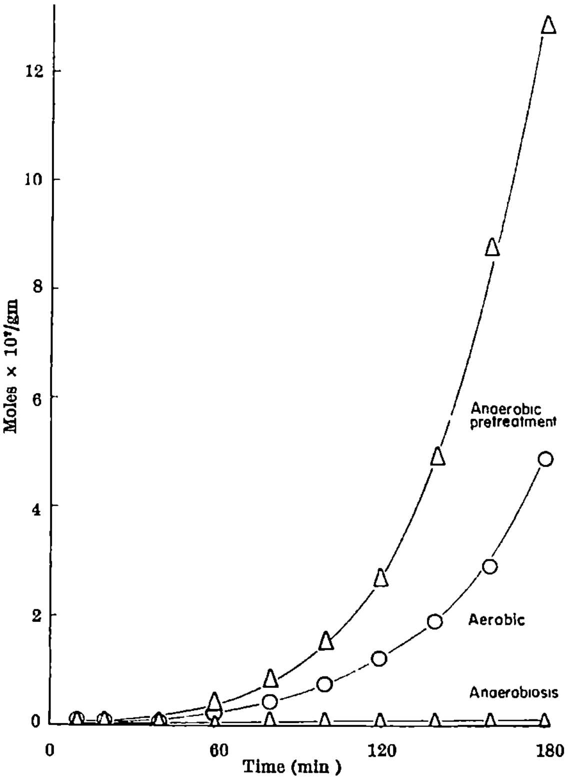

Fig 2 Transport of sodium ions to the shoots from 10^{-3} *M* solution at *p*H 5 0

Previously observed accumulation of sodium in plants under anaerobic conditions[1–3] over long periods of time was often considered in discussion as possible passive entry due to plant damage That the plants are not damaged by anaerobiosis in the present experiments is indicated by return to normal respiration after 60 min of anoxia and 15 further hours under aerobic conditions At the end of this treatment the rate of sodium accumulation in the shoots is increased but can be stopped by returning the plants to an anaerobic situation or by the use of inhibitors Anaerobiosis *per se* does not activate sodium uptake ; rather it increases the rate of sodium uptake on returning to aerobic conditions.

Activation of sodium uptake by shoots produced by anaerobic pretreatment of roots, either by bubbling nitrogen or carbon monoxide gas through the solution, occurs chiefly from the higher sodium concentrations (> 10^{-4} *M*). Two or more reactions appear to be involved in the uptake of sodium by roots[4] and shoots. The activation of anaerobic pretreatment is on the reaction for shoot accumulation which dominates at the higher sodium concentrations

The uptake of potassium and phosphate differs from sodium accumulation in respect to anaerobiosis

Both potassium and phosphate accumulation are immediately suppressed by anaerobic conditions and return only to the normal value in air (no enhancement). Accumulation was followed in all cases by the amount of radioisotopes appearing in the roots or tops.

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Effect of Environment on the Responses of Dwarf Groundsel to Gibberellic Acid

THE striking effects of gibberellic acid on internodal elongation in dwarf mutants and rosette plants of a number of species are now well established, and the effects of this substance on a single gene dwarf mutant, induced by γ-radiation in groundsel, *Senecio vulgaris* L., have recently been described[1]. The observations now reported show that the responses of this mutant to gibberellic acid may be considerably affected, both quantitatively and qualitatively, by environmental conditions. In fact, the phenotypic expression of both wild-type and mutant groundsel is greatly influenced by the environment: in certain unfavourable conditions the plants are stunted and the vegetative phase is prolonged. When the wild-type (tall) and mutant (dwarf) groundsel are grown in a greenhouse during the normal growing season, that is, from early spring to late summer, the transformation of the vegetative apex to an inflorescence apex occurs not later than 2 weeks after the emergence of seedlings from the soil, and fruiting begins when plants are 7–10 weeks old; but the apices of tall and dwarf plants grown in a growth cabinet under 'daylight' fluorescent tubes, 400 foot-candles, with a day-length of 10 hr., and a temperature of 22 ± 2° C., remained vegetative for 11 weeks.

Dwarf groundsel has shorter and fewer internodes than the wild-type plants of the strain from which it was derived. Mitotic activity is significantly less in the apical meristem and subapical region of the shoot in dwarfs than in wild-type plants. Treatment with gibberellic acid of dwarfs grown in a greenhouse leads to a conspicuous and precocious extension of the internodes due to enhanced mitotic activity and cell elongation in the subapical region of the shoot[1]. No evidence of any response of the tissues of the apical meristem was obtained, and there was no significant increase in the number of nodes.

Both tall and dwarf genotypes in the growth cabinet grew poorly and remained stunted. In these conditions, however, the genetic dwarfs responded proportionately more strongly to gibberellic acid than when they were grown in the greenhouse. Treated mutants were taller than untreated tall plants of the same age in the same environment, although they were not even as tall as untreated dwarfs grown in the greenhouse. An increase in the number of leaves, and thus of internodes, on the main axis of treated dwarfs in the growth cabinet was also observed (Table 1), and since leaf primordia originate on the apex, such an increase must reflect the influence of gibberellic acid on the apical meristem.

Table 1. EFFECT OF ENVIRONMENT ON THE HEIGHT AND NUMBER OF INTERNODES OF TALL AND DWARF GROUNDSEL AND THOSE TREATED WITH GIBBERELLIC ACID (GA)

Type of plant	Mean final height (cm.) Exp. *A*	Mean final height (cm.) Exp. *B*	Mean number of internodes Exp. *A*	Mean number of internodes Exp. *B*
Tall, control	40·4 (1·05)	2·2 (0·27)	14·2 (0·46)	15·5 (2·40)
Tall, GA-treated	45·2 (1·05)	8·0 (3·44)	13·8 (0·58)	17·6 (0·70)
Dwarf control	8·6 (1·01)	0·6 (0·16)	7·9 (0·16)	10·8 (1·27)
Dwarf, GA-treated	39·1 (1·52)	5·9 (2·53)	8·5 (0·63)	16·6 (1·56)

95 per cent confidence interval given in parentheses.

Exp. *A*, greenhouse. 18 plants in each group, measured at 47 days old when all plants were fruiting. Treated plants received 10 p.p.m. gibberellic acid at weekly intervals (6 treatments).

Exp. *B*, growth cabinet. 6 plants in each group, measured at 77 days old; most plants were still vegetative at the time of the final measurement, but appeared unlikely to survive to complete the life-cycle. Treated plants received 10 p.p.m. gibberellic acid twice weekly for the first three weeks, and once a week thereafter (13 treatments).

In order to obtain apical material from plants in the growth cabinet comparable with 8-day-old seedlings from the greenhouse, 20-day-old seedlings had to be selected, because of the slower growth and prolonged vegetative phase of plants in the growth cabinet. Observations of longitudinal sections of shoots from the growth cabinet which had been treated with 10 p.p.m. gibberellic acid twice weekly (6 treatments), showed that while the most striking morphogenetic effect of gibberellic acid was again on the sub-apical tissue of treated dwarfs, there was also a significant ($P = 0·05$) effect on the apical meristem. Treatment with gibberellic acid of dwarfs grown in these special conditions enhances cell division in the apical meristem and brings it to the level observed in normal tall plants grown in the same environment (Table 2). Since there were fewer than 10 mitotic figures in the apical and subapical regions of most shoots, tests of significance were carried out on data transformed by the method $\sqrt{X + 0·5}$, where X is the number observed[2]. As already stated, when groundsel seedlings were grown in greenhouse conditions where the vegetative phase is of relatively brief duration, no indication of any morphogenetic response of the apical meristem to gibberellic acid was obtained, and this is in accord with observations on other species[3,4]. When, however, groundsel seedlings were grown in an environment in which the initiation of the reproductive phase was delayed, permitting a longer period of gibberellic acid treatment while the apex was vegetative, there was clear evidence of a response of the apical meristem.

It may well be that stem elongation in normal and dwarf groundsel is modified by two major factors, namely, photosynthesis, which provides the basic substrates for growth, and gibberellin, which regulates the distribution of growth, and to the extent that the latter determines what proportion of the

Table 2. MEAN NUMBER OF MITOSES IN A MEDIAN PANEL 36μ IN THICKNESS (6 MEDIAN LONGITUDINAL SECTIONS, 6μ PER SECTION) OF SHOOT TIPS IN GROUNDSEL

Type of plant	Mean number of mitoses: Apical meristem Exp. *A*	Apical meristem Exp. *B*	Subapical region Exp. *A*	Subapical region Exp. *B*
Tall (wild-type) plant	6·0	3·8	4·6	3·0
Untreated dwarf	3·2	2·0	1·0	1·3
GA-treated dwarf	3·2	3·6	6·4	6·4

Exp. *A*. Greenhouse. 5 plants, 8 days old, treated with 10 p.p.m. gibberellic acid on alternate days (4 treatments).

Exp. *B*, Growth cabinet. 10 plants, 20 days old, treated with 10 p.p.m. gibberellic acid twice weekly (6 treatments).

products of photosynthesis is incorporated in leaf tissue, changes in it lead to changes in the former.

It seems likely that the heights of dwarf and normal plants grown in the greenhouse were limited, though to markedly different degrees, by deficiency of endogenous gibberellin, since both types of plant responded to applied gibberellic acid, the former strongly, and the latter relatively slightly. The growth of dwarf plants was also limited by the relatively deficient photosynthesis, since the number and size of leaves are characteristically less in dwarfs than in normal plants. Photosynthetic tissue was increased in greenhouse-grown dwarfs by gibberellic acid treatment, due to an increase in the size, but not in the number of leaves. (Photosynthetic efficiency, measured as net assimilation-rate, was similar in normal and dwarf plants, and was not affected by gibberellic acid[1].)

The growth and development of untreated and gibberellic acid-treated dwarf and normal plants grown under low light intensity in the growth cabinet was profoundly modified by reduced photosynthesis, and, in consequence, the morphogenetic response of the dwarf to gibberellic acid was modified, since during the protracted vegetative phase the apical meristem responded to gibberellic acid, and the treated dwarfs produced as many leaves as normal plants. Thus the effect of gibberellic acid on the photosynthetic tissue of the dwarf mutant was relatively greater when the plants were grown in the growth cabinet than when they were grown in the greenhouse, and this may have accounted for the relatively increased stem elongation of treated dwarfs in these conditions. Stem elongation was greater in these treated dwarfs than in untreated normal plants which were probably deficient in natural gibberellin, as indicated by the response of normal plants to treatment with gibberellic acid.

It is evident that the morphogenetic responses of *S. vulgaris* to gibberellic acid are considerably modified by environmental factors, and it seems probable that this is due to effects on both the photosynthetic capacity of the plants and their native growth substances, including gibberellin. It may well be that an investigation of the responses to gibberellic acid of dwarf mutants in other species grown in different environments would be of interest, and might lead, as in this case, to still more striking demonstrations of the effects of this substance.

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Volatile Mustard Oils of *Brassica juncea* Seeds

Brassica juncea Coss. and Czern. is an important cultivated mustard species. It is said to be a native of Asia and is grown particularly in China, Japan, India, Pakistan, Nepal and eastern Europe as a vegetable and an oil-seed crop, although it has also been introduced into North America and certain West European countries as an oil-seed crop and for use in the manufacture of condiments[5]. The species is of botanical interest in that it shows marked variation in external morphology, especially leaf form[1]. Variation, too, has been reported in the type of volatile oil produced on the hydrolysis of the seed glucoside; three conditions have been described: (*a*) the production of allyl isothiocyanate only[2,4], (*b*) the production of 3-butenyl (= 'crotonyl') isothiocyanate only[4], (*c*) the production of a mixture of allyl and 3-butenyl isothiocyanates[2].

In present investigations into variation in *B. juncea* in connexion with a programme of plant breeding, 96 samples of seed were obtained from a wide range of geographical sources, and grown in England; this collection was found to include all the main varietal forms of the species previously described in the literature. The volatile mustard oils of the seeds were identified using a paper chromatographical method[3]; in this analysis two controls were run, one of allyl thiourea and the other of phenyl thiourea (which gave a reference spot from which *Rph* values of other compounds were defined). Allyl isothiocyanate gave an *Rph* value of 0.26 and the value of the only other compound detected in the test, 0.62, indicated that it was 3-butenyl isothiocyanate.

All the three conditions of volatile oil production previously described were found during these investigations, and there appears to be a correlation between this variation and the geographical distribution of the species, a situation, so far as is known, not previously reported. Seeds from China, Japan, Nepal and Eastern Europe (49 samples) exhibited only condition (*a*), with the exception of three samples which produced very faint traces of 3-butenyl isothiocyanate in addition to allyl isothiocyanate. Conditions (*b*) or (*c*) defined here were shown only by seeds from India and Pakistan (twenty-one samples). The remaining twenty-six samples received from North America and western Europe had volatile oil production entirely consistent with their described origins from various countries, namely, six samples originating from India and Pakistan showed conditions (*b*) or (*c*), and twenty samples of other origin showed condition (*a*).

The possible amphidiploid origin of *B. juncea* from *B. nigra* (L.) Koch and *B. campestris* L. may account for these variations of volatile oil production, as *B. nigra* has been reported as giving only allyl isothiocyanate[6], which has been confirmed in the present investigations, and *B. campestris* has been found to produce only 3-butenyl isothiocyanate. The correlation with geographical distribution, together with other evidence from investigations of plant and seed morphology, might suggest that natural hybridization to produce *B. juncea* has occurred at different geographical loci.

The permission granted by Messrs. J. and J. Colman, Ltd., for the publishing of these results is gratefully acknowledged.

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Effect of Water Movement on Salt Movement through Tomato Roots

In recent years, there has been renewed debate concerning the effect of water absorption on salt absorption through the roots of intact plants. Salt absorption is regarded by many workers as involving active transport into the xylem by a mechanism dependent on energy released in respiration[1-3]. According to this view, water movement through roots affects salt movement only indirectly by removing salt from the root xylem and thereby producing conditions favourable for increased movement of salt by active transport. On the other hand, several investigators have concluded that, in rapidly transpiring plants, salt moves into the root xylem by mass flow as well as by active transport[4-6].

Estimates of the amount of salt moved by the two processes vary widely. The methods used in the present investigation made it possible to determine accurately that fraction of the total salt moved into the root xylem which was associated with water movement. Root systems of tomato (*Lycopersicum esculentum*, variety Marglobe) started in vermiculite and grown in Hoagland's solution were used in this work.

De-topped root systems were enclosed in a pressure chamber with their stumps projecting through the lid of the chamber. The roots were immersed in aerated half-strength Hoagland's solution which, in some experiments, contained phosphorus-32 as a radioactive tracer. Water was moved through the roots by increasing the air pressure in the chamber. The rate of salt movement through the roots was determined by measuring the volume and salt concentration of the exudate collected from the stumps. Rates of salt movement by active transport were determined by measuring the salt content of root pressure exudate collected before and after pressure applications. The effect of water movement on salt movement was determined by comparing the amount of salt moved through roots when water was moved through them under pressure, with the amount moved by active transport in the absence of external pressure.

The effects of applied pressure on rates of water and salt movement are shown for phosphorus in Fig. 1 and for total salt in Fig. 2. Figs. 1*A* and 2*A* show that, as the rate of water movement through the roots increased with increasing pressure, the phosphorus and total salt concentration of the exudate decreased. The amounts of phosphorus and total salt moved through the root systems increased with increasing pressure and rate of water movement except in the pressure-range of 10–20 lb. The amounts of phosphorus and total salt in root pressure exudate, and the effects of externally applied pressure on their rates of movement, are shown in Figs. 1*B* and 2*B*. The most important effect of increased water movement under pressure was an increase in the total amount of ions moved through the roots. This is shown by the fact that the amounts of phosphorus and total salt in the exudates were always greater during pressure applications than in root-pressure exudates in the absence of pressure. At 30 lb./sq. in., the amounts of phosphorus and total salt moved through the roots were 2·0 times and 2·4 times, respectively, the amounts moved by active transport in the absence of pressure. The amounts of phosphorus and total salt moved under pressure did not increase in proportion to the increased rates of water movement

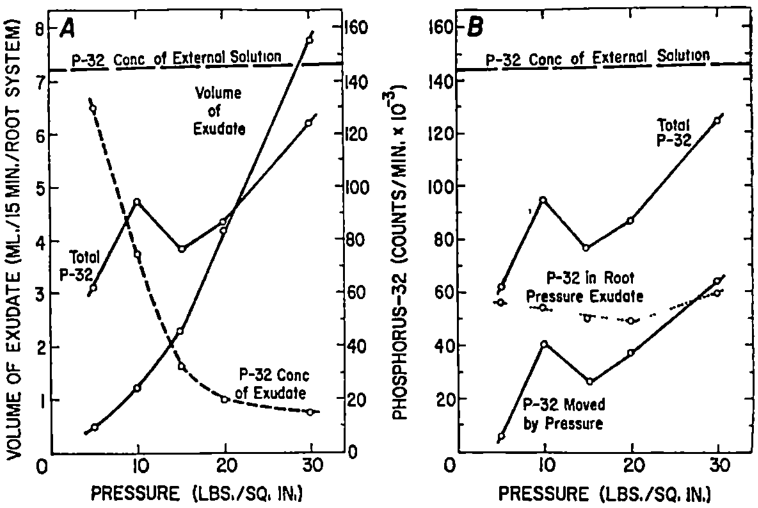

Fig. 1. *A*, Effect of pressure on rates of water and phosphorus movement through tomato roots. Phosphorus concentrations (dashed lines) are in counts/min./ml. *B*, Effect of pressure on phosphorus movement (total P-32 curve replotted from *A*). Phosphorus-32 in root-pressure exudate represents the amount of phosphorus-32 moved into the root xylem by active transport in the absence of applied pressure. Phosphorus-32 moved by pressure represents the fraction of the total phosphorus-32 which was associated with water movement under applied pressure. Points are averages of six root systems

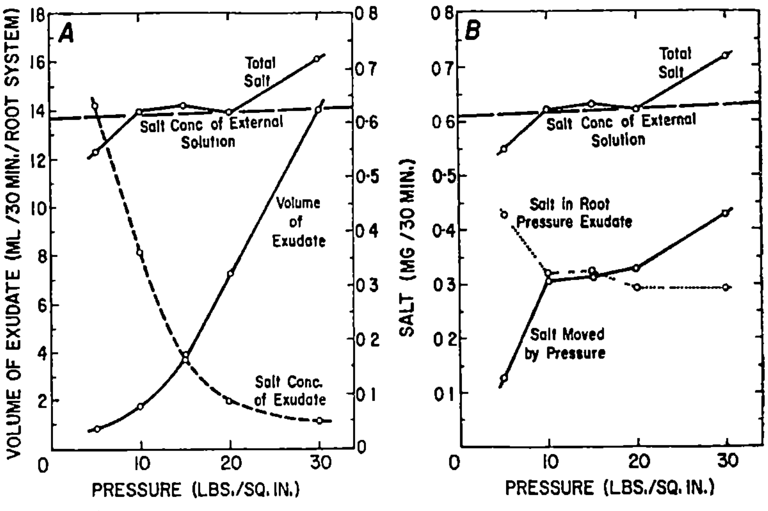

Fig. 2. *A*, Effect of pressure on rates of water and total salt movement through tomato roots. Salt concentrations (dashed lines) are in mgm./ml. *B*, Effect of pressure on total salt movement (total salt curve replotted from *A*). Salt in root-pressure exudate represents the amount of salt moved into the root xylem by active transport in the absence of applied pressure. Salt moved by pressure represents the fraction of the total salt which was associated with water movement under applied pressure. Points are averages of six root systems

Also, the salt concentration of exudates obtained under pressure usually was less than the concentration of the external solutions indicating the existence of a barrier in the roots which prevents free movement of ions into the xylem

These results show that rapid water movement through the roots greatly increases the rate of salt movement through the roots At 30 lb /sq in , more than 50 per cent of the salt moved through the roots was associated with water movement The manner in which increased water movement causes increased salt movement, however, is less certain As the rate of water movement through the roots increases, salt movement into the xylem by active transport may be accelerated by the development of a steeper salt concentration gradient across the root cells from the cortex to the xylem In addition, the change in the slopes of the total phosphorus and total salt curves at pressures of 15 and 20 lb /sq in suggests that the permeability of the roots to ions increased when higher pressures were applied Some ions might then be carried directly with water into the xylem An increase in ion permeability of root cells with an increasing pressure gradient across them was reported by Brouwer[7]

The results of these experiments indicate that at low rates of water movement salt movement is primarily by active transport However, at higher rates of water movement salt movement into the xylem probably occurs by both active transport and mass flow.

A detailed account of this work will be published elsewhere This investigation was supported by funds from the Atomic Energy Commission (Contract *AT*-(40-1)-1827)

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Freeze-drying of Bovine Spermatozoa

Since 1956, increased attention has been given to techniques for freeze-drying bovine spermatozoa as a method for long-term semen preservation[1-5] In one approach[1-3], spermatozoa were dried in vacuum after being diluted, glycerolated, equilibrated and slowly frozen Low survival-rates and frequent failures were encountered Leidl[3] suggested three possible causes (*a*) concentration of glycerol to toxic levels, (*b*) excessive removal of water, (*c*) morphological damage (occasional broken mid-pieces)

In a second approach, Yushchenko[5] freeze-dried spermatozoa suspended in a mixture of 'Freon' and heptane to circumvent the harmful effects of exposure to a eutectic mixture and to eliminate mechanical damage resulting from crystallization After glycerolation and equilibration in a synthetic medium, the diluted semen was centrifuged in test-tubes containing the 'Freon'-heptane mixture The spermatozoa were thereby transferred to the non-polar medium in which they were freeze-dried With this approach he observed 15–20 per cent revival of spermatozoa

A third technique was described by Meryman and Kafig[4] in which no glycerol was used Semen diluted in heated milk or egg yolk-citrate was taken up on a 1 × 3 in sheet of nylon gauze which was suspended in a desiccating chamber connected to a high-capacity vacuum pump (27 cu ft /min) Freezing-rate was controlled by constricting the pumping orifice Since the specimen presented a high surface area to volume ratio, very rapid drying was obtained They reported 40–50 per cent spermatozoan motility following reconstitution

These three approaches have been tested in this laboratory and no set of conditions resulted in successful freeze-drying of bovine spermatozoa The earliest attempts involved diluted semen containing glycerol, occasional motile cells and many tailless sperm and broken or bent mid-pieces were observed In addition to the deleterious effects suggested by Leidl[3], semen dried in the presence of glycerol resulted in a waxy residue which could not be reconstituted readily

The procedure of Yushchenko[5] could not be duplicated in that the transfer of spermatozoa from the polar to the non-polar medium was not accomplished When the two-phase system was centrifuged, the spermatozoa were still completely enveloped by the glycerol-containing polar medium after passing into the 'Freon'–heptane mixture As expected, freeze-drying this suspension produced the characteristic waxy residue pointed out previously and also resulted in severe morphological damage Incorporation of surface-active agents such as 'Tweens', 'Carbowaxes', and lecithin did not aid the transfer of cells from the polar to the non-polar phase

In the original work of Meryman and Kafig[4] a mechanical vacuum pump with a free-air pumping speed of 27 cu ft /min was used Later, H T Meryman (personal communication, 1959) suggested that a pump of larger capacity would provide greater versatility in the present work On this basis, a mechanical pump of 130 cu ft /min capacity was used in attempts to confirm their findings Samples were frozen and dried in a 4 × 9-in glass desiccating chamber connected to the pump by a 3-in vacuum line The line was 47 in long with one 90° bend A 3-in. globe valve was inserted in the line 16 in from the desiccating chamber to control cooling-rate, ultimate temperature of the frozen specimen, and drying-rate When full pumping capacity was utilized with this system (valve wide open), diluted semen suspended on a nylon gauze could be cooled to − 50° C solely by evaporation and sublimation

More than 250 trials were made using a wide range of cooling- and drying-rates by adjusting the size of the opening in the 3-in valve Heated skim milk diluent was used in most of the trials, however, egg yolk-citrate and undiluted semen also were tested Freezing- and drying-rates comparable with those of Meryman and Kafig[4] could be achieved readily However, they did not report quantitative data on desiccating chamber pressures and residual moisture of the specimen, and failure in the present work may be due to inability to satisfy these conditions In a few trials. occasional motile spermatozoa were obtained Further investigation indicated that the motile cells usually were associated with a thickened layer of material which accumulated at the bottom of the vertically suspended nylon gauze prior to freezing. The innermost part of this material was not dry and seemed to be the source of the motile spermatozoa When the membrane was kept under vacuum

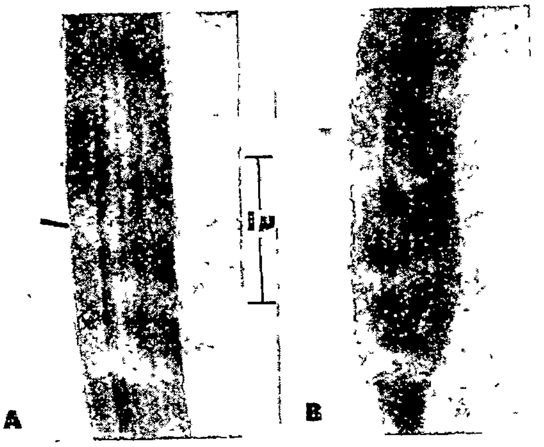

Fig. 1. Ultrastructure of the mid-piece of bull spermatozoa showing (*A*) the presence of the cytoplasmic membrane (arrow) in a control, and (*B*) its absence in a freeze-dried specimen (× *c*. 19,700)

long enough to assure complete drying of the semen, no motile spermatozoa were observed.

In contrast to cells freeze-dried in the presence of glycerol, both diluted and undiluted spermatozoa dried by the latter technique appeared morphologically normal when examined under a phase contrast microscope (× 660). Next, the ultrastructure of freeze-dried spermatozoa was studied with the electron microscope using thin sections. Freshly collected, pooled ejaculates of undiluted semen were freeze-dried on nylon mesh gauze in vacuum and reconstituted in a 2·9 per cent sodium citrate solution. Control spermatozoa were taken up on a nylon gauze and then resuspended in 2·9 per cent sodium citrate without freeze-drying or exposure to vacuum. Spermatozoa were centrifuged from the citrate solution into a loose pellet which was fixed with Caufield's modification[6] of Palade's fixative[7]. 'Araldite' was used as the embedding material and sectioning was performed with a diamond knife on a Porter–Blum ultramicrotome. Electron micrographs revealed that spermatozoa subjected to freeze-drying and reconstitution usually lacked the outer or cytoplasmic membrane (Fig. 1*B*), while control spermatozoa usually displayed an intact membrane (Fig. 1*A*). The presence or absence of the membrane was most apparent in longitudinal sections of the middle piece or principal piece. It is not known whether the same results would have been obtained if the spermatozoa had been diluted before freeze-drying. This possibility is being investigated.

The electron microscope also was used to examine undiluted spermatozoa frozen under vacuum to – 20° C. and thawed in citrate solution at room temperature and undiluted spermatozoa rapidly cooled to 0° C. by vacuum without freezing. As observed in freeze-dried spermatozoa the cytoplasmic membrane of frozen and thawed cells usually was lacking. Spermatozoa rapidly cooled under vacuum without freezing generally possessed intact membranes.

These observations on bull spermatozoa suggest that alteration or destruction of the cytoplasmic membrane represents one possible deleterious effect on survival after either freeze-drying and reconstitution, or freezing and thawing in the absence of glycerol. It is not known whether these treatments cause death and subsequent loss of the cytoplasmic membrance or vice versa. The importance of the cytoplasmic membrane and factors which affect its integrity are being investigated.

We wish to thank Dr. P. Grun and Mrs. Anne Radlow for their advice on the cytological techniques. This investigation was aided by grants from the Pennsylvania State Association of Artificial Breeding Cooperatives.

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Precipitating Antibodies in the Carpet Snake against Parasitic Nematodes

To our knowledge very little attention has been paid to the formation or presence of precipitating antibodies in the cold-blooded vertebrates. Indications of active antibody formation have nevertheless been reported in frogs, turtles and fish; however these estimations were made using either agglutination or anaphylaxis[1]. This communication is a survey of naturally occurring antibodies in the carpet snake (*Morelia spilotes variegatus*) which react positively with the body fluids of their nematode parasites, *Amplicaecum robertsi*, *Ophidascaris* sp., and *Polydelphis* sp., in interfacial precipitin ring tests.

The antigen was collected by puncturing the cuticle of these nematodes and drawing the fluids up a fine glass capillary, the body fluids from approximately fifty worms being pooled, in each case, to give sufficient antigen for these experiments. Blood was collected from the aortas of twelve snakes at autopsy, defibrinated and the serum stored frozen. Table 1 shows the results of interfacial precipitin ring tests using these sera versus the body fluids of the three snake nematodes and the presence or absence of the particular infection in the snakes at the time of autopsy.

In general these results indicate that circulating, precipitating antibodies exist in the sera of carpet snakes which react with the antigens of nematodes infecting them, and that the presence of antibody is probably related to the presence of the particular infection ($P \sim 14$ per cent). Certain expected discrepancies exist in these results, namely, antibodies are present in the absence of infection and infections also occur without detectable antibody. These discrepancies can arise since we do not know either the beginning or the duration of these infections or the number of previous infections (we have noticed that carpet snakes may regurgitate their partially digested food with some of their intestinal nematodes, which may explain how some infections are lost). The possibility also exists that the circulating antibodies are non-specific. To test the specificity of these antibodies the various sera were tested against the body fluids of *Ascaris suum*, from the pig, as antigen. It was found that three of the twelve sera gave positive reactions. This apparent lack of specificity in these

Table 1. THE PRESENCE OR ABSENCE OF ANTIBODY AND INFECTION IN CARPET SNAKES (*Morelia spilotes variegatus*) NATURALLY INFECTED WITH THREE PARASITIC NEMATODES

Parasitic nematode	Carpet snake *A*		*B*		*C*		*D*		*E*		*F*		*G*		*H*		*I*		*J*		*K*		*L*	
	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf
Amplicaecum robertsi	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	−	+	−	−	−	+	−
Polydelphis sp	−	−	+	+	+	+	−	−	−	−	+	+	+	−	−	−	+	−	+	+	+	+	+	+
Ophidascaris sp	−	+	+	−	+	+	+	−	+	+	−	+	+	−	+	+	+	+	+	+	−	+	+	+
Ascaris suum	+		+		−		−		−		+		−		−		−		−		−		−	

Ab, antibody, Inf, infection

three cases may be accounted for by the presence of antibodies versus Forssman antigen known to exist in certain helminths[2]. However, the carpet snake must also be able to produce specific antibodies against antigens from its three nematode parasites since the sera negative to *A suum* gave positive reactions against these worms ($P \sim 1$ per cent). Further work is being done on the natural antibodies found in the carpet snake

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Fibre Follicles in Romney Ewes

ORWIN has reported[1] the occurrence of what he describes as 'branching follicles' in skin from two-year-old New Zealand Romney ewes In his communication there appears to be some confusion concerning the evidence required to demonstrate conclusively whether follicles are branched, that is, have outgrowths of derived follicles, or are separate follicles which have coalesced Orwin's photographs show more than one fibre in a common sheath at sebaceous-gland level and at a higher level, this, however, in itself is not adequate evidence that one follicle has been derived from another by branching These follicles may equally well have been separately initiated and coalesced so that the fibres come to lie in a common sheath and a common follicular mouth

Auber[2] has indeed demonstrated the occurrence of wool follicles coalescing at a level below that of the sebaceous glands Serial skin sections at levels which include lower levels than that of the sebaceous glands should be made, the follicles traced up and observations confirmed by 'vertical' sections, before deciding whether a follicle has been derived from another one by branching or has coalesced with it. I do not suggest that follicles derived by branching do not occur in breeds of sheep other than the merino, however, in many thousands of serial skin sections from English Romney sheep and lambs that I have examined at all levels I have sometimes seen coalesced follicles, but have not seen truly branched follicles.

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Localization of Inhibitors of Neural Differentiation in Chick Embryos

IN testing the hypothesis of specific inhibition in cellular differentiation, I have shown[1] that early chick embryos, when cultured on a nutrient medium which has incorporated in it supernatants of adult and embryonic brain extracts, displayed homologous brain defects in a significant number of cases Since the preparation of subcellular components has become a standard technique for problems of cellular chemistry (see review by Allfrey[2]), this investigation was prompted with the hope of localizing the inhibitory material in the crude brain extracts to a particular cell fraction Accordingly, chick brains were homogenized and subjected to differential centrifugation in order to obtain mitochondrial, microsomal, and soluble protein fractions Earlier investigations of Braverman[3] and Lemcque[4] showed that the nuclear fraction is not involved The procedure used for culturing chick embryos was that of Spratt[5] Since I have found[1] that stages 4 and 5 (ref 6) are the crucial stages for treatment with brain extracts, the assay for inhibitory activity was restricted to the use of these stages

Whole brains from recently hatched chicks totalling 10–15 gm were homogenized in 2 vol (w/v) of 0 25 *M* sucrose–0 004 *M* calcium chloride in a Potter–Elvehjem homogenizer The nuclei and cellular fragments were sedimented in an International refrigerated centrifuge at 700*g* for 10 min The supernatant was then subjected to 15,000*g* for 30 min in a Servall centrifuge This resulted in a white pellet of mitochondria, which was dispersed in phosphate-buffered chick Ringer, *p*H 7 6–7 8 The supernatant was centrifuged at 105,000*g* for 60 min. in a Spinco *L* ultracentrifuge, rotor No 40 This yielded a microsomal pellet, and a supernatant consisting of the soluble protein fraction The former was dispersed in chick Ringer, while the soluble protein fraction was subjected to 18–24 hr dialysis against chick Ringer. All preparations were carried out in the cold room at a temperature of 0–2° C

Aliquots of the samples were taken for quantitative estimations of protein nitrogen using the Kjeldahl method The nutrient medium composed of 0 5 per cent agar, albumen extract, and subcellular fraction was made up in the ratio of 2 1 1 The concentration used in these experiments was 75–90 µgm of protein nitrogen of subcellular component per c c of culture medium

Controls using a myosin preparation obtained through the courtesy of Dr Yujiro Hayashi and adjusted to the equivalent concentrations as with the subcellular fractions in terms of protein nitrogen were used It was intended that the myosin sample, obtained from the leg muscles of the same chicks which were the sources for the brains, would serve as a control of mesodermal origin According to Dr Hayashi, the myosin samples, prepared by a modification of the method of Hasselbach and Schneider[7], showed a single peak when examined by both ultracentrifugal and electrophoretic methods Before use,

Table 1 EFFECT OF SUBCELLULAR FRACTIONS OF BRAIN ON NEURAL DIFFERENTIATION

	Subcellular fraction			
	Mit	Mic	Sol protein	Myosin
Total No of explants	53	67	80	45
Kinds of defects				
Normal	41 (77%)	54 (81%)	15 (19%)	8 (18%)
General defect	7 (13%)	5 (7%)	6 (8%)	—
No development	5 (9%)	5 (7%)	10 (11%)	5 (11%)
Brain limited defect	0 (0%)	3 (4%)	49 (61%)	6 (13%)
Mesodermal and general defect	—	—	—	26 (58%)

the myosin samples were dialysed against chick Ringer solution

The results of this investigation are presented in Table 1 It indicates that the inhibitory factor is localized in the final supernatant or soluble protein fraction For a discussion of the classification of defects see ref 1, since the same system was used in this work In regard to the myosin controls, at concentrations equivalent to the experimental series, that is, 75–90 μgm of protein nitrogen, it was found to be highly toxic for the developing embryos, and resulted in all embryos undergoing no development Consequently, the level has to be lowered considerably, to 15–20 μgm per c c of culture medium. It appeared that the mesodermal defects, which are manifested in circulatory abnormalities, concomitantly lead to generalized defects Thus, a high number of combined mesodermal and general defects were recorded This is not surprising in view of the fact that the myosin preparation represents a relatively purified sample, as compared with the fractions obtained from brain material, which are still rather crude preparations In view of this fact, future work is planned in an effort to characterize and isolate further the inhibitory properties of the soluble protein fraction In this connexion, fractionation of the soluble protein fraction may yield a more potent and pure source of the specific neural differentiation inhibitor.

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ENTOMOLOGY

An Apparatus for Observations on the Feeding Mechanism of the Flea

SEVERAL types of feeding chambers have been devised[1] for *in vivo* observations on the feeding mechanism of blood-sucking arthropods, but these chambers, designed to accommodate relatively large creatures (triatomine bugs, tsetse flies and ticks), have proved to be unsuitable for small insects and acarines, such as fleas, lice and mites Our interest in the mechanism of disease transmission by the flea led us to construct a small feeding apparatus by means of which we have been able to undertake extensive observations on the uptake of blood by this insect from the tissues of its host

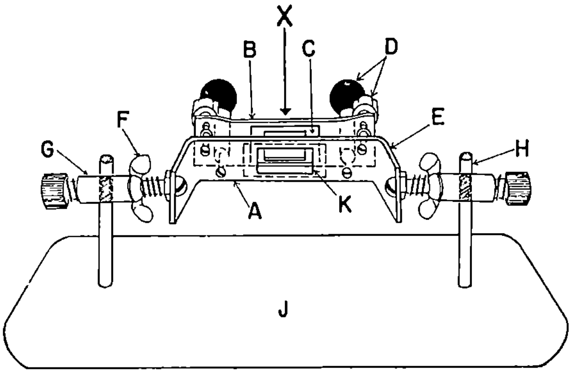

Fig 1 The feeding apparatus as seen from the microscope

The apparatus (Fig 1) consists essentially of two apposable, thin, steel plates (*A* and *B*), each with a centrally placed viewing window (*K*)

Plate *A*, 6·3 cm in length and 1 3 cm. in height, is extended at both extremities to form right-angled extensions (*E*), 1·5 cm. long Each extension is provided with a horizontal bar (*G*) which fixes into a vertical rod (*H*) attached to a carrying table (*J*). The carrying table is clamped to the reversed mechanical stage of a horizontally placed microscope as described by Griffiths and Gordon[2] for an earlier type of feeding chamber

Plate *B*, the same size as *A*, is joined to plate *A* by means of two pairs of adjusting screws (*D*) The adjusting screws (the upper pair is threaded through light springs) enable the plates to be apposed and tilted so that their lower edges can be approximated to enclose the ear of the host (Fig 2)

The viewing windows (*K*), 1 6 cm × 0 8 cm , are each covered by a coverslip (*L*) of a convenient size held in position by an adhesive applied to the edges of the window

An anæsthetized mouse is placed on the carrying table The upper portion of one of its ears, previously treated with a depilatory, is so arranged as to lie over the coverslip on plate *B*, and a strip of foam rubber is then introduced between the plates, one on each side of the window at position *C* (Fig 1) The adjusting screws are gradually tightened until the lower edges of both plates gently grip the ear, the upper edges being kept separated by the springs of the upper pair of screws. In this position plate *B*, carrying the ear, lies at an angle to plate *A* (which is vertical to the microscope tube) In order that the ear should present a flat microscopic field the apparatus is rotated by unloosening the wing nuts (*F*), until the plates have assumed the position shown in Fig 2, and the nuts are retightened

A flea (*N*) anæsthetized with carbon dioxide is then introduced into the chamber, and the upper part is closed off promptly with a strip of foam rubber (*M*) The space enclosed by the lower edges of the plates, the two vertical (*C*) and the horizontal (*M*) strips of foam rubber, forms a completely sealed cell (Fig 2) containing the tip of the animal's ear and the insect

We observed, using this simple apparatus, that *Pulex irritans*, *Xenopsylla cheopis*, *Ctenocephalides felis* and *Atyphloceras multidentatus* normally feed, in non-sensitized mice, directly from the lumen of small blood vessels, and thus may be described as capillary feeders On rare occasions we saw fleas attempting to feed from extravasations of blood resulting from the rupture of vessels, but they never took up any significant quantities of blood from this source

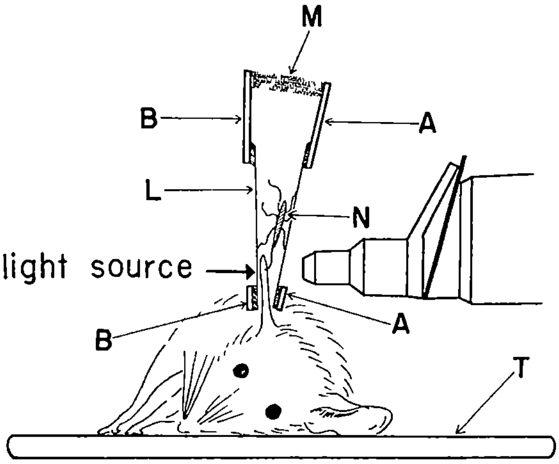

Fig 2 A cross-section through the feeding chamber at position X on Fig 1 (showing a flea feeding on a mouse's ear) after the apparatus has been rotated so that plate B, instead of plate A, is vertical to the tube of the microscope

Feeding appears to proceed in this way the flea introduces its biting fascicle[3]—the paired maxillæ and the epipharynx—into the skin of the host, the maxillæ acting as the cutting organs Having reached a suitable vessel, usually of small calibre, the tip of the epipharynx enters the lumen, the maxillæ (which form the salivary canals) remaining outside The flea then sucks up blood, emitting from time to time, as clearly visible puffs, droplets of saliva in the neighbourhood of the puncture in the vessel wall (but not into the vessel lumen) When the meal is completed the fascicle is rapidly withdrawn, there is surprisingly little damage at the point of introduction of the mouthparts into the skin, the withdrawal hæmorrhage being slight or absent

We wish to acknowledge the assistance of a National Institutes of Health grant, *E*-3571, in carrying out this project and the help of Frank Prince and his colleagues (of C D C station at San Francisco), who kindly provided us with a proportion of the wild and laboratory bred fleas we used in our experiments Histological material, which confirmed our direct observations, was prepared by Rosalie Gill of the Hooper Foundation, to whom we are indebted

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Intestinal Proteinases in an Insect, *Calliphora vomitoria* L.

THE properties of the proteolytic enzymes occurring in extracts of insect guts are generally thought to resemble those of vertebrate trypsin[1,2] Only proteinases of alkaline type, with maximum activity about *p*H 8, have been recorded in *Calliphora* adults[3], *Lucilia* larvæ[4], *Musca* larvæ[5], *Stomoxys* adults[6], and *Calliphora* larvæ[7] The only workers to suggest that proteinases in Diptera-Cyclorrhapha do not conform to the general theory, Greenberg and Paretsky[8], reported two distinct proteinases functioning in every stage of the life-cycle of *Musca* with *p*H optima at *p*H 3 and *p*H 8-9.

It has now been established by two techniques that a proteolytic enzyme (or enzymes) with an activity peak about *p*H 3 is produced by cells of the mid-gut of adult females of the blowfly species *C vomitoria*

Proteolytic activity of aqueous extracts of mid-guts of flies fed on meat was determined *in vitro*, over a selected *p*H range, by a colorimetric method Sheep hæmoglobin was used as substrate, prepared in citrate-phosphate buffer, and mixtures of buffered substrate and gut extract were incubated for 3 hr at 38° C After removal of undigested protein, by precipitation (with 10 per cent trichloracetic acid) and centrifugation, the mixture was made alkaline by the addition of sodium carbonate The presence of liberated tyrosine, and tryptophan, was demonstrated by the addition of Folin-Ciocalteau reagent, the intensity of the blue colour which develops being used as a measure of the degradation of the substrate Direct readings of colour intensities were made with an *EEL* portable colorimeter (*O R* 1 light filter) exactly 5 min after the addition of the reagent The graph in Fig 1 was prepared from means of three determinations of proteolytic activity at thirteen *p*H-levels between *p*H 2 5 and *p*H 8 5 The results indicate a high concentration of an enzyme (or enzymes) in gut extracts with an optimum *p*H about *p*H 3 and a lower concentration of an enzyme (or enzymes) with an optimum *p*H at least as high as *p*H 8 5 Controls were prepared at several *p*H-levels and tested for free tyrosine, etc , immediately after preparation, without incubation Readings for these controls approximated to those obtained from digests between *p*H 5 5 and *p*H 7, indicating that there is little enzyme activity within that *p*H-range Further tests

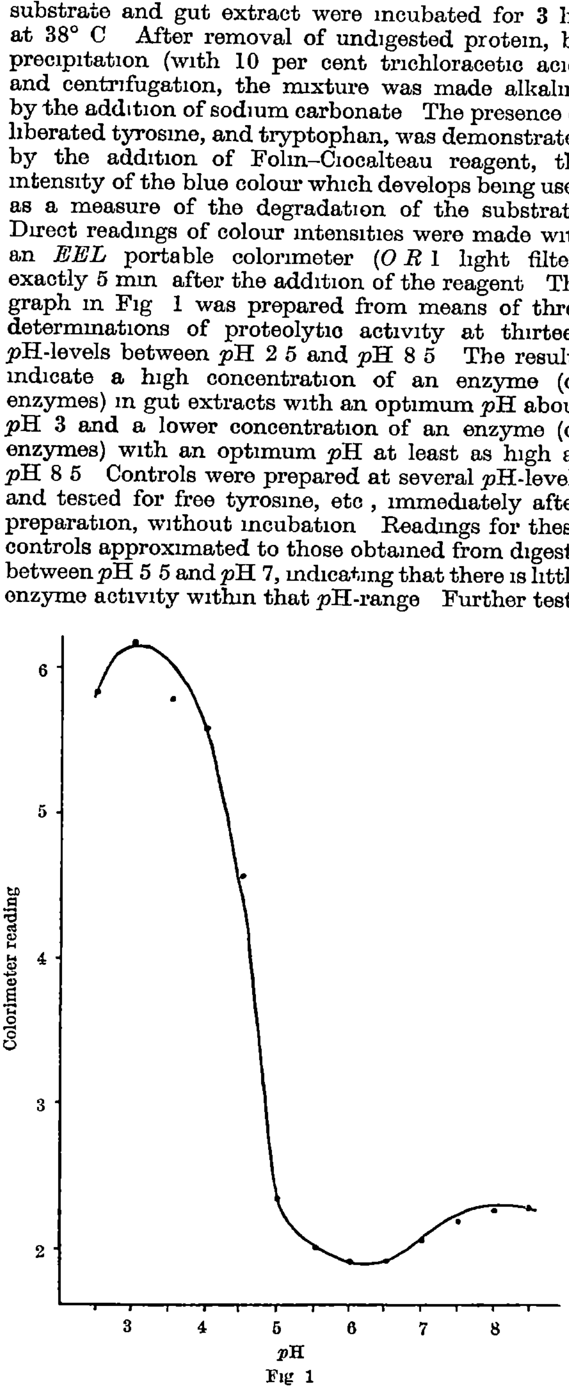

Fig 1

carried out at *p*H 3 with distilled water substituted for enzyme extract showed that the high reading at that *p*H was not due to autolysis of the substrate.

In the second demonstration of proteolytic activity at low *p*H, mid-guts were dissected from 5-day old flies which had fed on meat. The guts were split longitudinally and the peritrophic membrane tube and contents removed. Each gut was then divided into three parts corresponding to the three zones, fore-mid-gut, mid-mid-gut and hind-mid-gut, the contents of which are buffered (as shown by Waterhouse's range-indicator method[9]) at *p*H 5, *p*H 3·6, and *p*H 8. These fractions were then laid on the surface of a thin milk-agar plate of the type normally used for bacteriological work. The milk-agar medium, which contained thiomersalate to inhibit bacteria, was either buffered at *p*H 3·6 or *p*H 8. Preparations were incubated for 24 hr at 38° C. In the low *p*H plates a wide zone of clearing developed around the mid-mid-gut. There was also some evidence of an enzyme active at low *p*H diffusing from the last part of the fore-mid-gut in smaller quantity than that from the mid-mid-gut. Clearing occurred in the immediate vicinity of the hind-mid-gut in *p*H 3·6 plates, but this appeared to be restricted to the range of the gut cells' own buffering effect beyond which the hind-mid-gut enzyme was obviously inactive. In *p*H 8 plates a wide zone of clearing developed around the hind-mid-gut. Thus the results of the quantitative tests have been confirmed and it has been shown that the proteinase (or proteinases) active at low *p*H in the gut of *C. vomitoria* is principally produced by the cells of that zone the *p*H of which is adjusted to suit such an enzyme.

Female blowflies require protein in their diet in order to produce eggs. Males have no such need, but it is interesting to note that they will feed readily on meat, and the milk-agar plate method indicates the presence of the same enzymes in male as in female intestines.

We thank Drs. J. R. Norris and W. R. Rees of this University for valuable discussions and Dr. Ellen Thomsen, Copenhagen, for the initial suggestions.

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Home and Distribution of the House Cricket *Acheta domesticus* L.

THE house cricket, *Acheta domesticus*, is generally believed to have moved into Europe and North America from arid and semi-arid areas of northern Africa or south-western Asia[1]. However, during my work on *Acheta domesticus* and related species, I have found evidence which suggests that the question regarding the native country and distribution of *A. domesticus* is more complicated.

Colour and size are unreliable taxonomic characters for separating closely related species of crickets, and the species of the *domesticus* group are remarkably uniform in their outward appearance. However, among the male specimens from various parts of the world, identified as *Acheta domesticus* by Chopard, Uvarov, Dirsh, Kevan, Gurney and other specialists, I have found no less than five species which have no significant external morphological differences but can be clearly separated on the basis of their internal male genitalia. Indeed, two of these species have been experimentally shown to be reproductively isolated[2], but the anatomical reasons for this have only now become clear.

The first step to clear the confusion regarding the taxonomy of the house cricket is to fix the name *Acheta domesticus* L. for one of the five species distinguished in the course of the present studies. The name *A. domesticus*, described from Sweden, should be restricted to the house cricket commonly found in Europe and North America, including the Canadian strain[2]. The males determined on the basis of genitalia were compared with *domesticus* specimens from Sweden, and both sexes taken from the same laboratory-bred stock are being re-described.

Uvarov[1] described on the basis of colour and size *Acheta meridionalis* from Khartoum as a sub-species of *A. domesticus*, and Kevan[3] considered it a wild southern form of the same, but the examination of male genitalia shows that *A. meridionalis* (Uvarov) is a distinct species, known to me also from West Aden Protectorate, Egypt and Fezzan. The examination of the male genitalia shows also that the species referred to earlier as the Pakistani strain[2] is *Acheta hispanicus* Rambur, which I am able to record also from Morocco, Baghdad and West Pakistan. Three more distinct species of the *domesticus* group are known to me from Egypt, East Africa and India.

The specimens of all the species referred to above are preserved in the British Museum (Natural History), London, and their full descriptions, including a re-description of *Acheta domesticus* L. with full synonymy, are ready for publication. Once the identities of the species previously confused with *A. domesticus* are established, it may become possible to build up a better picture of the distribution of the European house cricket (*A. domesticus*), and to determine its native country with greater certainty. The correct identification of these species is also of great economic importance as at least two of them (*A. domesticus* and *A. hispanicus*) are serious pests of irrigated crops in the Indo-Pakistan sub-continent[4], and with the extension of canal irrigation in the arid and semi-arid regions their potential danger continues to increase. I shall be glad to receive material on these crickets either from houses or collected in the field for further studies.

This work was carried out while I held a research fellowship of the South East Asia Treaty Organization and I am grateful for it, and also to Sir Boris Uvarov and Dr. V. M. Dirsh for their advice and help.

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk * are open to the public*)

Monday, December 11

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W C 1), at 5 30 p m —Prof L S Penrose, F R S "A Theory of Self-reproducing Mechanisms"

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 6 p m —Prof W K Estes "Current Foci of Research in Mathematical Behaviour Theory" * (Last of three Special University Lectures)

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W C 2), at 6 45 p m — Mr G S C Lucas O B E "The Professional Engineer and an Expanding Industry" (Presidential Address)

Tuesday, December 12

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N W 1), at 5 p m —Scientific Papers

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W C 2), at 5 30 p m — Mr L Lewin "Diversity Reception and Automatic Phase Reception"

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Dr I D P Wootton "Retention of Aromatic Compounds in Acute Renal Failure" * (Last of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m — Mr J G G Hempson "Instrumentation Problems in Internal Combustion Engine Development"

UNIVERSITY OF LONDON (in the Beveridge Hall, Senate House, University of London, London, W C 1), at 6 p m —Sir Charles Snow Birkbeck College Foundation Oration

INSTITUTE OF WOOD SCIENCE (at Carpenters Hall, Throgmorton Avenue, London, E C 2), at 7 p m —Dr A C Copisarow "The Role of Forest Products Research in National Economy"

Tuesday, December 12—Wednesday, December 13

BRITISH BIOPHYSICAL SOCIETY (at the Royal Institution, Albemarle Street, London, W 1)—Symposia on "The Structure of Globular Proteins" and "The Function of Proteins", also a session on contributed papers

Wednesday, December 13

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 15 p m —Mr I D Hill "Sampling Inspection and Defence Specification DEF-131"

INSTITUTE OF FUEL (at the Royal United Service Institution, Whitehall, London, S W 1), at 5 30 p m —Mr A Sherry "Power Station Optimization" *

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (in conjunction with the BRITISH NUCLEAR ENERGY CONFERENCE, at Savoy Place, London, W C 2), at 5 30 p m —Mr J Henderson, Mr G F Kennedy and Dr K J Wootton "Electrical Aspects of Hunterston Nuclear Generating Station"

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W C 2), at 6 p m —Dr E S Hedges "Tin—Its Contribution to Social Development"

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S W 1), at 6 15 p m —Meeting on "Foods Low in Carbohydrates"

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMISTRY GROUP (at "The Feathers", Tudor Street, London, E C 4, at 6 30 p m — Discussion Meeting

SOCIETY OF INSTRUMENT TECHNOLOGY, CONTROL SECTION (at Manson House, 26 Portland Place, London, W 1), at 7 p m —Mr P K M'Pherson "Application of Complex Plane Methods to System Design"

BRITISH PSYCHOLOGICAL SOCIETY, MEDICAL SECTION (at the Royal Society of Medicine, 1 Wimpole Street, London, W 1), at 8 30 p m — Mr Stephen Black "Hypnosis in Physiological Research"

Thursday, December 14

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S W 1), at 2 30 p m and 5 30 p m —Special Meeting on "New Test Methods"

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY (in the Physics Building, Prince Consort Road, London, S W 7), at 5 30 p m —Sir Patrick Linstead F R S "The Prince Consort and the Founding of the Imperial College" (Special Lecture to commemorate the Centenary of the Death of H R H The Prince Consort)

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W C 2), at 5 30 p m —Dr E R Laithwaite "Oscillating Machines—Synchronous and Asynchronous", Dr E R Laithwaite and Mr R S Mamak "An Oscillating Synchronous Linear Machine"

BRITISH INSTITUTION OF RADIO ENGINEERS, TELEVISION GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street Gower Street, London W C 1), at 6 p m —Symposium on "Constant Luminance Colour Television"

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (in "The Feathers" Restaurant, Tudor Street, London, E C 4) at 6 30 p m —Seventeenth Annual General Meeting, followed by a Discussion Meeting on 'The Assessment of Anti-Aretherosclerotics"

RADAR AND ELECTRONICS ASSOCIATION (in the Lecture Hall of the Royal Society of Medicine, John Adam Street, Adelphi, London, W C 2) at 7 p m —Dr J Gough and Prof F Landgrebe "Loudspeaker Enclosures"

CHEMICAL SOCIETY (in the Lecture Theatre of the Royal Institution, Albemarle Street, London, W 1), at 7 30 p m —Prof C E H Bawn, F R S 'Stereospecific Polymerisation" (Liversidge Lecture)

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W 1), at 7 30 p m —Dr F I C Apted "Sleeping Sickness in Tanganyika, Past, Present and Future"

Friday, December 15

SOCIETY FOR ANALYTICAL CHEMISTRY (joint meeting with the INFRA RED DISCUSSION GROUP, at King's College, Strand, London, W C 2), at 11.10 a m —Meeting on "Applications of Infra Red Spectroscopy to Quantitative Analysis"

Saturday, December 16

BIOCHEMICAL SOCIETY (at the Wolfson Institute, Postgraduate Medical School of London, Ducane Road, London, W 12), at 10 30 a m —Scientific Papers and Demonstrations

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned

ASSISTANT EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER (preferably with a university degree) AT THE FRESHWATER FISHERIES LABORATORY, Department of Agriculture and Fisheries for Scotland, Pitlochry—Establishment Officer, Room 73, Department of Agriculture and Fisheries for Scotland, St Andrew's House, Edinburgh 1 (December 15)

ASSISTANT INSPECTOR (male, graduate in zoology, etc) OF FISHERIES in the Department of Lands, Dublin—The Secretary, Civil Service Commission 45 Upper O'Connell Street, Dublin 1, Republic of Ireland (December 15)

VERTEBRATE BIOLOGIST (with ethological training or experience), for a research post in a Mental Hospital—The Secretary, Birmingham Regional Hospital Board, 10 Augustus Road, Edgbaston, Birmingham 15 (December 16)

ASSISTANT IN THE DEPARTMENT OF GENETICS, for general duties and particularly to assist on work on the genetics of fungi—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (December 27)

LECTURER or ASSISTANT LECTURER IN PHYSICS—The Principal, Bede College, Durham (December 31)

LECTURER or SENIOR LECTURER IN GENETICS at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, December 31)

READER (with special qualifications in educational psychology) IN EDUCATION at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Brisbane and London, December 31)

HEAD (with high academic qualifications and experience in research and in teaching to university degree level) OF THE DEPARTMENT OF PHYSICS—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S W 3 (January 1)

LECTURER (with a registrable veterinary qualification and postgraduate experience in bacteriology) IN BACTERIOLOGY in the School of Veterinary Medicine—The Registrar, Trinity College, Dublin 2, Republic of Ireland (January 1)

BIOPHYSICIST (post-doctoral standing) IN THE ELECTRON MICROSCOPE SECTION, to take part in an investigation of the biological applications of the electron scanning microanalyser (X-ray microspectrometer)—The Secretary, Department of Physics, Cavendish Laboratory, Free School Lane, Cambridge (January 5)

CHAIR, and LECTURER or ASSISTANT IN PHILOSOPHY at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 15)

LECTURER (preferably with a special interest in historical geography) IN GEOGRAPHY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (January 15)

ASSOCIATE PROFESSOR IN EXPERIMENTAL PATHOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 17)

LECTURER (preferably with some experience in steroid and/or carbohydrate chemistry) IN ORGANIC CHEMISTRY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 17)

CHAIR OF AGRICULTURAL CHEMISTRY IN THE DEPARTMENT OF AGRICULTURE, University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 31)

CHAIR OF COLOUR CHEMISTRY AND DYEING—The Registrar, The University, Leeds 2 (January 31)

LECTURER (trained and experienced teacher with a degree in chemistry or biology) IN EDUCATION at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Hong Kong and London, January 31)

LECTURER (with at least an honours degree or equivalent qualifications, some postgraduate research experience, and preferably interests in mammalian or human biochemistry) IN BIOCHEMISTRY in the School of Biological Sciences, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W C 2 and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (January 31)

OFFICIAL FELLOW AND LECTURER (mathematician or theoretical physicist) IN MATHEMATICS—The Vice-Provost, Worcester College, Oxford (January 31)

SENIOR RESEARCH ASSISTANT (with a higher degree in chemistry or biochemistry, and several years experience in biochemical research) IN BIOCHEMISTRY, to be engaged full time in a research programme in the field of protein chemistry—The Director of Research, St Vincent's School of Medical Research, Victoria Parade, Fitzroy, N 6, Victoria, Australia (February 28)

ASSISTANT, ASSOCIATE OF FULL PROFESSOR OF PSYCHOLOGY and an ASSISTANT PROFESSOR OF PSYCHOLOGY—Prof J R Royce, Head, Department of Psychology, University of Alberta, Edmonton, Alberta, Canada

ASSISTANT PROFESSOR OF PHYSICS—Prof C E Challice, Department of Physics, University of Alberta, Calgary, Alberta, Canada

ASSOCIATE PROFESSOR OF ASSISTANT PROFESSOR IN THE DEPARTMENT OF PHYSICS—Head of the Department of Physics, Victoria College, Victoria, B C , Canada

HISTORICAL ARCHÆOLOGIST (graduate with specialization in archæology, approximately four years experience in conducting archæological investigations, including the excavation of historical ruins) in the Department of Northern Affairs and National Resources, Louisbourg, Nova Scotia, Canada, to undertake all archæological excavations in connexion with the restoration of the Fortress of Louisbourg National Historic Park—Civil Service Commission of Canada, Ottawa, Ontario, Canada

MASTER TO TEACH MATHEMATICS to public school scholarship standard—The Headmaster, Lathallan School, Montrose Angus

PHYSICISTS or ELECTRICAL ENGINEERS (preferably with experience in one or more of the following fields high vacuum, R F techniques (75 mc /s), high power rectifier and pulse circuits, general electronic instrumentation and controls) IN THE DEPARTMENT OF PHYSICS, to assist with the construction and commissioning of a large heavy ion linear accelerator—The Assistant Director, Linear Accelerator Laboratory, Physics Department, The University, Manchester

RESEARCH ASSISTANT (with a degree in engineering and some knowledge of mechanical vibration) IN THE DEPARTMENT OF ENGINEERING, for work on certain problems related to the vibration of turbine blades—The Secretary, The University, Old College, South Bridge, Edinburgh

RESEARCH FELLOW (recent or prospective Ph D graduate in biochemistry, physiology, nutrition or allied subjects) IN THE FIELD OF NUTRITION IN THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY—The Secretary and Registrar, The University, Southampton

SCIENTIFIC OFFICER (with an honours degree in chemistry or agricultural chemistry, A R I C or an equivalent qualification, and particularly interested in analytical chemistry) IN THE CHEMISTRY DEPARTMENT, to take part in researches on the composition of milk—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting Ref 61/32

SENIOR LECTURER IN ORGANIC CHEMISTRY—The Registrar, The University, Leicester

SENIOR LECTURER IN PHYSICS and a LECTURER IN PHYSICS—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N 7

SENIOR LECTURER or LECTURER (with a knowledge of West African livestock and feeding-stuffs, especially grass) IN AGRICULTURAL BIOCHEMISTRY AND NUTRITION at the University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1

SENIOR TECHNICIAN, for histological research—The Secretary, The Royal Free Hospital, Gray's Inn Road, London, W C 1

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Materials for Gas Chromatography Standards and Data for "Embaphase", Stationary Phases, and "Embace" Kieselguhr Second edition Pp 68 (Dagenham May and Baker, Ltd , 1961) [259

The Industrial Diamond Information Bureau Diamonds in Industry an Introduction Pp 12 The Diamond Research Laboratory Pp 4 (London The Industrial Diamond Information Bureau, 1961) [259

Radiation Botany, Vol 1, No 1 (September, 1961) (An International Journal devoted to Plant Radiobiology and Closely Related Fields) Pp 100 Annual subscription rates (A) For libraries, government establishments, research laboratories, etc , 140*s* , 20 dollars (B) For individuals who place their orders directly with the publisher and certify that the journal is for their personal use, 70*s* , 10 dollars (London and New York Pergamon Press, 1961) [259

Philosophical Transactions of the Royal Society of London Series B Biological Sciences No 710, Vol 244 (21 September, 1961) A Study of a Natural Population of *Phytodecta Olivacea* (Forster) (Coleoptera, Chrysomeloidea) By O W Richards and Nadejda Waloff Pp 205–257 (London Royal Society, 1961) 18*s* [259

Falklands Islands Dependencies Survey Scientific Reports No 26 The Geology of the South Shetland Islands 1 The Petrology of King George Island Pp 28+3 plates 10*s* net No 27 The Geology of the South Shetlands Islands 2 The Geology and Petrology of Deception Island By D D Hawkes Pp 43+4 plates 16*s* net (London H M Stationery Office, 1961 Published for the Colonial Office) [259

Other Countries

American Academy of Arts and Sciences, Boston Records of the Academy (including new members elected 10 May, 1961), 1960–1961 Pp 157 (Boston, Mass American Academy of Arts and Sciences, 1961) [259

United States Department of the Interior Geological Survey Bulletin 1058–H Geology of Part of the Craig C–2 Quadrangle and Adjoining Areas, Prince of Wales Island, Southeastern Alaska. By C L Sainsbury Pp v+299–362+plates 33–39 Bulletin 1087–I Relation of Uranium Deposits to Tectonic Pattern of the Central Cordilleran Foreland By Frank W Osterwald and Basil G Dean Pp iii+337–390+plates 28–34 Bulletin 1116–E Index to Geophysical Abstracts 180–183, 1960 By James W Clarke, Dorothy B Vitaliano, Virginia S Neuschel, and others Pp ii+637–695 25 cents Bulletin 1117–A Geochemistry of Minor Elements on Coals of the Northern Great Plains Coal Province By Peter Zubovic, T Stadnichenko, and Nola B. Sheffey. Pp iv+58+plate 1. 50 cents (Washington, D C Government Printing Office, 1961) [259

United States Department of the Interior Geological Survey Water-Supply Paper 1498–D Effect of Depth of Flow on Discharge of Bed Material By Bruce R Colby Pp iii+12 15 cents Water-Supply Paper 1522 Quality of Surface Waters of the United States, 1957 Parts 7 and 8 Lower Mississippi River Basin and Western Gulf of Mexico Basins Prepared under the direction of S K Love Pp x+499 Water-Supply Paper 1539–C Ground-Water Reconnaissance of Winnemucca Lake Valley, Pershing and Washoe Counties, Nevada By C P Zones Pp iii+18+plate 1 45 cents Water-Supply Paper 1539–R Selected Bibliography on Evaporation and Transpiration By T W Robinson and A I Johnson Pp ii+25 Water-Supply Paper 1581 Ground-Water Potentialities in the Crescent Valley, Eureka and Lander Counties, Nevada By C P Zones Pp iv+50+plate 1 Water-Supply Paper 1610–A Waterpower Resources of the Bradley River Basin, Kenai Peninsula, Alaska By F A Johnson Pp iv+25+plate 1 (Washington, D C Government Printing Office, 1961) [259

Canada National Research Council, and Medical Research Council Report on University Support, 1960–61 Pp 123 (N R C No 6390) (Ottawa National Research Council, and Medical Research Council, 1961) [259

Tokyo Astronomical Observatory Tokyo Astronomical Bulletin, Second Series No 123, (January 25, 1961) A Total Eclipse of the Sun, July 20, 1963 and its Circumstances in Hokkaido, Japan By Shinko Aoki and Yoshiko Endo Pp 1695–1706 No 140, (February 20, 1961) A Method of Improvement of the PZT Star Places By Masahisa Torao, Shigetaka Iijima, and Naoyuki Matsunami Pp 1759–1769 No 142, (March 5, 1961) Polar Tube Observations during 1960 By Baosuke Sekiguchi and Jun'itsu Matsumoto Pp 1901–1902 No 143, (March 20, 1961) On the Auto-Compensating Effect in Travel Time of Radio Signals By Shigetaka Iijima Pp 1903–1916 No 144, (February 5, 1961) A New Photo electric Photometer with Electronic Refrigerator By Masaaki Huruhata and Masatoshi Kitamura Pp 1917–1922 (Tokyo Tokyo Astronomical Observatory, 1961) [259

Occasional Papers of the California Academy of Sciences No 30, (August 31, 1961) The Immature Stages of *Callomina*, with the description of a New Species of this Genus (Diptera Platypezidae) By Edward L Kessel Pp 10+1 plate (San Francisco California Academy of Sciences, 1961) [259

Annals of the New York Academy of Sciences Vol 94, Article 1 Plasma Proteins in Health and Disease By George T Dimopoullos and 40 other authors Pp 1–336 (New York New York Academy of Sciences, 1961) [259

National Academy of Sciences National Research Council Nuclear Science Series NAS-NS 3038 The Radiochemistry of Tellurium By G W Leddicotte Pp v+42 (Washington, D C Office of Technical Services, Department of Commerce, 1961) 50 cents [259

Canada Department of Mines and Technical Surveys Geological Survey of Canada Geological Maps—Preliminary Series Map 9–1961 Kettle Rapids, Manitoba, (Sheet 54D) Map 10–1961 La Loche, Saskatchewan, (Sheet 74C) Map 16–1961 Firebag River Area, Alberta and Saskatchewan, (Sheets 74E (E½) and 74F (W½)) Geological Maps Map 1068–A Collins Point, West of Principal Meridian, Manitoba, (Sheet 63 N/3, West Half) (Ottawa Geological Survey of Canada, 1961) [259

Administration of South-West Africa. Marine Research Laboratory Investigational Report No 2 The Pilchard of South-West Africa (*Sardinops ocellata*)—Age Studies and Age Composition of *Sardinops ocellata* in the Commercial Catches, 1952–1958, and a New Method for the Determination of the Age of *Sardinops ocellata* By O Nawratil Pp 43 (Windhoek Secretary for South-West Africa, 1961) [259

United States Department of Agriculture Farmers' Bulletin No 1893 Control of Grape Diseases and Insects in the Eastern United States By J R McGrew, G W Still and Howard Baker Pp i+32 (Washington, D C Government Printing Office, 1961) 20 cents [259

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO , LTD
ST MARTIN'S STREET, LONDON, W C 2
Telephone Number Whitehall 8831. Telegrams Phusis Lesquare London

Advertisements only should be addressed to
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PROFESSIONAL CIVIL SERVANTS IN BRITAIN

IN his speech at the sixteenth annual delegate Conference of the Institution of Professional Civil Servants last May, Mr Stanley Mayne, who was then retiring from the office of general secretary, commented at some length on the position of three scientific classes in the Civil Service He noted a feeling of frustration in the experimental officers class, and emphasized that the scientific officer class was not attracting the numbers it needed In particular, promotion from assistant engineer to executive engineer was no longer a matter of selecting an officer best fitted for the job, but of trying to persuade someone to accept the post, because the reward for promotion was so insignificant and there was no acceptable compensation for any consequent removal and disruption of social life. Mr Mayne also referred particularly to the position of those in high professional posts in the Service, where salaries were still disproportionate with the responsibilities and with those paid for the corresponding professional positions outside

Mr Mayne made it clear, in pressing these claims, particularly those of certain leading professional posts, for salaries at the same level as those of 'permanent secretaries', that the Institution of Professional Civil Servants recognized that it was not simply a matter of pay but of status also The professional and scientific Civil Service had suffered too long because its leading members had been valued too cheaply by the Service, and because science and technology are in general valued too cheaply With admirable restraint and a firm appeal to reason, as well as a deep sense of responsibility to the community, Mr Mayne pressed the Institution's position, while urging that it should never forget that it was part of a larger world

That speech alone demonstrates the existence of a very significant element of morale in the questions of salaries for professional Civil servants, and perhaps especially as regards the scientific classes Because of that, the application of the pay-pause announced by the Chancellor of the Exchequer at the end of July has seriously strained staff relations in the Service, and Mr Richard Nunn, who has succeeded Mr Mayne, points out that because of the continued delay in dealing with claims submitted much earlier, professional Civil servants are beginning to believe that the Treasury is using the pay-pause not only to delay salary increases but also to stop negotiations altogether From what he has written in recent issues of *State Service*, it appears that confidence in the staff consultation organization within the Civil Service is being seriously undermined.

Clearly questions of morale are deeply involved, and although Mr N Walker claims that his book, published a short time ago*, is no more than a factual study of the psychological aspects of employment as a desk worker, morale in the Civil Service can scarcely be considered in relation to one particular class of workers alone Moreover, Mr. Walker points out at the start that his concern with morale arises from an attempt to study the efficiency of office work and to offer suggestions for improving it This is not merely a matter of pay, of working conditions, or of improving the chances of promotion, although these can all be contributory factors, if adjustment is limited to possibilities

Mr Walker's book has little of direct interest to the scientist or technologist, except from the point of view of a study of human relations and of problems with which the manager of any sizable department, whether technical or not, may be confronted He does not altogether ignore the professional classes of the Civil Service, though his questionnaire touched comparatively few of these, and only three of many professions in the Service Among those in the small Ministry which he studied, and whose work to some extent was creative, he found a high degree of satisfaction with their work, but those in a large Ministry were much more critical of the system Perhaps even more significant, professional workers in both Ministries showed little confidence in the administrators Mr Walker himself recognizes that this may be a symptom of the belief that decisions taken by laymen who are administrative colleagues or by their Ministers have too little regard to the technical advice or needs of the professional classes

This is part of the problem of relations between the administrative and the scientific and other professional grades, and lay outside the scope of Mr. Walker s investigation While he makes some interesting comparisons between Civil servants and the office staff of private companies to whom his questionnaire was also circulated, and discredits some common hypotheses while confirming others, it leads to little in the way of conclusions He failed to find any reason for young male clerical officers resigning in such large numbers, or for the increase in the sick-rate with the number of workers in the office building, or to suggest any sort of training which might make office workers better at managing subordinates On morale itself, it seems rather to raise the unanswered question What makes work interesting ?; and although the differences between the office staff in the two Ministries and in the private firms covered by Mr. Walker's investigation do not suggest that any one is preferable to the others, one important difference is again brought out which can scarcely be over-emphasized The office worker in the Civil Service is not altogether uncritical of the Service itself and its efficiency, frequently he is critical of laxity in discipline, where

* *Morale in the Civil Service a Study of the Desk Worker* By Nigel Walker Pp viii+302 (Edinburgh At the University Press, 1961 Distributed by Thomas Nelson and Sons, Ltd) 30s net

this is reflected in inefficiency The Service, however, is highly sensitive to criticism from outside, especially ill-founded or ill-informed criticism, and the bearing of this on morale and efficiency in the Service generally is possibly closer than Mr Walker himself indicates It could well encourage the group loyalty or *esprit de corps,* often so important in efficiency, to lead to a blindness to faults which undermines efficiency in other ways

Apart from the comments on the need for restraint and fairmindedness in public criticism, the two most important points made in Mr Walker's book are the reminders that we need to know much more about the causes which lead to satisfaction in work and also about the relations between the professional and administrative officers There is still the feeling that the work of the former is neither properly valued nor clearly understood, and that Mr Walker directs even passing attention to this serious problem is sufficient reason for the scientist and technologist to take note of his book

While those professional Civil servants who occupy very senior positions are given both status and remuneration more in accordance with their responsibilities, as is shown by the annual lists of special promotions, there must be many others whose conditions of enployment could be much improved Perhaps Mr Walker's book might be put in the hands of the administrative classes of the Civil Service, where its readers would learn something of the outlook of their opposite numbers on the scientific side, to the ultimate benefit of the whole of the Service

THE HALLEY BAY EXPEDITION OF THE ROYAL SOCIETY

The Royal Society International Geophysical Year Antarctic Expedition

Halley Bay, Coats Land, Falkland Islands Dependencies, 1955–1959 General Editor Sir David Brunt Vol 1 Introductions, Aurora and Airglow, Geomagnetism Pp xv + 405 + 12 plates 140*s* , 23 dollars

THE origin, organization and execution of the project known as the International Geophysical Year, and in particular the participation of the United Kingdom, are well known[1] Full details concerning the Royal Society's International Geophysical Year Expedition to Halley Bay, Antarctica, during 1955–59, are given in the introduction by Sir David Brunt, former Physical Secretary of the Royal Society, to the volume under review The British National Committee for the International Geophysical Year was set up by the Council of the Royal Society on December 11, 1952, and formal approval was given by this Committee on March 1, 1955, to the general proposals for setting up a station in the Antarctic The necessary support from H M Treasury and the Colonial Office was forthcoming, and the advance party, with Lieutenant-Commander D G Dalgleish as leader, left Southampton on November 22, 1955, a landing was made on January 11, 1956, at a suitable point *c* 75° 31′ S , 26° 37′ W , which was later named Halley Bay

The main party left England during November 1956 under the leadership of Colonel R A Smart, in the M S *Magga Dan,* along with the main party of the Commonwealth Trans-Antarctic Expedition The Antarctic Sub-committee set up by the British National Committee had planned in detail a programme of observations on meteorology, geomagnetism, aurora and airglow, ionospheric physics, glaciology and seismology, including an extensive programme of radio-astronomical observations A further visit was made to the base during the Antarctic summer 1957–58, when Colonel Smart, following his wish expressed at the time of his appointment in 1956, returned home and was succeeded as leader by J MacDowall

An outstanding criticism of the International Polar Year expeditions of 1882–83 and 1932–33 had been the long delay in publishing some of the observations This consideration was doubtless in the minds of the Antarctic Analysis Subcommittee set up to supervise the publication of the scientific results of the present expedition, and it is commendable that Volume 1, dealing with aurora and airglow, and geomagnetism, should have appeared so soon The observations themselves are preceded by a vivid narrative account of the establishment and maintenance of the base, this account is illustrated by a series of excellent photographs, which in particular show the measures taken to combat the accumulation of snow, amounting even on a flat drift-free surface to 3 ft /year

The visual and photographic observations of the aurora are discussed by S Evans and G M Thomas, an all-sky camera was used whenever conditions were favourable Some very beautiful photographs, together with a table of movements of auroral features, are given in this volume An airglow photometer, sensitive to a band of infra-red wave-lengths of 7,400–11,300 Å , was operated as conditions permitted, the report by G M Thomas, who was observer of auroral and airglow phenomena to the main party, lists the mean nightly airglow intensities, which constitute a valuable record inasmuch as it was possible in general to recognize those occasions on which the measured zenithal intensity was enhanced by light from auroræ

The geomagnetic observations occupy by far the greater part of the volume The laboratory was a non-magnetic hut with its centre line along the observed magnetic meridian, it was at least 110 m from any large ferrous objects, and tractors were not allowed within 100 m of the hut The observatory was built on a floating ice-shelf which moved slowly and steadily seaward The foundations for the recording and absolute instruments were wooden piles driven 6 m into the snow Continuous registration of the horizontal force (H), the declination (D) and the vertical force (Z) were made by two independent recorders of the La Cour type The absolute instruments were three quartz-fibre horizontal-force magnetometers, two balance magnetometers for vertical force, and a portable unifilar magnetometer-inductor The discussion by J MacDowall and A Blackie of the geomagnetic work is too detailed to summarize here It contains the diurnal variation (including a harmonic analysis of H, D and Z), a detailed examination of K-indices, Q-indices and the absolute daily range Diagrams are given of the vector field changes, and tables are included listing (*inter alia*) the hourly mean values of the three components of geomagnetic force for every day, the 3-hourly K-indices, and the Q-indices for consecutive intervals of 15 min The volume concludes with reproductions of the complete series of magnetograms from April 30, 1957, to December 31, 1958

In all, this work presents the results of a prodigious amount of labour It is well set-out, and the typography is excellent The Royal Society and the authors are to be congratulated on their fine achievement
R STONELEY

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[1] *Nature*, 188 529 (1960)

THE TEACHING OF QUANTUM MECHANICS

Introduction to Quantum Mechanics

By Prof Robert H Dicke and Dr James P Wittke Pp xi+369 (Reading, Mass , and London Addison-Wesley Publishing Company, Inc , 1960 66*s*)

Problems in Quantum Mechanics

By I I Gol dman, V D Krivchenkov, V I Kogan and V M Galitskii Pp iii+394 (London Infosearch Limited, 1960 Distributed by Cleaver-Hume Press, Ltd) 42*s*

Problems in Quantum Mechanics

By I I Gol'dman and V D Krivchenkov Edited by Prof B T Geilikman Translated from the Russian by E Marquit and E Lepa Authorized revised edition Pp viii+275 (London and New York Pergamon Press 1961) 50*s*

AFTER many years of famine in the supply of quantum mechanics text-books, there is now an abundance and more titles keep appearing It is possible therefore to be fairly critical in deciding which text-books to recommend to students Quantum theory is gradually taking a larger part in undergraduate training in general and theoretical physics, and the type of book required at the undergraduate level is not for example, Dirac's, which is too difficult, or that of Landau and Lifshitz which is too big and thorough

Prof Dicke and Dr Wittke have written their text for the first postgraduate physics year at Princeton, and it covers much the same ground as that at many British universities (it should be all), in the third year of honours physics All the essentials of quantum theory are covered, and the late chapters of the book cover the effects of strong fields on atoms, scattering theory, the effect of statistics on the two and many-body systems, and on quantum statistical mechanics There is a surprising and serious omission from the contents since the uncertainty relation between energy and time is never mentioned On the whole it is well written, with the difficulties that a beginner experiences well aired Useful sets of examples are appended to each chapter But it can be criticized in that several mathematical statements are not precise and the same lack of precision shows up in several of the discussions of points of principle For example Why should Hermites polynomials have to terminate ? Why should they be polynomials at all ? Why should the reader accept, without proof, that $(\exp ikr)/r$ is the Green function for scattering and not $(\exp - ikr)/r$? The discussion of orbital and spin angular momentum make these appear only trivially different, and in discussion of what Dirac calls the Schrodinger and Heisenberg 'pictures', the word representation is confusingly used to mean picture as well as its mathematical meaning with respect to a basis There are several other minor inaccuracies and obscurities, and a general tautening up of statements could be made throughout the book without making it longer or more difficult In spite of these drawbacks it is an attractive book and beautifully produced

The two collections of examples present a curious situation for they are effectively the same book, being translations of a set of problems with full solutions, used by Drs Gol'dman and Krivchenkov in teaching a course of quantum mechanics based on Landau and Lifshitz's text The volume from Infosearch has added further problems from a comparable set by Kogan and Galitskii, while that from Pergamon is based solely on Gol'dman and Krivchenkov, though from an extended and revised version The latter states it is the 'authorized version', which may account for the price difference As it stands the Infosearch edition is considerably larger and clearly the 'best buy' These problems cover much more ground than say Dicke and Wittke, and are suitable for theoretical specialists With the book of Landau and Lifshitz they throw up a powerful challenge This is how quantum mechanics is taught to theorists in Moscow Are British theorists taught as thoroughly ? Moreover, in those universities where they are, do the students also reach the same standard in hydrodynamics, physical kinetics, elasticity, etc , as the introductions to the Landau and Lifshitz texts assure us the Russian theorists do Regretfully one must give the answer that scarcely anywhere in Britain is this standard aimed at, let alone achieved.

S F EDWARDS

THEORY AND TABLES OF THE BLUE SKY

Tables related to Radiation emerging from a Planetary Atmosphere with Rayleigh Scattering

By Kinsell L Coulson, Jitendra V Dave and Zdeněk Sekera Pp x+548 (Berkeley and Los Angeles University of California Press, London Cambridge University Press, 1960) 116*s* net

ONE of the best-known of the many fundamental contributions to science made by Lord Rayleigh during his long career was his discovery of the law of molecular scattering (named after him) and its application to the explanation of the colour and polarization of the sunlit sky The problem of the diffuse reflexion and transmission of sunlight in an atmosphere scattering it in accordance with Rayleigh's law has since become one of the classical problems of mathematical physics, of particular interest to meteorology Many efforts were made toward its solution—culminating in the well-known work by Chandrasekhar, who succeeded in constructing its exact solution in terms of the so-called X- and Y-functions satisfying four pairs of simultaneous integral equations—but, alas, these equations turned out to be non-linear and of the type which resolutely defies any possibility of an analytical solution

Yet, in order to learn what these functions are capable of revealing, it is necessary to evaluate them numerically and insert in various relevant expressions The construction (by successive approximations) of numerical solutions of the respective equations has indeed been undertaken recently by the authors of the volume under review, on an impressive scale, using the facilities of the Watson Scientific Computing Laboratory in New York and of the Institute for Numerical Analysis of the U.S. National Bureau of Standards at Los Angeles. From the functions X and Y so obtained, the Stokes parameters of the radiation emerging in different directions at the top and at the bottom of the atmosphere have been computed for different elevations of the Sun and different reflectivities of the ground. The results of these computations, performed by the authors at the Data Processing Centre of the University of California at Los Angeles, are then presented in this book.

The volume itself consists largely of an impressive set of tables, containing accurate data which should meet any conceivable problem encountered in the field of atmospheric optics for a long time to come, and represents a permanent contribution to the literature on physical meteorology. We can indeed agree with Chandrasekhar's words in the foreword to this book that “ with the publication of this volume, comprising the work of Dr. Sekera and his collaborators, the problem that was formulated by Rayleigh in 1871 has now at last found its complete solution”.

Zdeněk Kopal

STRUCTURE, LIFE-HISTORY AND DISTRIBUTION OF *GNETUM*

Gnetum

By P. Maheshwari and Vimla Vasil. (Botanical Monograph No. 1.) Pp. xii+142. (New Delhi: Council of Scientific and Industrial Research, 1961.) Rs 20.00, 40s.

GNETUM is a genus consisting of rather more than thirty species of which most are woody climbers, but some are shrubs and small trees. It is confined to certain humid regions in South-east Asia, West Africa and the north-eastern parts of South America. It was at one time classified with *Ephedra* and *Welwitschia* in the order Gnetales, but it is now generally accepted that these last two genera are not very closely related to *Gnetum*, so that the order Gnetales is now thought of as consisting of this one genus. The chief botanical interest of the genus is that all its members exhibit, in their floral morphology, embryology and in the structure of their vegetative organs, a mixture of angiosperm and gymnosperm characters. In consequence the genus has been much discussed whenever the origin of the flowering plants is under consideration. To those botanists who are familiar with *Gnetum* mainly on account of its phylogenetic interest it may come as a surprise to learn that *Gnetum gnemon* is cultivated for the sake of its edible seeds, that fibre from the bark is used to make fishing nets and ropes, and the wood of old trees is used in cooperage.

The book under review is the first of a series of botanical monographs that is to be produced by the Indian Council of Scientific and Industrial Research. The senior author, who is professor of botany at Delhi, is already well known for the extensive programme of research carried on in collaboration with his pupils and associates, on the morphological aspects of the embryology of the flowering plants. His *Embryology of Angiosperms* is also much used as a work of reference and by students. In the monograph now under review the authors integrate and summarize all that has been done on the geographical distribution, morphology, anatomy and embryology of *Gnetum*. The relationships of the genus to the angiosperms and gymnosperms and also with *Ephedra* and *Welwitschia* are also fully discussed. The book is well illustrated by distribution maps for the species, as well as by line drawings and photographs, all of which are of excellent quality and well reproduced, many of these illustrations being original. There are a comprehensive bibliography and a single index to authors, plant names and subjects.

The book, which is well printed and reasonably well bound, can be recommended to research workers, teachers and students who will no doubt welcome this modern treatment of a topic that has been of interest for many years. Readers will find, however, that the authors are cautious in drawing phylogenetic conclusions, for the text ends with these words: “Thus *Gnetum* remains largely a phylogenetic puzzle. It is gymnospermous, but possesses some strong angiospermic features”.

C. R. Metcalfe

WORLD FOOD PROBLEMS

World Review of Nutrition and Dietetics

Vol. 2. Edited by Geoffrey H. Bourne, assisted by Eileen M. H. Wilson. Pp. viii+245+14 plates. (London: Pitman Medical Publishing Co., Ltd., 1960.) 60s net.

THE second volume in this series is slightly shorter than the first, but, as the editor remarks in the preface, it “spreads its international net even wider”. There are two contributions from the United States and one each from France, Sweden, South Africa, New Zealand, Switzerland and the United Kingdom. The subject-matter again covers a wide range, as the titles of the following four contributions out of a total of nine indicate: “Proteins and Hæmatopoiesis”, “The Effect of Malnutrition on the Eye, with Special Reference to Work with Experimental Animals”; “The Microbiology of Digestion”, “The Role of Carotene and Vitamin A in Animal Feeding”. The reviews are written by well-informed and experienced workers, who between them list some 2,000 references.

In the preface to Volume 1 the editor expressed the hope that each review would “provide a critical evaluation of the field” and added that “the author's interpretation, his views and his theorising will be welcomed as an integral part of his article”. These aims have not been fully achieved. Some of the authors draw some conclusions from their extensive reviews, other confine themselves to a survey of the literature which ends abruptly without summary or comment. It would surely be useful if authors of multi-reference scientific reviews would attempt more often to summarize the salient points and briefly discuss the key question: Where do we go from here? This might help towards discarding literature which has contributed at some stage to the advance of knowledge, but no longer needs to be consulted.

With this second volume, the *World Review of Nutrition and Dietetics* is fully launched and on the way to establishing itself as a publication of importance to research workers in nutrition and associated fields throughout the world. It is well printed and produced. The editor is to be congratulated on the high standard attained.

W. R. Aykroyd

Biological Education in American Secondary Schools, 1890-1960

By Paul De Hart Hurd (Biological Sciences Curriculum Study Bulletin, No 1) Pp ix+263 (Washington, D C American Institute of Biological Sciences, 1961) 4.75 dollars

TO help in the preparation of new material for American schools, Dr Paul De Hart Hurd of Stanford University was invited to make a historical critique of the development of secondary school biological education in the United States His report describes the investigations that he has made of the teaching of biology in the American high school covering the period 1890–1961, and is limited to consideration of curriculum development and investigations of classroom and laboratory learning. Training of teachers, professional training of biologists and special programmes for high-school students are not considered. The work would be of interest to students of the history of biological education Teachers of biology in Britain could find many parallels to the questions which Dr Hart Hurd raises about contemporary teaching in the United States , they would also wish that some of the answers had been forthcoming

Histologie und Mikroskopische Anatomie des Menschen

Von Prof. W. Bargmann Dritte, verbesserte Auflage Pp xvi+820. (Stuttgart. Georg Thieme Verlag, 1959) 69 60 D M

THIS is the third edition of Prof Bargmann's monumental work, which originally appeared in two volumes In keeping with—as the author says—"the renaissance in structural biological research" which we witness at the present time, the original text has been revised in many respects. This is particularly so in the chapters dealing with cells and tissues generally Accounts of ultra-structure as revealed by electron microscopy have been added *per se* or skilfully woven into the text The neurone theory has been restored to its appropriate place, full descriptions are given of the fine structure of synapses, sensory epithelia, Ranvier's nodes, motor end-plates and of the myelin sheath. The reality of bile capillaries and Disse's space is attested and the 'sex chromatin' fully discussed, to mention only a few items, but 'amitosis' as a mode of cell proliferation still looms unduly large With the fast advance being made in the field of electron microscopy, keeping up to date is almost impossible, but it is regrettable that some of the exciting and illuminating pictures which have been obtained with the electron microscope of skeletal muscle are not included

Where the book scores over the many others available in the field of histology is in the treatment of the microscopic anatomy of the connective tissue apparatus, its intimate architecture and the relationship of particular designs to the function of individual organs. First-class descriptions are given of complex mechanical, elasto-muscular arrangements and, in some instances, also of intricate vascular patterns

The compass of the book being exceptionally wide, it includes many embryological data The value of these could be enhanced by some more illustrations of what are, after all, very complex situations and events, and, sometimes, a single picture would aid and clarify a very condensed description Similarly, the chapter on the central nervous system would gain by additional illustrations Valuable information is given on post-natal developmental processes and on senile changes.

The masterly style bears the stamp of the dynamic personality of the author, makes fascinating reading and brings microscopic anatomy very much to life There can be no doubt that the interest of the student must be aroused and highly stimulated by such a vivid account, at the same time, the student would do well to keep an open mind towards many problematic issues which have remained somewhat hidden in this presentation Key references to the literature are given at frequent intervals, and photographs of famous microscopists and brief footnotes here and there direct attention to historical aspects of the subject. F JACOBY

Coir

Its Extraction, Properties and Uses Pp v+54 (New Delhi Council of Scientific and Industrial Research, 1960) Rs 6 00, 10*s*

THIS small book of some fifty pages gives a good account of the Indian coir or coconut fibre industry, India being one of the main producers of this important commercial fibre. The book is the result of a decision made by the All India Board of Technical Studies in Textile Technology in 1950 to collect and collate available information on the coir industry This has now been presented in condensed form under such headings as extraction of coir, structure and properties of the fibre, spinning, weaving, dyeing and printing, ropes and cordage, other uses, marketing and trade

The value of coir as a fibre lies in its resilience or elasticity and its durability and damp resistance, properties which render it so well suited for matting or floor-covering and certain types of cordage One of the features of its production is the traditional retting process with the drawbacks (evil smell) that go with it It also involves a considerable loss of potential plant food (potassium and phosphorus). Reference is made to various alternative methods of extracting the fibre from the husk, mechanical and chemical, that have been put forward from time to time. For various reasons, however, the old method prevails in what is still essentially a cottage industry in India A number of interesting photographs and a short bibliography are included

Indian Tobacco

A Monograph Pp xxii+413+154 photographs (Madras Indian Central Tobacco Committee, Ministry of Food and Agriculture, Government of India, 1960) Rs 36.00, 72*s* , 10 dollars

THIS book, which contains a great deal of useful information on the cultivation, production and use of tobacco in India, owes its origin to the Indian Central Tobacco Committee and is the work of several authors, including past and present directors of tobacco research As the Indian Minister for Agriculture points out in his foreword, India is faced with a land shortage or crisis, with simultaneous pressure from food and non-food crops Tobacco falls into the latter category and cannot claim priority. Steps to increase yield from the existing acreage are therefore all the more imperative This comprehensive work should play a useful part in this connexion

Tobacco cultivation in India differs from that in most other important tobacco growing countries because in India the crop has to be grown under monsoon conditions The book may be of special value to those concerned with tobacco cultivation in other parts of the world where monsoon or similar climatic conditions prevail It is divided into three parts In the first part soils, climate, botany, cultivation, curing, pests and diseases, etc, are dealt with Part 2 deals in detail with the five basic types of tobacco produced in India, namely, flue-cured tobacco, bidi tobacco, cigar and cheroot tobacco, hookah tobacco, chewing and snuff tobacco The third part is concerned with marketing

The book is not without certain errors, one of the most lamentable being the statement in the second paragraph of the introduction that Christopher Columbus discovered tobacco in his voyage from Persia to Spain on the island of Tobago!

Transactions of the Society of Rheology

Vol 5, 1961 Edited by E H Lee Pp ii+382 (New York Interscience Publishers Inc, London Interscience Publishers, Ltd, 1961) 10 50 dollars

THE 1961 volume comprises the majority of the papers presented at the thirty-first annual meeting of the (American) Society of Rheology in Pittsburgh (Pa) and includes symposia on the mechanics of continua and on the rheology of suspensions as well as nearly twenty papers concerned with other topics I remember criticisms at the first meeting (1929) that rheology was too mathematical for the practical man, but, more than thirty years later, it seems that workers in many industries are quite able to cope with triple integrals and tensor notation

Like all sciences, rheology has its fashions At the moment, normal stresses ('Weissenberg effects') and dynamic mechanical properties lead in the theoretical field, and die entry and extrusion problems on the practical side High polymers are still in the lead among the materials of interest, but a paper on the rheology of blood reminds us of increasing attention to biological applications

The Bingham Medal for 1960 has been awarded to Dr B H. Zimm for outstanding work linking optical with rheological methods in the study of macromolecules and the determination of their molecular weights

Many of the papers are very short, but the authors' addresses are given and readers will doubtless write for reprints of longer articles

The *Transactions* are, as always, well printed and produced but the price for a volume of this size is rather high

G W SCOTT BLAIR

Technical Data on Fuel

Edited by H M Spiers Sixth Edition, completely revised Pp xi+360 (London British National Committee, World Power Conference, 1961) 60s net

THIS sixth edition of a well-known and much-used reference book constitutes a marked advance on its predecessors, for it contains more information in the form of both text and tables and the size of the page has been increased This may be a disadvantage to the worker who wishes to have a readily portable book of useful handiness, but the greater wealth of information compensates amply for the change in character The tables and diagrams are brought into better relation with the text and most of the tables are placed on single pages In general it will be agreed that the changes will be approved by fuel technologists. The contents of the book have been extensively revised, tables have been recalculated where necessary, or replaced on the basis of more recent or more trustworthy data, and the conversion factors and the symbols for units have been brought into conformity with British Standard specifications These features that have been confirmed in use constitute a worthy improvement The sections that have been extensively revised or completely re-written include many subjects in which technical advances are bound to move with the times, and the new information is therefore all the more welcome

Of the entirely new topics included in this new edition mention should be made of information relating to atomic and nuclear fuels, the viscosity blending of oils and flow in fluidized systems A table of additive viscosity functions derived from the usual type of viscosity blending chart enables the viscosity of a blend of several fuel oil components to be estimated with considerable accuracy Since the use of fluidized beds is coming more into use in fuel technology the information provided is typical of the general character of the advancement shown in this edition The general legibility of the text, charts and tables is a noteworthy feature of the book

R J SARJANT

Symposium on Pilot Plants in Metallurgical Research and Development

Held at the National Metallurgical Laboratory, Jamshedpur, India, February 15th to 18th, 1960 Edited by R M Krishnan (Organized by the National Metallurgical Laboratory, Jamshedpur) Pp 304 (Calcutta The Technical Journals of India Private, Ltd., 1960) n p

THIS symposium drew a large gathering of scientists and metallurgists from ten overseas countries as well as equally important representatives from metallurgical science and industry from India Formal papers presented totalled 29, of which 16 were by foreigners and 13 by Indians There were also three lectures by visitors All papers and lectures are reprinted in full together with the discussions

The National Metallurgical Laboratory at Jamshedpur is concerned with ferrous and non-ferrous problems, and while part of its activity is devoted to laboratory research in order to exploit to the full the mineral resources of India, a major emphasis has been placed on the design, erection and operation of pilot plants The scope of these pilot plants ranges from the basic chemistry of metals to financial evaluations of ambitious metallurgical processes Many of these experiences were reported

Some of the papers directed attention to the uses and limitations of pilot plant studies and others described in detail many of the successful pilot plants operating in the world These included coking studies, cupola and blast-furnace operation, ore sintering, oxygen steel-making, refractories, continuous casting, mineral dressing, etc

It is clear from the papers and discussion that most people present fully appreciated the gulf which exists between initial laboratory findings and economically attractive commercial operations They also realized the vital part that well-planned pilot plant investigations can play in bridging this gap This book forms a valuable collection of experiences on this topic and covers a wide range of industries

E W VOICE

ANALYSIS OF THE RELATIONSHIP BETWEEN DEPOSITION OF LONG-LIVED FISSION PRODUCTS AND LEVEL OF ACTIVITY IN MILK

By Dr. W ANDERSON, L. K BURTON and J. O. CROOKALL

Physics Department, Institute of Cancer Research Royal Cancer Hospital, London, S W 3

IN recent years attention has been focused on the mechanisms of transit of fall-out radionuclides in food chains, particularly the steps leading to the contamination of milk One of the main issues to be resolved is the extent to which the levels of activity in foods are determined, on one hand, by the deposit of activity accumulated over the years in the top of the soil, and, on the other hand, by the proximate deposition The evidence available on strontium-90 and cæsium-137 concentrations in milk produced in Great Britain indicates that, up to the present time, the activity-levels have been determined largely by the rate of fall-out at or near the time of production of milk[1-4] However, the relative importance of the contributions of activity from the cumulative and current deposits may be expected to change with the changing fall-out conditions, and it would therefore be useful to obtain a more quantitative expression of these relationships than is available at present Since marked changes in the rate of fall-out occurred during the period 1958–60, it is now possible to tackle this problem by correlating the levels of activity for milk, for various open-pasture feeding months, with the corresponding cumulative and current amounts of deposition

In the present article we report the levels of strontium-90 and cæsium-137 occurring in milk produced in west Cardiganshire, Wales (Felinfach creamery) throughout 1960, and examine these results in relation to the situation for the same series of samples in 1958–59 It should be noted that the activity values reported here refer to levels in milk produced in a single area and they should not be taken to represent the average levels for Britain as a whole On the other hand, except in the case of special areas where the rate of growth of herbage is abnormally low[4], the variation in levels of activity for milk between different regions of the country is largely attributable to difference in rainfall[1-3,5] This indicates that the relationships between levels of activity for milk and deposition-levels in the different regions are broadly similar in nature to those discussed below for the Felinfach region

The milk sampling and measuring techniques used in this work have already been described[3,6,7] Samples of dried skimmed milk, each representative of a total day's production, were obtained at weekly intervals, and measurements were made on bulked samples, compounded from four consecutive weekly samples Thus, each measured value represents approximately the mean level of activity in the milk drawn from the Felinfach area, averaged over a 4-weekly period It may be noted that the full open-pasture feeding period in the area extends from May to October

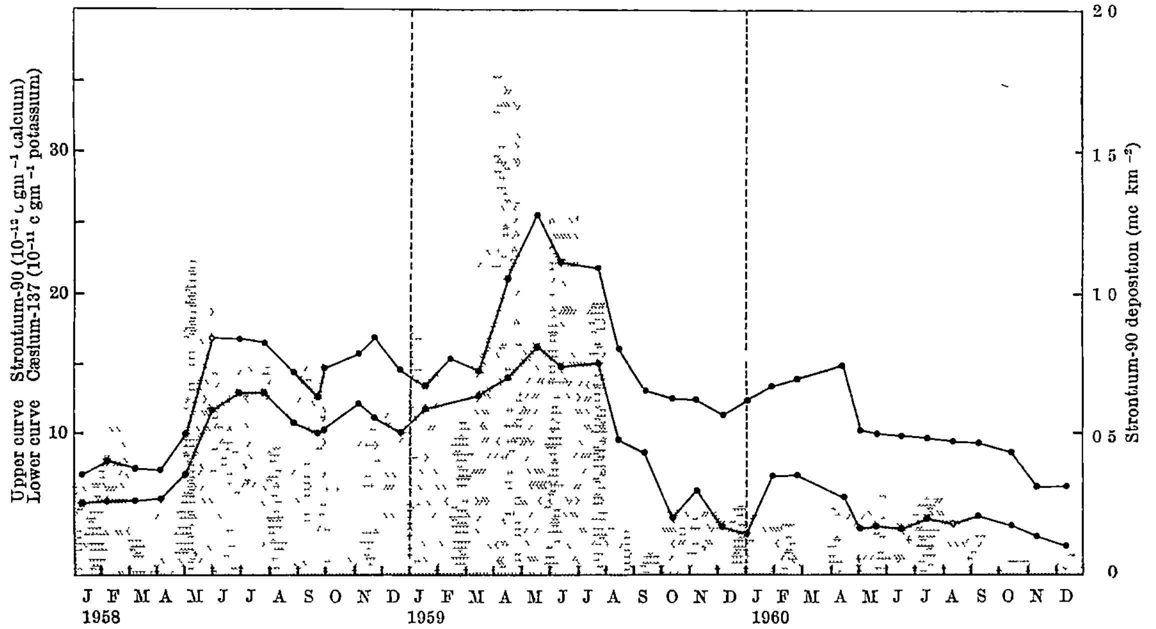

Fig 1 Levels of strontium-90 (upper curve) and cæsium-137 (lower curve) in milk from Felinfach, west Cardiganshire Wales (1958–60) The histogram shows the monthly deposition of strontium-90 in the Felinfach area (ordinate on right)

Table 1. MEAN LEVELS OF STRONTIUM-90 AND CÆSIUM-137 IN FELINFACH MILK DURING SUCCESSIVE MAY–OCTOBER PERIODS (1958–60)

Year	Strontium-90 ($\mu\mu$c /gm calcium)	Cæsium-137 ($\mu\mu$c /gm potassium)	Cæsium-137/ strontium-90 ($\mu\mu$c /L/$\mu\mu$c /l)
1958	15·2	114	10·0
1959	18·5	113	8·1
1960	9·5	36	5·0

inclusively. The results for strontium-90 and cæsium-137 levels in the milk during 1958–60 are presented in Fig. 1, together with a histogram showing the monthly strontium-90 deposition in the Felinfach area. The trend of the monthly cæsium-137 deposition was broadly similar to that shown for strontium-90, with an average cæsium-137/strontium-90 ratio of 1·7.

Looking at the 1960 results (Fig. 1) it will be seen that the levels of both strontium-90 and cæsium-137 in the milk rose during the early months of that year. This reflects the change-over to winter feeding conditions which involved the use of feeding stuffs harvested during a period of relatively high fall-out rate in 1959. When we come to the 1960 open-pasture feeding period (May–October) there is a sharp change to lower steady values. In Table 1 the average level of activity in the milk during the May–October period of 1960 is compared with those for the corresponding periods of 1958 and 1959. It will be seen from Table 1 that the level during the 1960 open-pasture period was markedly lower than those for the two preceding years. A glance at Fig. 1 shows that the trend of the levels of activity for milk was broadly similar in direction to that of the rate of deposition. In contrast, the cumulative total of deposition rose by a factor of 1·75 between mid-1958 and mid-1960 (Table 2). Thus, the view[3] that the sharp increases of activity in the milk during 1958 and 1959 were due chiefly to corresponding rises in fall-out rate, is strongly confirmed.

Table 2. DATA ON STRONTIUM-90 AND CÆSIUM-137 LEVELS IN MILK SAMPLES AND ON RATE OF DEPOSITION AND CUMULATIVE DEPOSITION IN THE FELINFACH AREA DURING 1958–60

	Strontium-90			Cæsium-137		
Month*	Activity in milk (10^{-12} c gm $^{-1}$ calcium)	Rate of deposition (mc km $^{-2}$ mth $^{-1}$)	Cumulative deposition (mc km $^{-2}$)	Activity in milk (10^{-12} c gm $^{-1}$ potassium)	Rate of deposition (mc km $^{-2}$ mth $^{-1}$)	Cumulative deposition (mc. km.$^{-2}$)
1958						
Jan.		0·328			0·623	
Feb.		0·506			0·961	
Mar.		0·148			0·251	
April		0·212			0·360	
May	13·5	1·070	13·9	95	1·819	25·8
June	17·2	0·707	14·6	123	1·273	27·0
July	16·3	0·727	15·3	135	1·236	28·2
Aug.	15·1	0·443	15·7	113	0·797	28·9
Sept. +	13·8	0·719	16·4	103	1·079	29·9
Oct. +	15·2	0·294	16·6	112	0·529	30·4
Nov.		0·492			0·738	
Dec.		0·663			0·995	
1959						
Jan.		0·900			1·620	
Feb.		0·451			0·586	
Mar.		1·153			1·960	
April		1·690			2·873	
May	25·5	0·834	22·5	161	1·168	39·9
June	22·2	1·190	23·7	147	1·547	41·3
July	21·8	0·852	24·5	149	1·363	42·6
Aug.	16·0	0·156	24·6	96	0·312	42·8
Sept.	13·0	0·073	24·6	87	0·073	42·8
Oct.	12·5	0·227	24·8	40	0·431	43·2
Nov.		0·257			0·488	
Dec.		0·246			0·517	
1960						
Jan.		0·219			0·372	
Feb.		0·198			0·376	
Mar.		0·105			0·147	
April		0·243			0·413	
May	10·0	0·284	26·0	35	0·483	45·3
June	9·8	0·211	26·1	32	0·317	45·6
July	9·7	0·256	26·4	39	0·384	45·9
Aug.	9·4	0·211	26·5	35	0·338	46·1
Sept.	9·2	0·132	26·6	40	0·211	46·2
Oct.	8·7	0·059	26·6	35	0·094	46·2
Nov.		0·101			0·162	
Dec.		0·071			0·114	

* The time unit used in the computation of the rate of deposition was the calendar month. The periods covered by five of the bulked milk samples in their original grouping (refs. 2, 3) (Fig. 1) did not correspond closely to a calendar month. In these cases we have re-grouped the single samples and have made fresh determinations. In two cases, indicated by +, the bulked samples each comprised only two single samples.

A previous interpretation of the strontium-90 level in Felinfach milk samples during 1958 indicated that less than 35 per cent was derived from the reservoir of activity accumulated in the soil[7]. In 1960, with a larger soil reservoir and a much-reduced rate of fall-out, it might be expected that the levels in milk would be determined to a greater extent than before by the soil reservoir activity. This appears to be borne out by the fact that in 1960 the strontium-90 level in milk did not drop in the same degree as did the rate of fall-out (Fig. 1). In comparison, the trend of cæsium-137 levels in milk followed the fall-out trend more closely, indicating that in this case the contribution from the soil reservoir was less important. The demonstration (Table 1) that the cæsium-137/strontium-90 ratio of activities in the milk decreased progressively during 1958–60 is consistent with this interpretation. In the analysis which follows these various relationships are assessed more closely.

Attempts have been made previously[8–10] to establish quantitative relationships between the levels of activity in milk, on one hand, and the rate of deposition and the cumulative deposition, on the other, using equations of the type

$$C = a\,D_p + b\,D_t \qquad (1)$$

where C represents an activity-level in milk, D_p an amount of activity deposited over unit area of ground during a short selected time period (rate of fall-out), D_t the accumulated total of activity deposited over unit area until any given time (cumulative fall-out), and a and b are proportionality factors. These attempts have been hampered by a lack of adequate data and in addition there has been an unfortunate tendency to use yearly average values for the C and D_p parameters. Since yearly average values for C incorporate winter values which reflect in large measure levels of contamination in stored fodder, the meaning of such correlations is often difficult to assess. In the present analysis, we restrict ourselves to consideration of levels of activity in samples which were produced during May–October periods, and we use monthly average values for strontium-90 (10^{-12} c gm $^{-1}$ calcium) and cæsium-137 (10^{-12} c gm $^{-1}$ potassium) activities in milk. Our values for activities in milk are listed in Table 2, together with estimated values for the monthly rates of deposition (mc km $^{-2}$ month^{-1}) and the cumulative totals (mc km $^{-2}$) at each month. The deposition figures have been computed from published information on the strontium-90 and cæsium-137 concentrations in rain-water collected at Milford Haven (50 miles south-west of the Felinfach area[11–13], and recently at Sutton in Surrey[14,15], together with the average of rainfall data from five stations located at different altitudes in the Felinfach area. The cumulative totals listed in Table 2 have been corrected to allow for the radioactive decay occurring in each monthly increment of fall-out between the time of deposition and the time at which the total is recorded. We

Table 3. COMPARISON OF COEFFICIENTS OF CORRELATION FOR VARIOUS REGRESSIONS BETWEEN RATE OF FALL-OUT (COMPUTED IN DIFFERENT WAYS) AND MILK ACTIVITY-LEVEL

Periods* used to compute rate of fall-out		(Pn)	P_0	P_1	P_2	P_0+P_1	$P_0+P_1+P_2$	$P_0+P_1+P_2+P_3$	$P_0+P_1+P_2+P_3+P_4$
Coefficients of correlation based on regression analyses made according to equation (2)	(r_{12})	Strontium-90	0.73	0.91	0.75	0.94	0.95	0.94	0.91
		Cæsium-137	0.74	0.83	0.75	0.91	0.95	0.94	0.90

* P_0 corresponds to the month during which the milk was produced, P_1 to the month immediately preceding milk production, and so on for the other Pn. In the analyses which used combinations of fall-out from different months, the numbers taken to represent the rate of fall-out were the arithmetical averages of the sums of the deposits (mc. km.$^{-2}$) for the months involved.

now consider the information contained in Table 2, according to the methods of correlation analysis[16,17].

In considering the correlation of activity-levels in milk with the average of deposition figures over comparatively short periods of time, it is important to take into account factors which may introduce a lag correlation effect. Earlier reports, showing that strontium-89/strontium-90 ratios for both herbage[1] and milk[7] were smaller than those for rain-water collected during the same month, pointed to some degree of integration of recently deposited activity in the herbage, but in the absence of full knowledge of the role of the cumulative deposit it was not possible to express the extent of this effect in a simple fashion. In the present analysis we approach this problem by examining the degrees of correlation which hold between, on one hand, the activity-levels in milk (X_1) for eighteen open pasture feeding months (Table 2), and on the other, various combinations of the monthly amounts of deposition, which may correspond more or less to an effective rate of fall-out (X_2). These relationships are treated as simple linear regressions according to the equation

$$X_1 = a + b_1X_2 \qquad (2)$$

A preliminary examination of scatter diagrams for the two variables indicated the presence of a similar degree of positive correlation when the X_2 values were represented either by the deposition occurring during the month (P_0) in which the milk was produced, or during the previous month (P_1), or during the second previous month (P_2). When deposition values for months before P_2 were used, there was a significant and progressive decrease in the degree of correlation. This result applied equally to strontium-90 and cæsium-137 data. Various combinations of depositions from two or more months were now used to represent the X_2 values, and correlation coefficients were calculated in several important cases. These coefficients are presented in Table 3. It may be noted first that the degree of correlation obtained using the deposition of the month P_1 was higher than in all other cases for which single monthly depositions were used. Secondly, it may be noted that a significant increase in the degree of correlation occurred when certain combinations of monthly depositions were used, the maximum being obtained with depositions from P_0, P_1 and P_2 grouped together. The correlation result for this latter combination is illustrated in Fig. 2 for strontium-90 and in Fig. 3 for cæsium-137. It appears from the analysis that the rate-dependent component of activity in the milk is largely determined, both in the case of strontium-90 and cæsium-137, by the deposition in the month preceding milk production together with smaller contributions from deposition during the current and second previous months.

We now consider the extent to which the levels of activity for milk may be influenced by the reservoir of activity accumulated in the top of the soil. It appears that both strontium-90 (ref. 1) and cæsium-137 (ref. 18) may be largely held within the top few inches of the soil for comparatively long periods of time. A number of factors[4] may lead to a progressive reduction in the availability of this activity to the herbage, but at the present time the rate at which this process occurs is not well established. For the present purpose we assume that the effective soil reservoir is proportional to the cumulative deposit and we denote the levels at each month (Table 2) by X_3. The effective rate of fall-out (X_2) is taken to be the arithmetic average of the sum of the depositions during the months P_0, P_1 and P_2. Analysis of the data to include the second independent variable requires the use of a multiple regression equation which may take the linear form

$$X_1 = a_{1\cdot 23} + b_{12\cdot 3}X_2 + b_{13\cdot 2}X_3 \qquad (3)$$

Application of this regression equation to the data provides a basis for evaluating the proportionality factors appearing in equation (1).

$$(X_1 = C,\ X_2 = D_p;\ X_3 = D_t,\ b_{12\cdot 3} = a;\ b_{13\cdot 2} = b)$$

Restricting the regression plane to pass through the origin ($a_{1\cdot 23} = 0$), we obtain the following results

$$\text{for strontium-90} \quad C = 15.1\,D_p + 0.28\,D_t \qquad (4)$$

with a standard error of estimate (S) = 1.8×10^{-12} c. gm.$^{-1}$ calcium and coefficient of multiple determination (R^2) = 0.85, and

$$\text{for cæsium-137} \quad C = 84.1\,D_p + 0.36\,D_t \qquad (5)$$

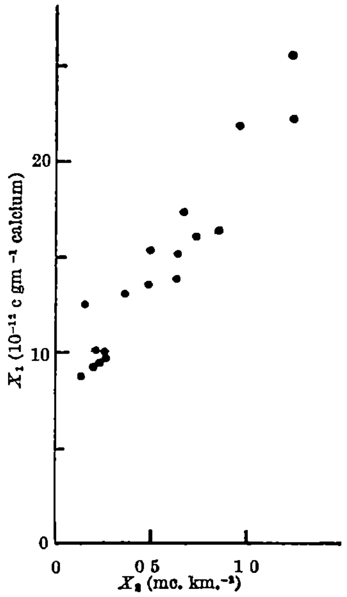

Fig. 2. Correlation chart of rate of strontium-90 fall-out (X_2), taken as average of sum of depositions during months P_0, P_1 and P_2, and monthly strontium-90 level in milk (X_1)

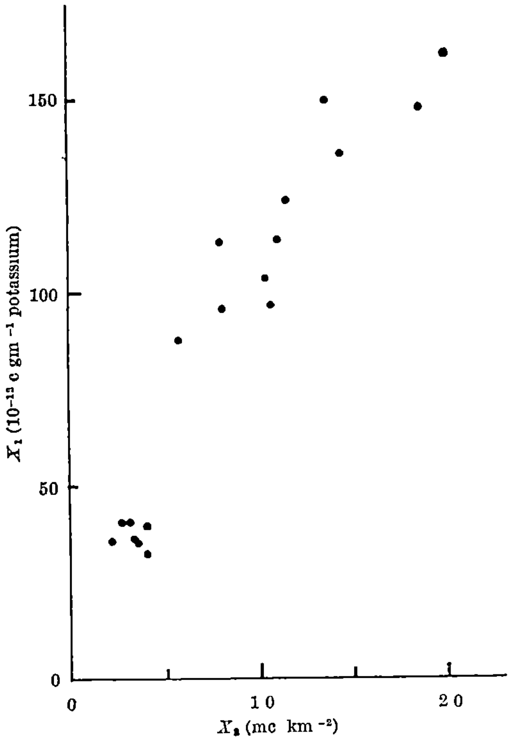

Fig. 3. Correlation chart of rate of cæsium-137 fall-out (X_2), taken as average of sum of depositions during months P_0, P_1 and P_2, and monthly cæsium-137 level in milk (X_1)

with $S = 16·0 \times 10^{-12}$ c gm^{-1} potassium and $R^2 = 0·88$.

The values of the standard errors and the coefficients of determination show that equations (4) and (5) provide a fairly close description of the strontium-90 and cæsium-137 data, respectively, for the open-pasture periods of 1958–60. An application of equation (4) to earlier strontium-90 data also gives estimated levels close to the observed values ((1) October 1957, Felinfach area[6]—observed 9·7 × 10^{-12} c gm^{-1} calcium, estimated 8·1; (2) May–August 1957, pastures at Carmarthen adjoining Felinfach area[19]—average value observed 7·0, estimated 7·8; (3) October 1956, Carmarthen[1]—observed 8·0, estimated 7·5). An evaluation of the rate and cumulative components of the equations suggests that during May–October 1960 the soil reservoir activity accounts for about 70 per cent of strontium-90 in the milk and about 35 per cent of cæsium-137. It may also be noted that the ratio of the proportionality factors in the equation for strontium-90 ($a/b = 54$) is markedly different from that for cæsium-137 ($a/b = 234$). A change in deposition-levels would not therefore be expected to have an equal effect on the level of strontium-90 in milk compared with that of cæsium-137. This provides an explanation of the changes observed in the cæsium 137/strontium-90 ratios for the milk samples produced during 1958–60 (Table 1).

The results of the present analysis apparently provide a useful guide to the degree of contamination which might be expected to arise in milk under a given set of deposition conditions, and at the same time indicate the extent to which this contamination might persist. The results are strictly applicable only to the Felinfach area, but, as noted at the outset, it appears likely that the corresponding relationships for other regions in Britain are broadly similar in magnitude to those shown in equations (4) and (5).

The trend in levels of activity of milk during 1961 will depend partly on the rate of transfer of activity from the stratospheric reservoir to the troposphere, and also on the extent to which direct additions of activity may be made to the troposphere during the new series of weapons tests that was begun in September 1961. Measurements of the concentration of strontium-90 in rain-water collected at our laboratory during the early part of 1961 give values which are about 1·5 times smaller than the corresponding values for 1960. These results, together with the rainfall data available at present, give a preliminary indication that the rate of fall-out during January–August, 1961, will be smaller than that for the corresponding period of the previous year. Consequently, the rate-dependent components of activity in the milk may also be expected to decline. An increase will occur in the cumulative deposition between mid-1960 and mid-1961 but the increment should be less than 5 per cent of the mid-1960 total. Further, this increase may be more than offset by depletion of the effective soil reservoir due to small changes in the distribution of activity within the top layer of the soil. This is suggested by the results reported by Squire[4] of experiments on plants grown in soils treated with strontium-90, where it was found that the availability of the activity to the plants decreased by about 25 per cent over a period of 3 years. The available evidence therefore indicates that a further decrease of activity-levels will be observed in milk produced during the summer and early autumn of 1961.

We thank the Milk Marketing Board for the supply and preparation of the milk samples, and also the Meteorological Office and the Welsh Plant Breeding Station (Aberystwyth) for information on rainfall. Valuable assistance in the chemical analyses was given by Mrs. A. Perry. We are indebted to the director of this Department, Prof. W. V. Mayneord, for his constant support and guidance.

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FISH IN NUTRITION

THE Fisheries Division of the U N Food and Agriculture Organization, in collaboration with the Divisions of Nutrition and of Animal Production and Health, were responsible for organizing a conference which, at the invitation of the Government of the United States, and with generous financial assistance from the Division of General Medical Sciences through the agency of the National Academy of Sciences, was held in the new conference auditorium of the Department of State, Washington, D C, during September 19–27 Various North American commercial fish organizations also combined to make the gathering a success

The conference, which was attended by more than three hundred official participants and various experts and industrialists from more than thirty countries, was opened by Dr D B Finn, the director of the Fisheries Division, as representative of the Director-General, after an address of welcome by the Hon Stewart L Udall, Secretary of the Interior There was also a message of greetings from President Kennedy

The chairman of the conference was Mr Donald L McKernan, director of the Bureau of Commercial Fisheries of the Fish and Wildlife Service of the U S Department of the Interior, and five main topics were discussed, each under a distinguished chairman, with a rapporteur and secretary, covering exhaustively the various biological, chemical, technological, nutritional and economic aspects of fish and fish products For each topic a number of review papers, thirty-four in all, had been invited from well-known authorities, supplemented by thirty-five selected original short research papers These were all circulated beforehand, the latter as abstracts only, and contributors briefly introduced their papers The edited proceedings, including the various recommendations and conclusions, are to be published by Arthur J Heighway Publications, Ltd , 110 Fleet Street, London, by arrangement with the Food and Agriculture Organization, next year

As it is impossible in the course of a short article to cover adequately such a broad and detailed agenda, a personal selection has had to be made of the various important issues that were discussed by the numerous experts present

Productivity of the Seas

There was a clash of opinion among the marine biologists on the maximum harvest that could be expected from the seas Some took the conservative view that knowing the quantity of solar energy that can be fixed by marine plants and making certain assumptions as to the number and efficiency of the conversion steps in the food chain, one cannot hope for much more than double, or at most treble, the present catch of slightly less than thirty million tons of marine fin fishes per annum Others pointed out that landings from the oceans have already nearly doubled in the past ten years, and thought that there was reason for believing that, even using present methods of fishing, five or even ten times the present yield could be achieved and sustained

At present commercial fishing operations are largely determined by market considerations Many edible species, such as sharks, are often not brought home even when caught A two million ton per annum fishery of anchovies used for fish meal has sprung up off the coast of Peru in less than a decade Some American biologists believe that a similar potential exists off the coast of California, and that there are also untapped resources of saury, which would be despised as a food fish in the United States although since the War it has been canned on a large scale in Japan Improved methods of preventing decomposition, such as freezing at sea, is enabling Russian and Japanese ships, for example, to exploit fishing grounds all over the world

Again, greatly improved methods of fishing can be envisaged In spite of much effort on the part of the Food and Agriculture Organization and Governments, there is still a several hundred-fold difference between the highest and lowest productivity per fisherman The suggestion was revived of a planned ploughing of the sea through the convectional effect of nuclear reactors, by means of which nutrient-rich ocean depths could be brought into the life-cycle of the upper layers Finally, also looking well into the future, there is the prospect of fish farming on a scale as yet unimaginable, perhaps involving the intelligent utilization of the organic material at present prodigally wasted in sewage

However, these distant vistas do not dispose of the fact that here and now in certain more easily accessible areas there is a decided falling off in yield due to uncontrolled fishing, with uneconomic results The future yield in such cases is not determined only by Nature, but depends in large measure on the decisions of man himself Man acts not only as a predator and disturber, but also as an intervener in natural systems, so that the fish stock of the future, in the words of Dr G L Kesteven (Cronulla, New South Wales), "is likely to be so much more or less than it is now, as man makes it to be"

It therefore seems that, although there is no justification for pessimism, more work will be needed before the basis exists for even an approximate estimate of the potential yield of the oceans

Unequal Distribution of Fish Supplies

Added to the uneven development of the present world fish catch is the inequality of its distribution In this respect there is a general lack of realization of the implications of the tremendous gap that exists between the 'have' and 'have-not' nations

Although the world dietary deficit in calories is relatively slight, so that probably relatively little actual starvation occurs, there is a general protein under-nourishment over much of the globe which greater consumption of fish could do much to remedy Even where national statistics which are reliable indicate adequate average diets, deficiencies are known to exist in special regional or socio-economic groups Children in the age group 1–4 years have special protein needs but often do not receive a proper share of the right foods Kwashiorkor and marasmus are protein-deficiency conditions of infancy which cause many fatalities, particularly in Africa and South America Women frequently pregnant and lactating equally require high animal protein intake, but all too often men get priority Protein under-nourishment, even where not fatal, is responsible for untold misery, debility, disease, and retarded growth and development.

Although fish accounts for only about 12 per cent of the total animal protein available, it nourishes far more than this percentage of the world population. For more than 1,000 million people it constitutes the chief source of animal protein. Milk and meat products, although bulking much larger, feed far fewer millions. Fish is the protein of the poor, nationally and globally.

Over North America and most of Western Europe the consumption of other animal products is so much greater than that of fish that the latter could practically disappear altogether from the diet without being missed. However, fish is equally nourishing for farm animals, the requirements of which have received much closer examination than those of human beings. The high and increasing output of meat products in the West is facilitated by converting about 5 million tons of edible fish annually into feeding meal for animals. Only a small proportion of this fish meal comes from recovery of industrial waste. The bulk is from whole, edible fish caught entirely for reduction, much of it in Africa and South America. 95 per cent of this trade goes to produce milk, bacon and eggs in the countries least requiring extra nutrition. For example, one third of the broiler production in the United States can be attributed to the sea.

One of the topics which received greatest attention at the conference was the part that the polyunsaturated fatty acids of fish oil could play in the prevention of obesity and coronary and cerebral thrombosis, associated with excessive meat consumption, which are now among the major killers in the 'over-developed' nations (as they were termed). It is a grim irony that a contributory factor to such 'over-nourishment' (itself a special form of malnutrition) is the fish protein concentrate drawn from the very continents where protein under-nourishment is so rife.

But quantities that are essentially only of marginal interest to the West represent the difference between health and disease for many millions of people on the verge of starvation. Thus, if the enormous fish production of Peru, at present exported as fish meal, were consumed in South America instead, it could raise the nutritional status of that continent to the level of that of Italy.

The inequitable distribution of fish protein resources is further flaunted in the 300,000 tons of raw fish used yearly to produce fur on the mink farms of the United States and Scandinavia for a form of apparel used not so much for the warmth as the adornment of the wealthy. To quote the report issued at the end of the conference: "It is clear that 400 million people cannot continue to get the overwhelming part of the world's animal protein, while 2,500 millions are neglected and deprived of this essential nutrient". The situation is made even more acute by the expected doubling of the world's population to 6,000 millions in the next 40–50 years.

Utilization of Fish

Great disparities also emerged in considering the efficiency of utilization of fish as food for man and beast.

There was a general consensus of opinion that fish is a first-class food, rich in high-quality protein, valuable lipids and most of the fat- and water-soluble vitamins and minerals. However, there are appreciable losses of nutrient in handling, processing and storage which, in extreme cases, can amount to a sheer wastage of as much as three-quarters of the substance of the fish before it is consumed. Wastage occurs in trimming raw fish, by which much of the edible flesh is left on the bone, to the benefit of fish meal again. Protein and water-soluble vitamins escape during salting, and both can suffer as much as 50 per cent loss of quality during severe drying processes. Over much of the world insects cause further loss, as much as 50 per cent being reported from Nigeria.

Furthermore, there is, even in well-developed countries, the imponderable consideration of the effect on fish consumption of loss of palatability on account of chronically careless handling of a very perishable commodity between catching and consumption.

Although there is much more that could be found out by further research, it is not lack of knowledge that is holding up technical advance so much as the lack of application of established knowledge and experience. In many cases the formulation and adoption of suitable standards of processing the product are seen as a means of providing the impetus for technical improvements.

There was a difference of opinion about the toxicity of oxidized fish oils, attributed to the extreme reactivity of hydroperoxides. Such oxidation, associated with many traditionally cured products, is also partly responsible for their characteristic flavour, which is often greatly relished in a cereal diet in circumstances where fish is regarded more as a condiment than as a source of protein.

Refrigeration and canning, which are serviceable stock methods of preservation under a wide variety of conditions, result in little or no loss nutritionally.

There did not seem to be much hope that any of the newer methods of preservation, however satisfactory nutritionally, could be expected to assume such importance that proper care and control over established processes would soon be unnecessary. Vacuum freeze-drying of fish seemed unlikely to compete in quality with freezing in the West, or in cheapness with the traditional methods used elsewhere. The possibilities of sterilization by radiation, too, seem to have been greatly over-rated. On the other hand, the recent development of a large Japanese industry for manufacture of fish sausage was noted with interest, and thought to be a means of extending the outlet for fish in many other areas. Consideration would, however, have to be given to the advisability of encouraging the use of the chemical preservatives permitted in Japan.

Much discussion centred around the possibility of producing a tasteless fish flour that could be introduced particularly into the diets of young children in needy areas. The technical difficulties and achievements to date were reviewed and standards proposed for products of acceptable quality and nutritive properties. While some saw in fish flour "the basic fishery product of the future", others felt that much more could be done here and now to improve nutrition by means of the application of knowledge and experience already available for types of fishery product known to be generally acceptable.

Economic and Educational Problems

Without denying the need for immediate emergency schemes for using fish as nourishment, other animals are not necessarily competitors with man for the fish supplies A good case can be made out for the use of fish for animal feeding as part of a planned protein economy, although at present this is of more interest to the West than to the rest of the world Much vegetable protein, in the form of grass, for example, is made available to us because cattle diets are supplemented with a small amount of fish meal Although there is a loss of energy at each step in the food-cycle, protein may be converted with 50 per cent efficiency in ideal circumstances Essential amino-acids such as lysine and methionine may be recovered at efficiency-levels up to 70 per cent in animal products such as hen's eggs Ruminants, particularly, can make better use of fish products regarded as of low quality for other purposes

However, it was admitted that animal feeding is a very opportunist operation, which makes use of whatever products and by-products are available at suitable prices If the fish at present used for fish meal were to be upgraded to, say, fish flour, for human use, the industry would be forced once again to look for alternative sources of supply and would have to rely on industrial fish waste and such feeds as soya bean meal supplemented with synthetic amino-acids, although at somewhat higher cost

In the world of commerce it is more profitable to make fish meal or raise mink than it is to make and distribute food for children of low-income groups However, there are circumstances in which nations cannot afford to count the cost, and a concerted attack on freeing the world from hunger is surely a worthy cause The Food and Agriculture Organization has, in fact, initiated such a campaign which it is the duty of the more fortunate nations to support to their utmost However, even if economics were over-ridden in some such manner, there are technological and educational problems to tackle in getting fish consumed where it is needed It is true that this is only one aspect of accelerating a general expansion in production and standard of living in under-developed countries But more organized technical assistance needs to be given before many countries are in a position fully to help themselves The Organization needs further support in making their self-help more effective Training of personnel at all levels should have greater priority Fishermen and others have to be taught to use better methods of catching and preservation Consumers need education in the development of better dietary habits Technical personnel, research workers and administrators all need training before programmes of fisheries expansion can be implemented

Obviously this cannot be done overnight However, it was felt by many at the conference that more effort should be put into education by providing expanded facilities for technicians to be trained to understand and utilize improved methods based on present knowledge and experience

Finally, there was considerable support for the thesis that, although admitting that much of what is already known is not being made use of, increased support for fundamental research is essential for the most efficient utilization of fish in the future

C L Cutting

THE INTERGOVERNMENTAL OCEANOGRAPHIC COMMISSION

THE oceans, which cover some 70 per cent of the surface of the Earth, are perhaps one of the greatest fields for scientific investigation They contain unlimited areas of study in practically every scientific discipline and yet, measured with the yardstick of our knowledge of the Earth and the atmosphere, it must be admitted that we have to learn much more about the sea before we can hope to understand its vagaries and make full use of its resources

We know enough about it to appreciate the complexity and vastness of the problems which confront us, and there can be no doubt that many of them can only be solved when scientists co-operate on an international scale Although one is apprehensive in condoning the establishment of more international organizations labelled with forbidding combinations of the alphabet, there is no doubt that the establishment of the Intergovernmental Oceanographic Commission (I O C), which held its first meeting in Paris during October 19–27, fulfils a long-felt need and promises better co-ordination in the future for marine science

International organizations dealing with the science of the sea have existed for many years, but they have been either of a regional nature or confined to a limited scientific field In 1957 the International Council of Scientific Unions foresaw the necessity for a wider scale of co-ordination of activity, both geographically and disciplinary, and it established a Special Committee on Oceanic Research (S C O R) to consider such problems The activities of the Committee are, however, primarily scientific, and although, as one of its projects, it has initiated an international expedition to the Indian Ocean, it still remains a non-governmental organization solely dependent on co-operation with national academies or similar scientific organizations

The Intergovernmental Oceanographic Commission, on the other hand, now makes possible a direct approach to participating governments and forms a very necessary link where projects involving considerable expenditure are contemplated At its first meeting, Dr Anton Brunn, of Denmark, a leading biological oceanographer, was elected chairman. Dr William M Cameron, director of oceanographic research for the Canadian Department of Mines and Technical Surveys, and Vice-Admiral V A Tchekourov, head of the Hydrographic Service at Leningrad, were elected as vice-chairmen The Commission appointed as its secretary Dr Warren Wooster, director of the Unesco Office of Oceanography, which will act as secretariat

The initial task of the new body was to define its own responsibilities and the manner in which it would function, particularly in relation to existing organizations The decisions reached show that it is prepared not only to receive the advice of existing inter-governmental and non-governmental organizations which at present advise Unesco on oceanographical matters, but also the hope was expressed that the Food and Agriculture Organization, the World Meteorological Organization and other specialized agencies of the United Nations would co-operate actively with the secretariat of the Commission.

Numerous proposals for joint international oceanographic programmes were made by member States and a list of these was selected for particular consideration Many of the proposals amounted to regional surveys of interesting and important parts of the ocean, but it is also encouraging to note that some novel ideas were put forward The proposal from the United States for a synoptic survey of the North Atlantic Ocean emphasizes a long-felt need in oceanography, which could only be accomplished by the ships of several nations working together Some proposals by the U S S R included plans for studying the time changes in physical characteristics of the oceans, while others were aimed at the study of limited fields of currents which might be expected to yield more information about the mechanism of the processes occurring in the sea Such fundamental studies are an essential corollary to survey work if any real understanding of water movements is ever to be reached

An awareness of the difficulties facing large co-operative programmes was shown by a resolution submitted by the French delegation, who noted with concern the present shortage of experts in the marine sciences This shortage could well delay the whole development of marine science for many years to come, and it was proposed that an urgent appeal should be made to Governments to create the necessary permanent posts and assured careers for young scientists and technicians who are attracted by the marine sciences

Another barrier to advancement noted by one of the working groups set up by the Commission was the present lack of standardization and intercalibration of oceanographical methods and equipment With a number of nations participating in co-operative programmes, the necessity for more standardization and intercalibration becomes more urgent and the Commission has requested the Special Committee on Oceanic Research to appoint working groups of scientists qualified in the specialized fields to examine the present methods and make recommendations about the appropriate steps necessary to make them universally effective

In its planning for the International Indian Ocean Expedition, the Special Committee on Oceanic Research has already commenced this task Scientists from different countries have already met together to intercalibrate methods of measuring primary organic production, nutrient chemical analysis and zooplankton sampling methods Agreement has been reached on a standard plankton net for use on the Indian Ocean Expedition

The question of the co-ordination of activities in the International Indian Ocean Expedition was also the subject of discussion, and it was felt that although this had been most satisfactorily and energetically carried out by the Special Committee on Oceanic Research and its co-ordinator, Mr R G Snider, in New York, the responsibilities of that body were primarily scientific, and the co-ordination of the participating nations in such expeditions should more rightly lie with the Intergovernmental Oceanographic Commission and its secretariat The question whether the latter should take over these activities for the Indian Ocean Expedition was considered by the Special Committee on Oceanic Research at its meeting in Monaco, following the meeting of the Commission While appreciative of the principle, the Committee considered that it would be a slow and complicated task to divide and transfer the co-ordination, but a mechanism was set up to take advantage of the new possibilities

A further subject of discussion was a proposal for an international oceanographic research and training vessel to be sponsored by Unesco While sea-training is essential, opinion was very divided on the practicability of operating such a vessel In view of existing facilities for international exchange of scientists between national vessels, it was felt that there is no really outstanding need for a jointly owned ship, but that the idea should be kept in mind for consideration at some future date

To assist in the urgent question of accurate positioning at sea, the working groups recommended close co-operation with the International Hydrographic Bureau and the International Civil Aviation Organization to see what can be done to expedite the development and establishment of navigational systems which will meet the requirements of detailed oceanographical investigations (for example, an accuracy requirement of ± 0 25 nautical mile)

The intended use of unmanned equipment in the study of the sea also raises a problem of communication and telemetering information from remote parts of the ocean Existing frequencies in radio communications are rapidly becoming absorbed and the bureau of the Commission has been authorized to specify requirements which can be put to the next meeting of the Administrative Radio Conference

Perhaps the one outcome of the meeting of the new Commission of most immediate interest to practising oceanographers will be suggested improvements in disseminating information Not only should these improve the exchange of data, but also publicize plans of projected cruises with information on the facilities available to visiting scientists Such information will be circulated in a newsletter from the Unesco Office of Oceanography

Although a good deal of the meeting of the Intergovernmental Oceanographic Commission was occupied with putting its own house in order, it is clear that action has been initiated in a number of urgent problems The Commission is in a position to place the needs of marine science before the Governments of member countries and will no doubt adopt a balanced attitude to the various disciplines involved In this respect, the biologists attending the meeting felt some degree of apprehension as their own subject, although extensively studied, is ill-co-ordinated at an international level After an informal discussion, they concluded that to ensure that the Commission should receive the best scientific advice concerning biological phenomena in the sea, it would be of the greatest importance to set up a forum which can give the proper international backing to biological oceanography and they have undertaken, with the approval of the Special Committee on Oceanic Research, to work for the formation of an International Association of Biological Oceanography.

Towards the conclusion of the meeting of the Intergovernmental Oceanographic Commission, a session was devoted to the memory of one of the greatest oceanographers, Dr Fritjof Nansen, on the occasion of the centenary of his birth on October 10, 1861 A most eloquent tribute was paid by Prof A D Dobrovol'skiy, who described many of Nansen's achievements in oceanography and exploration Dr G Bohnecke spoke of his personal memories of Nansen, and Prof H Mosby concluded the session by showing some of Nansen's own lantern slides

RONALD I CURRIE

CHEMISTRY IN THE SERVICE OF MEDICINE

THE second British Congress on the History of Medicine and Pharmacy, organized by the Faculty of History of the Worshipful Society of Apothecaries of London in co-operation with the Chemical Society and sponsored by Glaxo Laboratories, Ltd., was held in London during September 27–29.

Prof. D. McKie, who occupies the chair of the history and philosophy of science at University College, London, was president of the congress, which had as its theme "Chemistry in the Service of Medicine". The twelve papers given provided an authoritative historical review of the services which chemistry has rendered to the development of medicine from the sixteenth century to the present day.

The inaugural session was held at Apothecaries' Hall on September 28, when Dr. A. G. Debus (Harvard University and University of Chicago) opened the proceedings with an account of Paracelsian doctrine in English medicine up to 1660. Prior to that date chemical methods and terminology were widely used in England on practical and theoretical levels. Chemical remedies and analysis did not originate with Paracelsus but were closely associated with the work of the iatrochemists. The impact of chemistry can be discerned in Paracelsian views on the origin of matter and in premature attempts to explain life processes in chemical terms. Theory was less popular in England than practice, but the present close bond between chemistry and medicine can be traced to the Paracelsian and Helmontian iatrochemists of the sixteenth and seventeenth centuries.

Dr. F. W. Gibbs (Royal Institute of Chemistry) emphasized the importance of Herman Boerhaave (1668–1738) who impressed a new, scientific, pattern on medical theory and practice in which chemistry had a central position. His *Elementae Chemiae* (1732) is a classic, the first volume being largely physical in outlook while the second is devoted to physiological, biochemical and clinical applications. The characteristics of his method were described with the aid of examples taken from his work on heat, pretended alkaline cures for gout and the stone, fermentation processes, and body fluids.

Prof. D. McKie spoke on "Chemistry in the Service of Medicine, 1600–1800" and described some of the many text-books of medical chemistry which appeared in that period. Notable among these was the *Cours de Chymie* of Nicholas Lemery, which appeared in many editions between 1657 and 1757. Boerhaave's work had transformed chemistry into an academic science *per se* and rid it of its pharmaceutical bias. The next line of progress was exemplified by Van Helmont's work on 'gas', the experiments of Boyle on combustion and respiration, and the remarkable theories of Hooke and Mayow, the influence of which was deferred by the rise of the phlogiston theory. With Black's discovery of 'fixed air' as an 'air' chemically distinct from common air, and with similar studies by Cavendish, Priestley and Scheele, this problem approached solution with the work of Lavoisier, whose discovery of the compositions of air and water, together with his calorimetric studies on respiration, opened the way to physiology.

The afternoon session at the Royal College of Physicians, with Dr. Charles Newman in the chair, was opened by Prof. F. G. Young (Department of Biochemistry, University of Cambridge), who traced the rise of biochemistry between 1800 and 1900. The idea that the chemical processes in living materials were subject to laws different from those applicable to the inorganic world lingered until almost mid-century. By the time it was largely rejected, the term 'organic chemistry' began to lose its original connotations and to be applied to synthetic compounds of carbons whether or not such substances had originally been derived from living matter. The controversies about fermentation and decomposition between Liebig and others, who included Schwann and Pasteur, were gradually decided in terms of the existence of intracellular enzymes. Ehrlich's early investigation on intracellular utilization of oxygen emphasized the role of activation of oxygen in the tissues for purposes of cellular combustion. The reasons why British chemists so largely ignored the possibility of applying their science to biology in general, and to animal metabolism in particular, are obscure, but may be found in the love of household pets (with dislike of animal experiments), the dominance of mathematical studies in British scientific education, and the controversies which followed the publication of *The Origin of Species*.

Speaking on the early history of pharmacology, Prof. W. D. M. Paton (Department of Pharmacology, University of Oxford) began by comparing the knowledge of drugs at the beginning of the nineteenth century, as shown in J. A. Paris's *Pharmacologia*, with that in Binz's *Lectures in Pharmacology* at the end. The main difference lay only in the general scientific background, although some important new drugs had been found in the intervening period the real revolution in therapeutics had scarcely begun. There was some exemplary work on the analysis of drug action and in structure/action relationships, but there was no consistent impetus or drive. When, therefore, did experimental pharmacology really get under way and what period could be defined as its 'early history'? Prof. Paton sought to answer this by studying the rate of change in the pharmacopœias, by tracing the rise and fall of different types of preparation and the introduction of substances pure enough to inject, by following the development of new 'unnatural' chemical structures, and by charting the rate of establishment of fully fledged departments of experimental pharmacology. All this type of evidence indicated that pharmacology was only now reaching maturity and that the main contribution which it would make to medicine, as well as to general biological science, was still to come.

Dr. W. D. Foster, of Dudley Road Hospital, Birmingham, and formerly of St. Thomas's Hospital, London, spoke on chemical pathology, which began as an extension of two ancient diagnostic procedures uroscopy and the inspection of blood shed at therapeutic phlebotomy. Between about 1680 and 1740, men such as T. Willis, J. Locke, R. Boyle and B. Langrish conceived that useful diagnostic information might be obtained by chemical analysis as well as inspection of blood and urine, but because of the limitations of chemistry obtained no useful results. A simpler approach to blood analysis in which the weight of fibrin, red cell mass, serum protein and water was determined, enabled Andral, in the 1840's, to establish that disease might produce chemical changes in the blood The first really useful diagnostic test in chemical pathology was for the detection of albuminuria and was introduced largely through the work of three British physicians, W C Wells, J Blackall and R Bright, in the early nineteenth century.

The modern development of chemical pathology began during the first decade of the twentieth century, and depended on the introduction of venepuncture to obtain adequate blood samples and the development of colorimetric analysis which was both sufficiently sensitive and simple enough for clinical purposes

At the third session, held at the Royal College of Surgeons on Friday morning, the help given by chemistry to the elucidation of certain important medical problems was discussed Sir Charles Dodds traced the growth of endocrinology and of organic chemistry, the former from Addison's great work on the suprarenal capsules (1855) and Claude Bernard's work on glycogen, followed by Oliver and Schafer's experiments with an extract of the suprarenals in 1895, the latter beginning with Wohler in 1828 and taking a great leap forward to synthetic organic chemistry with the contributions of Kekulé, Cooper, Van't Hoff and Le Bel The synthesis of adrenaline was the first milestone, the second was the synthesis of thyroxine by Barger and Harington (1926), following its isolation by Kendall (1916) and the clinical work on myxœdema by Murray in 1891 In a remarkable diagram Prof Dodds showed all the chemical activity which has stemmed from Addison's paper in 1855 Among the substances isolated and characterized from the suprarenals, and yet others derived from these substances, are ascorbic acid, adrenaline and the sympathome drugs (for example, amphetamine), œstradiol and its analogue stilbœstrol, cortisol and the derivatives triamcinolone and prednisolone, aldosterone and its derivatives, progesterone and analogous compounds, testosterone and the anabolic steroids, and, finally, noradrenaline and the whole group of antihistaminic drugs which stem from it The stimulus for it all came from medicine and biology, and particularly from endocrinology

Chemistry and cancer was the theme of the paper by Prof Alexander Haddow, who discussed the role of chemical substances in cancer pathology and therapeutics In 1775 Percival Pott had written a classic account of cancer of the scrotum in chimney-sweeps which definitely incriminated soot as the causative agent During the industrial revolution, coal-tar, mineral-oil and pitch were added to soot as materials capable of causing a great deal of industrial and environmental cancer, as exemplified in Joseph Bell's description of skin cancer among workers in the Scottish shale-oil industry The term 'carcinogen' was first used in 1853 by Sir James Paget, who cited Lebert (1851) as proposing that a chemist must aid the pathologist in research on cancer After many attempts, experimental cancer was produced artificially by coal-tar by Yagamura (1915) and during 1921–26 Block showed that cyclic hydrocarbon was the active carcinogen At about the same time the work of Cook and Kennaway vindicated Lebert's forecast and provided, using artificial tars, virtual proof of the carcinogen hypothesis, which was further extended by the implication of 3 4 benzpyrene, certain biological alkylating agents and other substances It was stressed that there was no antithesis between the study of viruses and chemical carcinogens, for chemistry was essential to both Chemotherapy, which had begun with Lissauer and the use of arsenic in the treatment of leukæmia, was already developing rapidly, as shown by the use of stilbœstrol in breast cancer and of other useful products such as myleran

Dr T F Macrae, speaking on chemistry and nutrition, said that in the development of improved nutritional standards, especially in the past thirty years, chemistry has played a major part Increases in crop yields have largely been due to synthetic fertilizers, adequate supplies of which have been ensured by the early discovery of nitrogen fixation More recently further advances have accrued from the wide range of pesticides now available

In the field of nutritional deficiencies the work of chemists on vitamins has been particularly beneficial All the important vitamins are now available synthetically at costs far lower than from natural sources Indeed, in advanced countries at least, vitamin deficiencies are no longer encountered. Similar developments are likely with the amino-acids, it now being a simple matter analytically to determine the adequacy or otherwise of a protein source Already in animal nutrition it has in some instances proved more economic to provide part of the nutritional requirements in synthetic form.

There still remains, however, the problem of inadequate nutrition, especially of protein, in the under-developed countries This is in part technological since, though we can identify the deficiency and, in chemical terms, propose the remedy, we still have to find sufficiently cheap means of providing this remedy in a form acceptable to the populations concerned

In the final session, held at the Chemical Society's rooms with Sir Robert Robinson in the chair, Prof D Catcheside reviewed the relationship between chemistry and genetics, beginning with the rediscovery of Mendel's work in 1900 and the speculation about the nature of his 'elements' Chemical knowledge of their action is more advanced than that of the genes themselves It began with the study of the differences between phenotypes determined by single Mendelian differences, such as the flower pigments of snapdragons by Muriel Wheldale (Onslow) This showed that a gene controlled the onset of a specific chemical reaction But the conception of a gene-enzyme-chemical reaction system was due to Garrod's work on alcaptonuria and other inborn errors of metabolism in man He considered that the abnormal gene resulted in absence or inactivity of the enzyme that normally cleaves homogentisic acid, in turn giving rise to disordered metabolism and disease The theory was revived by Beadle and Tatum, following work on the eye pigments of *Drosophila*, when they reversed the procedure by searching in *Neurospora* for gene mutations that influence known chemical reactions This has led, with the aid of the analytical techniques of Pauling, Sanger and Ingram, to the demonstration of specific amino-acid substitutions in abnormal hæmoglobins, and, with the development of microbial genetics, to attempts to correlate genetical and chemical detail for several proteins in fungi, bacteria and bacteriophage As regards the genes themselves, geneticists in the first place showed that they were in the chromosomes, but direct implication of deoxyribonucleic acid, knowledge of the chemistry of which is a recent event due to Wilkins, Watson and Crick, resulted from microbial genetics First, the demonstration that deoxyribonucleic acid is the sole substance concerned in transformation of *Pneumo-*

coccus (Avery *et al.*), and then that it is the vehicle of heredity in bacteriophage (Hershey *et al.*). More recently, attention has been directed to the coding problem and to the mechanism of transfer of specific information from deoxyribonucleic acid to protein.

A survey of progress "from antisepsis to antibiotics" was offered by Dr. W. A. R. Thomson, who said that although antiseptics had a long history, the antiseptic principle was first established by Lister in 1867, an event which led to a long and successful search for bactericidal drugs. Landmarks in that search were Ehrlich's '606', demonstrating the possibility of treatment by specific drugs, the introduction of the sulphonamides and, third, of the antibiotics. The usefulness of the sulphonamides had not yet been fully exploited and 'the antibiotic rush' of the fifties may in retrospect be seen as fundamentally unscientific. The isolation of 6-aminopenicillanic acid in 1959 marked the opening of a new and genuinely scientific era in antibiotic research. Dr. Thomson concluded with a word of caution; indiscriminate use of antibiotics had upset the balance of Nature and "the mass-murder of micro-organisms" was not necessarily a good thing. The careful use of antibiotics combined with immunotherapy offered a wiser course for the future.

Dr. F. L. Rose took one of the important developments of the same period for closer investigation in his account of the origin and rise of the synthetic drugs. With one or two exceptions, he said, all the medicaments available until the mid-nineteenth century were natural products. At that point in time, the coincidental discovery of the first synthetic dyestuffs and of micro-organismal origin of infectious diseases soon brought about a rapid change. The former because it caused an almost explosive growth of synthetic organic chemistry in a way which invited its direction into biological pathways, and the latter because it provided the motive and means for examining the effect on disease of the compounds poured out by the chemists. Thus the evolution of the modern antimalarial agents can be traced back directly to the observed staining of the malaria parasite by methylene blue. Methylene blue is a derivative of phenothiazine, itself widely used as an anthelmintic, and from which stem some of the most effective tranquillizing agents. Similarly, the modern trypanocides owe their beginning to the feeble therapeutic action shown by the early direct cotton dyestuffs, and the medicinal properties, initially antiseptic but later trypanocidal, found in the first azine and cyanine dyestuffs. Likewise, the appearance of the first azosulphonamide drug coincided with interest in the use of the sulphonamide group as a component of azo dyestuffs. These drugs were additionally important in that their technical development directed attention to the sulphones, now widely used in leprosy, while minor side effects were exploited to produce highly effective diuretic and hyperglycæmic agents.

A dinner was held in Apothecaries' Hall when the guest of honour was Sir Howard Florey, and the president of the Chemical Society, Sir Alexander Todd, gave the after-dinner address. He discussed the long-standing relationship between chemistry and medicine and stressed that the indebtedness was mutual, for medicine and its needs had provided a very great impetus to the growth of chemical research. He found in the title of the congress a reflexion of the orthodox separation of the basic sciences, a situation which was probably incapable of fruitful development. Many important problems required workers with many kinds of scientific skill, and although research institutes may provide this kind of team, research was still best undertaken by the universities. The barriers between departments must be broken down to enable the constant influx of young minds to cross the barriers between the sciences.

The papers given at the congress are to be published in volume form. It has already been decided that the meeting next year will be held in London during September 27–28, and will have as its theme "The History of British Hospitals". F. N. L. POYNTER

THE AUSTRALIAN NATIONAL RADIO ASTRONOMY OBSERVATORY

AUSTRALIA made an early start in radio astronomy at the end of the Second World War, and has remained ever since among the leading countries in this rapidly advancing field. Australian radio astronomers now possess a radio telescope which is the foremost of its type in the world at the present time—a 210-ft. steerable paraboloid, with a surface accuracy of a few millimetres.

The new telescope, which is located at Parkes, New South Wales, 230 miles west of Sydney, was commissioned on October 31 by the Governor-General of the Commonwealth of Australia, His Excellency the Right Hon. Viscount De L'Isle, and it will be operated by the Division of Radiophysics of the Australian Commonwealth Scientific and Industrial Research Organization. The telescope is the first instrument of the Australian National Radio Astronomy Observatory, which was inaugurated at the same time.

The plan for a large steerable paraboloid originated seven years ago, when the first specifications were drawn up by Dr. E. G. Bowen, Chief of the Organization's Division of Radiophysics. His vision and enthusiasm have been largely responsible for the successful completion of the project.

A large donation from the Carnegie Corporation (250,000 dollars) enabled the design work to start, and other generous donations were afterwards received from the Rockefeller Foundation (357,000 dollars), and from a number of private donors in Australia. These sums were matched on a pound-for-pound basis by the Australian Commonwealth Government. The total cost of the project has been about £800,000 (Australian) equivalent to £640,000 (sterling).

The novel design was produced by Freeman, Fox and Partners, consulting engineers, of London, with Mr. Gilbert Roberts in charge of the responsible group. The construction was carried out by Maschinenfabrik Augsburg-Nürnberg, A.G., of West Germany, which submitted the best tender, both in price and delivery time, and was awarded the prime contract. The principal sub-contractors were Askania Werke, of West Berlin, Associated Electrical Industries, Ltd., of Manchester, and Concrete Constructions Pty. Ltd., of Sydney.

The telescope is mounted in azimuth-altitude style, on a single concrete tower, which also contains the telescope controls and the radio receivers. The whole structure rotates in azimuth on a circular track which is set into the top of the concrete tower; a range of 450° is available, limited only by the cable-twisting requirements. The altitude motion, between zenith angle 0° and 60°, is obtained through a large rack gear. A wide range of speeds is available in each co-ordinate.

A unique feature of the instrument is the control system for driving the telescope in equatorial co-ordinates. The heart of this system is a small 'master equatorial' unit, which is mounted at the intersection of the altitude and azimuth axes, on a special concrete support, completely isolated from the rest of the structure. This unit controls the motion of the telescope through a servo system which contains an optical link.

The 'dish' is supported by a structure of 30 radial trusses, which carry a system of ring girders and two sets of intersecting spiral members. The reflecting surface consists of wire mesh, with wires 1 cm. apart, except for the inner 54 ft., which is covered with steel plates. The inner solid section was adjusted to fine limits during construction; but the individual panels of the mesh surface carry screw adjustments, which can be reset at any stage.

The aerial feeds and pre-amplifiers are supported by a large cabin, which is mounted on a tripod above the reflector. The cabin is large enough to carry test equipment and observing personnel during special tests, though normal operations will of course be controlled from below. There are a lift in one tripod leg and ladders in the other two, for access to the focus cabin.

The telescope is now structurally complete, and is at present undergoing a rigorous series of tests, through theodolite surveys of the shape of the dish and radio observations of cosmic sources. Some progress performance figures are now available, and very satisfactory results are being obtained.

The original specification called for a surface accuracy of ± 9 mm. With the dish looking vertically, most of the adjustment points on the mesh surface are within 2 mm. of the required parabolic shape—with additional deviations of the same order inside the individual panels.

Some early figures have been obtained for the radio performance at wave-lengths of 21 and 10 cm. At 21 cm., the beam-width is 13½ min. of arc or less, and the side-lobe level is in the region of − 25 db. At 10 cm., the first measurement gave a beam-width of 7½ min., and the side-lobe level is again very low. Moreover, these figures were obtained with the dish 40°–60° from the zenith, where any distortions would be at their maximum.

On the opening day, there was a widely representative gathering of nearly 500 guests at the Observatory, many of whom had come from Sydney, Canberra and Melbourne in a special airlift. Before the ceremony, the visitors inspected the structure, and then were shown a large photographic and descriptive display which had been specially set up for the occasion.

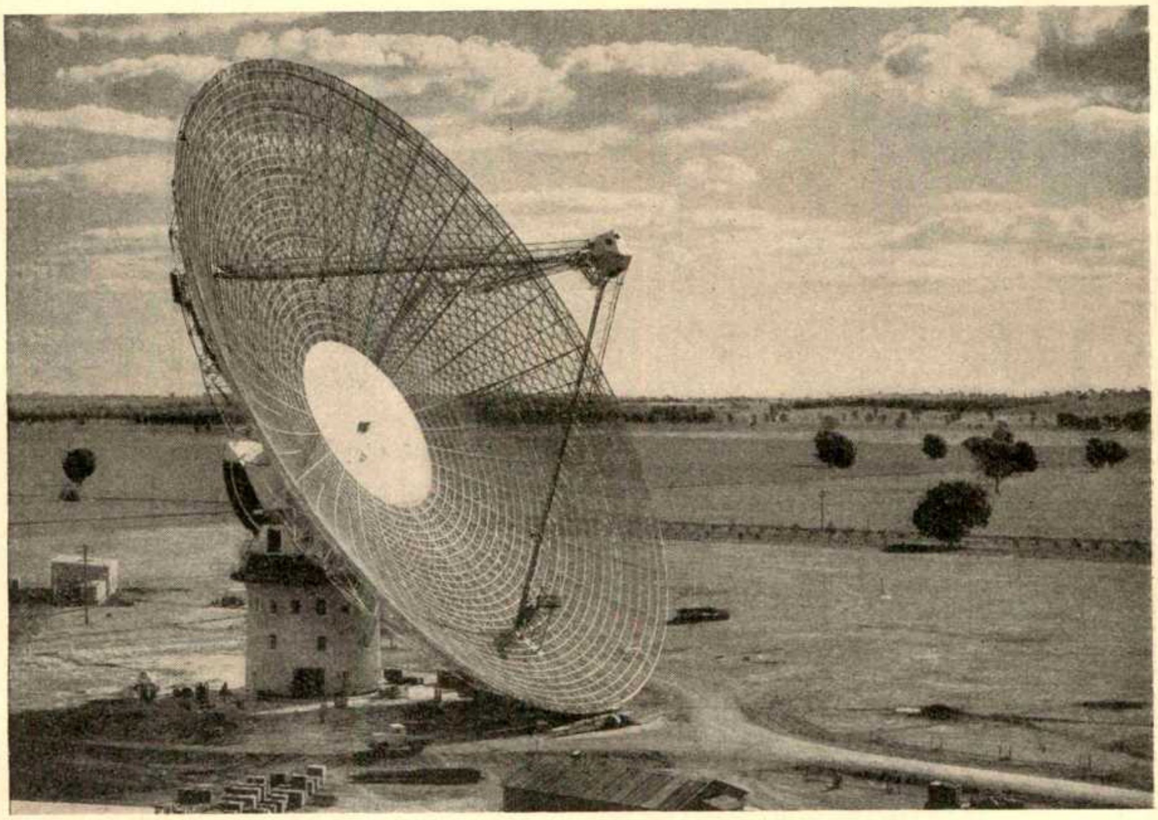

Fig. 1. The completed telescope, tilted down to its lowest position, 60° from the zenith

After being welcomed by Dr F W G White, Chairman of the Commonwealth Scientific and Industrial Research Organization, the Governor-General began by reading a message from H R H The Duke of Edinburgh

"The development of radio astronomy has been one of the great events of modern Science, and the scientists of C S I R O have played a leading part in this important work Their studies of the Sun and the exploration of extraterrestrial space to the remotest parts of the universe have gained the admiration of astronomers everywhere and have brought great credit to Australia

"To-day the radio astronomers of Australia are to be rewarded by the inauguration of a magnificent radio telescope which will enable them to penetrate still further the secrets of the cosmos This occasion marks the culmination of much planning and hard work and I send you my best wishes for the success of your investigations with this new instrument "

His Excellency then discussed the way in which realization of this "Giant Radio Telescope" had come about as the result of a happy conjunction in Australia of several distinct, but all of them propitious, circumstances "Thus conceived and encouraged, the enterprise has leapt forward at a pace which I found both admirable and refreshing " He closed in whimsical mood with a quatrain which ' seemed to float into my mind from an extra-planetary source"

"Twinkle, twinkle radio star
We can't see you you're too far
Now we hear from outer space
How you run your cosmic race "

The next speakers were Dr The Hon D A Cameron, the Minister-in-Charge of the Commonwealth Scientific and Industrial Research Organization, and The Right Hon Lord Casey, who was Minister-in-Charge of the Organization during the early critical stages of the project, and has continued his connexion with it as chairman of the Radio Astronomy Trust

At this stage the Governor-General performed the commissioning ceremony, and a large Australian flag was broken out above the focus cabin, the highest point of the structure Unfortunately, wind gusts of more than 50 m p h interfered with a plan to tilt the dish down towards the assembled crowd The area is normally very calm, which in fact was one of the reasons for its choice, but on this ceremonial occasion the weather was unkind, with wind-speeds at their highest level for several years

Dr Bowen closed the proceedings by outlining some of the plans for the future use of the instrument A number of important observational programmes are ready to start as soon as the present period of extensive tests is completed These include work on the identification of extragalactic sources, and studies of galactic structure, the Magellanic Clouds, bright hydrogen regions, and the planet Jupiter

The new instrument will make a powerful addition to the facilities available to the Australian radio astronomers, but it is also a notable gain for the astronomical world at large To quote Dr Bowen's concluding remarks "When the first urgent tasks of the next few months have been performed, it will be thrown open to astronomers from every part of the world with worthwhile problems to attack We pledge ourselves to work in close collaboration with our fellow scientists from any country and will make this Observatory a rallying point for equipment and information, for techniques and intellects which, together, will lead to further great advances in the science of astronomy"

OBITUARIES

Prof Robert Boutflour, C.B.E.

Prof Robert Boutflour, who had resigned as principal of the Royal Agricultural College in 1958, died on October 27 in Cirencester at the age of seventy-one He was educated at Barnard Castle School and Armstrong College—now King's College, Newcastle upon Tyne

Boutflour's first appointment was lecturer in agriculture with the Lancashire County Council, this was followed by a similar post in the University of Leeds, and in 1921 he was appointed agricultural officer for the County of Lindsey In 1922 he was made chief agricultural officer to the Wiltshire County Council, where he remained until 1926, when he was appointed the first and last national director of dairy husbandry

He is best known for his work on the breeding and management of dairy cattle This he started in Wiltshire, where it was realized that he was a man of sound progressive ideas, and in fact, many years ahead of his time, which is now proved inasmuch as his ideas are now accepted in general practice His fame extended abroad as well as at home, and he is well known to many farmers of the territories he visited in the United States, Canada, Kenya and Australia

While national director of dairy husbandry, he was attached to the Harper Adams Agricultural College, where he established his famous Friesian herd of dairy cattle This enterprise proved to be an outstanding success and enabled him to put into practice those points of management that he had so strenuously advocated to farmers throughout the United Kingdom

Boutflour was an impressive author and wrote many works on farm management in general, and the subject of dairy cattle in particular While his success as a scientist was largely due to his keen powers of observation, coupled with patient and inspired reasoning, his ability to impress audiences or individuals was the result of his enthusiasm, alert mind and logical arguments coupled with a quick wit In character and speech he was always most forthright, but his caustic remarks were usually offset with a characteristic twinkle in his eyes

His appointment as principal of the Royal Agricultural College in 1931 gave him the necessary scope for his ability in organization and administration, and particularly to associate with young people, to whom he was always willing to give advice and encouragement When he retired from the principalship in 1958 he had the satisfaction of knowing that during his term of office he had increased the number of students from 48 to more than 450

During the Second World War, as executive officer for food production in Gloucestershire, he adopted many revolutionary methods to increase the output of food One of his most daring ventures was to plough up the Slimbridge pastures adjoining the River Severn, which for centuries had been the habitat of wild geese His critics said that with this terrific fertility no cereal crop would 'stand', but once again he proved them wrong He was created C.B.E in 1952, and in 1955 he received the Sir Thomas Baxter Prize and Gold Medal for outstanding work in dairy husbandry

However, it is by his personal character that so many will remember him, and among his personal characteristics was a 'human' appreciation of his fellow men and the possession in a very large measure of that great virtue—tolerance His wife, who survives him, was the greatest help to him throughout his varied career, by her companionship and understanding The passing of this colourful character has created a void which will be difficult, if not impossible, to fill, and to the older generation who knew him—and there are so many—Bob Boutflour will always remain in their memory with affection as a generous, likeable and very 'human' person of outstanding ability, while the younger generation will reap the benefit of his scientific work, and the valuable contribution he made to British and, in fact, world agriculture W T PRICE

Dr A. E. Western

ALFRED EDWARD WESTERN, who died on October 11 at the age of eighty-eight, was one of the few who have been able to carry on mathematical research while fully engaged in another profession in his case, that of a solicitor He was educated at Marlborough and at Trinity College, Cambridge, which he entered with an open scholarship in 1892 He was seventh Wrangler in the Mathematical Tripos in 1895, the year in which Bromwich was Senior Wrangler and E. T Whittaker second Wrangler

Dr Western published several memoirs on the theory of algebraic numbers, and one on the theory of groups, during the years 1898–1911, and showed himself to be thoroughly at home in a subject which was then little cultivated in Britain His standing as a mathematician was recognized by the award of the degree of Sc D by the University of Cambridge in 1910 He gradually became more interested in questions of numerical computation, and published several notes giving the numerical evidence which he had found for various conjectured formulæ of Hardy and Littlewood relating to the distribution of primes, to the occurrence of primes of the form $n^2 + 1$, and to Waring's problem

During the last years of his life he was engaged (jointly with Dr J. C P Miller) in completing a table projected by the late Lieut -Colonel A. J C Cunningham, with whom he had formerly collaborated This table, which is essentially a continuation of Jacobi's *Canon Arithmeticus*, is shortly to be published with the help of a bequest made by Cunningham to the London Mathematical Society for the purpose

Dr Western was closely associated with the London Mathematical Society over a long period, and rendered great service to it He became a member of the Council in 1900, and treasurer in 1913 in succession to Sir Joseph Larmor He retired from this office in 1950, and many members will recall the delightful speech he made at a dinner held at that time

As a solicitor, Dr Western was a pioneer in initiating 'legal aid', and it was largely from his work that the legal aid scheme used in courts in Britain developed

He will be remembered by many as a man of great kindliness and of transparent sincerity and honesty of purpose H DAVENPORT

NEWS and VIEWS

Geology at Trinity College, Dublin: Dr R. G. S. Hudson, F R S

THE chair of geology and mineralogy at Trinity College, Dublin, vacated by Prof W D Gill on his appointment to the chair of oil technology at the Imperial College of Science and Technology, London (see *Nature*, 191, 651, 1961), has been filled by the appointment of Dr R G S Hudson, Iveagh Research Fellow in geology at Trinity Dr Hudson, who studied geology at University College, London, under the late Prof E J Garwood, joined the staff of the Geological Department at Leeds in 1922, became professor of geology in 1939 and during 1940–45 acted as consulting geologist to various companies engaged in the search for oil in Great Britain During these years he published mainly on the Dinantian and Namurian of the north of England, demonstrating the rhythmic succession of the Yoredale Series, the stratigraphical relationship of the limestone of the Craven Reef Belt, the pre-Namurian age of the Mid-Craven Fault, and helped in mapping a large part of the Craven Lowlands He also worked with W S Bisat on the goniatite faunas of the Namurian and, in particular, detailed the goniatite succession in the Alport Boring in Derbyshire During 1946–58 he was a senior research geologist and palæontologist with the Iraq Petroleum Company, leading field exploration parties in Kurdistan, Iraq (1949, 1950) and in the Oman Mountains of Arabia (1951, 1952), and publishing on the stratigraphy and faunas of these areas Much of the present knowledge of the Middle East Mesozoic faunas, in particular the stromatoporoids and hydrozoa, is due to his work He has received the Foulerton Award of the Geologists' Association and the Wollaston Award and Murchison Medal of the Geological Society He has also been president of the Yorkshire and the Liverpool Geological Societies and the Palæontological Association, and a vice-president of the Geological Society In 1959 he was made an honorary member of the Société Belge de Géologie et Paléontologie, and in 1961 he was elected to the Royal Society.

The British Nuclear Energy Society

ON January 1, 1962, the British Nuclear Energy Society will be established in succession to the British Nuclear Energy Conference The successful use of nuclear energy demands the application of many divisions of science and technology and a constant interchange of knowledge and experience between those trained primarily in these divisions. It was to provide a channel for this interchange that in 1955 the British Nuclear Energy Conference was established by the Institution of Civil Engineers, the Institution of Mechanical Engineers, the Institution of Electrical Engineers, the Institute of Physics and the Institution of Chemical Engineers, which were joined later by the Iron and Steel Institute, the Institute of Metals, the Institute of Fuel and the Joint Panel on Nuclear Marine Propulsion. The Conference has sought to fill its role principally in three ways: (1) by publishing under one cover, in the *Journal*, all the more important papers on nuclear energy presented to the constituent societies; (2) by keeping members of the individual societies informed of those meetings of the other societies that are devoted to aspects of nuclear energy and making such meetings open to them; (3) by sponsoring meetings on nuclear energy organized either for the Conference by the appropriate societies or, where the subject involves the application of several disciplines, by the Conference itself.

The time has now come to provide a central forum for the discussion of nuclear energy, and it is for this purpose that the British Nuclear Energy Society has been established. The Institution of Civil Engineers has made its premises in Great George Street, Westminster, available as a meeting place, and a regular programme of papers, lectures, symposia and informal discussions is being planned. The Society will (like the Conference) publish a quarterly *Journal* which in addition to reprinting a wide range of important papers will also contain original papers and discussion on them, information and authoritative scientific and technological comment on developments in nuclear energy throughout the world, and reports on the proceedings of meetings of the Society. The first issue of this *Journal* will be in January 1962.

Membership of the new Society will be open to members of all classes of the constituent societies of the British Nuclear Energy Conference and to all who satisfy the Board that they are (to quote the Society's constitution) "actively engaged in the professional, scientific, or technical aspects of the application of nuclear energy and ancillary subjects". Further information can be obtained from the Secretary, British Nuclear Energy Society, Institution of Civil Engineers, Great George Street, London, S.W.1.

Changes in Times of International Time Signal

For more than one hundred years, the Royal Greenwich Observatory has been responsible for providing exact time signals for a wide variety of users both in Britain and abroad. In recent years this service has become increasingly important in various fields of scientific research where extreme accuracy is essential. In order to provide the various users with more frequent opportunities for checking the time, the present twice-daily transmissions from Rugby were increased to four as from December 1. This will mean that the transmissions on the low frequency of 16 kc./s. now radiated at 10 a.m. and 6 p.m. will be superseded by signals at 3 a.m., 9 a.m., 3 p.m. and 9 p.m. There will, however, be no change in the form of the signals. To ensure a world-wide coverage, the 10 a.m. and 6 p.m. broadcasts have also been transmitted on short wave. This service will continue after December 1, but the times will be changed to 9 a.m. and 9 p.m. As long ago as 1833, the Royal Greenwich Observatory provided hourly time signals for the operation of 'time balls', which were devices consisting of a large ball secured to the top of a mast and released by a special catch at a precise time. One such ball is still in use in the grounds of the old Observatory at Greenwich. The hourly transmissions continued for many years, but this service has now diminished to almost negligible proportions and, in fact, the only user is the G.P.O. for the Talking Clock (*TIM*) which has been in operation since 1936. The reason for the falling-off of the requirement for the hourly Greenwich transmissions was the inauguration in 1924 of the B.B.C.'s Greenwich time signal, which is claimed to be as accurate as possible.

The Federal Council for Science and Technology

According to *Scientific Information Notes* (3, No. 5, October–November 1961), the U.S. Federal Council for Science and Technology will in future, to help scientific periodicals meet the cost of publishing research results, pay, as a necessary part of research costs under Federal grants and contracts, page charges for publication of research results. The research papers concerned must report research work supported by the Government and the charges must be levied impartially on all research papers published by the periodical. Payment of such charges must not be a condition for acceptance of manuscripts by the periodical, and the periodicals must not be operated for profit. The new policy was stimulated by the National Science Foundation. The National Science Foundation has also placed a contract for computer-aided research with the Ramo-Wooldridge Corporation, Canogen Park, California, to further the investigation of new techniques for processing language data. Under the contract, 300,000 words of Russian text, representing five fields of study, will be used for dictionary compilation. Concurrently, the translation programme will be used for mechanical translation research in which the translation will be automatically compared on the computer with the results of a previous revision of the dictionary. Part of the investigation will be to consider the introduction of modifications and supplements into the programme. At the Institute for Scientific Information, Philadelphia, molecular formulæ of several hundred randomly selected chemicals have been calculated on an electronic computer, using a newly constructed recognition grammar of chemical nomenclature, and the new grammar enables the computer to understand the chemical names fed to it by a typist. The linguistic analyses indicate that the procedure can be used manually by chemists in training clerks, librarians and indexers to calculate a molecular formula from a chemical name, and the new computer technique is being incorporated into the preparation of *Index Chemicus*.

National Central Library

The forty-fifth annual report of the Executive Committee of the National Central Library (Pp. 24. London: National Central Library, 1961) covers the period March 1, 1960–March 31, 1961, in which, on an average, applications for loans increased by 4.6 per cent each month over the corresponding month in the previous year, while the percentage dealt with successfully increased from 72·22 to 74·13. Volumes added to the general stock by purchase rose from 1,129 to 1,952, largely in foreign languages, and nearly 18 per cent of requests were met directly by loans from the Library's own stock, compared with 16 per cent the previous year. Satisfactory progress is reported in the first stage of the project for dealing with the arrears of cataloguing, but further funds are necessary for dealing with the later stages of the project. The Library's British National Book Centre has now extended its service to include foreign libraries, and some 19,977 specially requested books and periodical volumes and parts were allocated to 126 foreign libraries, while in return 3,987 offers of foreign material were made by the foreign libraries benefiting. Of the total issues of 90,612 volumes, 16,555 were to university libraries, 10,255 to special outlier libraries, 2,653 to Government departments, 3,414 to industrial and research organizations, and 4,520 to overseas libraries. The university libraries lent 29,826 books, compared with 28,611 in the previous year. The Library itself supplied 641 microfilms and 326 photostat copies, and of these 491 microfilms and 187 photostat copies came from co-operating libraries in the United Kingdom. Foreign libraries supplied 732 microfilms and photostat copies.

The Institute of Personnel Management

THE annual report of the Council of the Institute of Personnel Management for 1960–61 records an increase of 441 in membership to 4,938, and the Information Service is now handling about double the number of inquiries in 1959 (Pp. 31. London: Institute of Personnel Management, 1961). During the year, views on the proposed Government Bill on Health, Welfare and Safety in Shops, Offices and Railway Premises were submitted by the Institute to the Ministry of Labour. A paper by A. B. Cherns on priorities for research in the social services in industry was published in the Institute's journal, and during the year a sub-committee on research was formed which convened a meeting of representatives of research bodies in this field to advise it, at which the proposal that the Institute should develop its research role was welcomed. Two important new broadsheets have been published, one by H. E. Roff and T. E. Watson, dealing with job analysis, and the other by J. R. Armstrong on supervisory training, and an address by Sir Ben Lockspeiser, "Man and his Machines, Automation and Computation—their Origins and Effects", given at the 1960 National Conference, has been published as an occasional paper. Particulars of this conference and of the regional conferences held during the year are also given in the report.

Science and Public Health

THE annual report for 1960 of the scientific adviser, Dr. S. G. Burgess, of the Scientific Branch, Public Health Department, London County Council, at present organized in eight sections, records that 42,090 samples were examined during the year (Pp. 60. London: London County Council, 1961. 1*s*. 3*d*.). Some 90 per cent of the increase of 6,800 samples is due to the analysis of air samples necessitated by safety precautions against the leakage of inflammable sludge gas at the Northern Outfall Works. During the year the resources of the Board were made available to the Metropolitan borough councils, and investigation of noise, and nuisance arising from noise, in a large area of London was commenced in collaboration with the Department of Scientific and Industrial Research. A light-weight concrete based on an expanded aggregate was used for the construction of a thirteen-storey block of flats, and the Scientific Branch co-operated with the architect in solving many problems associated with the design. The practicability of laying hot asphalt over an expanded polystyrene roof insulation was also investigated, a new washing technique developed which offers considerable savings in laundry processes, while other special investigations were concerned with methane gas detectors, impurities in sludge gas and deposits in sludge gas lines and flame traps. New laboratories were completed at the Northern Outfall Works and a new Building Materials Laboratory at County Hall, London.

Corrosion Science

THE first quarterly issue of a new international journal devoted to the science of corrosion and protection of metals and entitled *Corrosion Science* made its appearance in August (1, No. 1. An International Journal of the Science of Corrosion and Protection of Metals. Pp. 76. (London and New York: Pergamon Press, 1961.) Annual subscription (*A*) for libraries, Government establishments and research institutions, 140*s*., 20 dollars; (*B*) for individuals who write directly to the publishers certifying that the journal is for their personal use, 70*s*., 10 dollars.) The journal is published by Pergamon Press under the guidance of an executive editorial board consisting of C. Edeleanu, J. G. Hines, T. P. Hoar and L. L. Shrier of Great Britain, M. Pourbaix of Belgium and M. J. Pryor of the United States. The standard of reproduction is of the usual high quality associated with Pergamon Press. The editorial notice mentions, among other things, that papers on corrosion are at present published in many diverse chemical, metallurgy and engineering journals, and expresses the hope that the new journal will help to concentrate this information. That there will be an adequate flow of papers of appropriate quality to the new journal seems assured, in view of the continually increasing amount of work in this field.

The first issue of a new journal always presents some difficulties for both publisher and reader, for the former in deciding how to obtain appropriate papers, and for the reader in deciding whether the first issue is likely to be representative of the eventual standing of the journal. In the present case there are five papers occupying 75 pages, with sufficient breadth of content to offer something of interest to the majority of corrosion scientists. The composition of the executive editorial board is such that the journal should establish itself as of high repute and with a continuing breadth of appeal.

The Australian Museum, Sydney

THE September issue of the *Australian Museum Magazine* (13, No. 11, September 15, 1961) contains illustrated articles on the boomerang, insect migration, vampire bats—true and false—the piranha and meteorites. It is recorded that Mr. F. A. McNeill retired from the curatorship of the Department of Crustacea and Cœlenterates after 47 years service. He was a specialist on Australian Crustacea. An exhibit on whales and whaling has been completed. It contains models of fifteen different species, of whaling ships and of whales being flensed on deck. Maps show the areas in the Antarctic in which whaling is carried out, the location of shore stations, and the areas in which whales are protected.

Automatic Data Processing

A PUBLICATION issued by the Department of Scientific and Industrial Research (*Automatic Data Processing*. By Derek Wragge Morley. Pp. iv+76+8 plates. London: H.M.S.O., 1961. 6*s*. net) seeks to provide guidance to the public about available techniques and machines in this field. The booklet is well produced, but the journalistic style and lack of critical appreciation of the history and implications of the subject will make it considerably less valuable than might otherwise have been the case. A particular shortcoming is the complete absence of any reference to more authoritative literature—the reader is directed to various manufacturers for further information. While this may be suitable for intending purchasers, it is unlikely to be helpful for the layman who wishes to gain a fuller understanding of the subject.

The latest issue of the *Philips Technical Review* (23, 1, October 1961) contains a description of a new digital computer *Pascal*. This machine, installed at the Philips Computing Centre at Eindhoven, has a well-balanced, parallel operation, arithmetic unit the speed of operation of which varies between 10 and 73 μsec for the various operations. The machine is a binary one with optional floating-point facilities. As may be imagined from its origin, it has a large magnetic core store as well as drums, tape and a plugboard. Input and output are in punched tape or cards and on magnetic tape and the speeds considerably exceed those of standard telegraph and business machine practice. The components used in the machine include valves, transistors and diodes since, in addition to being a useful calculator, the machine will provide information on component performance.

Technical Papers of the Sandia Corporation

THE bibliography *SCR*-255 is a revision of *SCR*-150 and consists of a cumulative numerical list of technical papers of the Sandia Corporation, Albuquerque, which are available to the public (Pp. 120. Albuquerque, New Mexico: Sandia Corporation. Obtainable from the Office of Technical Services, Department of Commerce, Washington 25, D.C., 1961. 2.50 dollars.) The prices of printed or photographic and microfilm copies are included in most cases. The title list includes in addition a section on engineering drawing and specifications on sale through the United States Atomic Energy Commission's Engineering Materials Section at Oak Ridge. The contents of the bibliography is divided into eight sections, dealing with the reprint and monograph series, technical reports, development reports, technical memoranda, divided into annual series covering 1953–60 inclusive, manuscript releases, miscellaneous papers, and engineering materials. The Sandia Corporation is a prime contractor to the Atomic Energy Commission.

Plasma Physics

REPORT No. 18 of the Danish Atomic Energy Commission Research Establishment, Riso, contains the essential part of the material presented in the form of lectures or at the seminars during the international summer school on plasma physics held there during August 1–12, 1960 (Pp. viii+645. Riso, Roskilde: Danish Atomic Energy Commission Research Establishment, 1961. 60 D. Kr.) The lecturers were distinguished scientists from Denmark, France, Germany, The Netherlands, United States of America and Great Britain. The director was Dr. M. N. Rosenbluth (General Atomic, San Diego, California) and 130 students from twenty-four countries attended. The contents of the report, compiled by C. F. Wandel (Riso Establishment), consist of the short general introduction to plasma physics given by the director, followed by thirty-six articles grouped under the headings: single-particle motion, magnetohydrodynamics, fundamental equations, stability, relaxation, radiation, thermonuclear aspects, pinch and shock apparatus, concepts covering the stellarator and the mirror machine, and plasma waves and diagnostics. Not all the articles are in the form of lectures, some are reprints of published papers. The standard of the lectures, though representing a good introduction to and clear statement of the basic principles of plasma physics, nevertheless is somewhat more advanced than, for example, Lyman Spitzer's book. The report should prove an admirable text for postgraduate students and research workers who want to pursue an advanced course in plasma physics before proceeding to tackle the latest publications on the subject.

British Chemicals and their Manufacturers

THE Association of British Chemical Manufacturers has recently issued a guide, *British Chemicals and their Manufacturers*, which supplies information on all chemical products made by its members (Pp. 219. London: Association of British Chemical Manufacturers, 1961.) The booklet is far more than a guide, and will be invaluable as a reference book to purchasers and producers alike. It will certainly prove to be indispensable in all academic and industrial research departments throughout the United Kingdom. The booklet consists essentially of five sections including the grouping of chemical industries, a directory of members of the Association, classified lists of British chemical products (with a special section on indicators and microscopical stains), lists of proprietary and trade names and proprietary and trade marks. The information is in each case presented in the clearest possible way. The first section provides information on the nature of the products, that is, their grade, whether heavy or fine, their origin, for example coal-tar distillation products, and their type such as dyestuffs, explosives, fats, glycerine, greases, soaps, etc., and those suitable for pest control. The directory, besides giving the name and address of members, also supplies telegraphic addresses, telephone and telex numbers. All in all, 366 industries are listed, and supplemented by a list of affiliated associations. Products are listed under their chemical names, and coded references give means of tracing the producer. In each case it is indicated whether the chemicals are of commercial grade or fine, pharmaceutical, pure, analytical and similar grades. The lists of proprietary and trade names will certainly be of great assistance to all editors who have to decide what is, and what is not, a registered trade name, and hence what should have quotation marks.

Autonomic Nervous System and the Occurrence of Visceral Lesions

Hiroshi Itoh, Teizo Kasahara, Kazua Amano, Koh Okamoto and Samon Tanabe, of the Department of Pharmacology, Yokohama University School of Medicine, have shown histologically that a chronic chemical stimulation on a defined region of the skin produced lesions in both renal cortices (*Yokohama Med. Bull.*, 12, No. 3; June 1961). They have now followed the development of these lesions by functional examinations over a period of four months. The state of intrarenal circulation was investigated to determine the pathogenesis of the lesions, and the renal function was examined in several ways. The test for albuminuria and Volhard's test proved to be positive, but disorders of renal function could not be detected by other methods. Albuminuria was confirmed at two stages of the experiment, namely, on the twenty-eighth and fifty-fifth days Investigations made on the fifty-fifth and seventy-sixth days on the renal cortical region suggested some disorder of the intrarenal circulation Over a period of four months the systolic blood pressure did not rise significantly above the level in the pretreated period The authors suggest that the lesions may have been caused by impulses generated in the afferent nerve, following repeated chemical stimulation on the skin, passing through a reflex arc involving the higher central nervous system These continue to the intrarenal arteries by way of the sympathetic nerve, and the resulting ischæmia in the renal cortex may be the cause of the degeneration of the renal parenchymal cells, especially those of proximal convoluted tubules The functional changes of the kidney are slight in the period before the lesions advance but they are readily perceived by histological examination

An Inventory of Abstracting Services

The Fédération Internationale de Documentation, 7 Hofweg, The Hague, Netherlands, is preparing, with financial assistance from the National Science Foundation in Washington, D C, a comprehensive inventory of abstracting services covering the fields of science, technology and social sciences It is expected that the study will take two years The data will be collected by means of a questionnaire to be distributed at the beginning of February 1962 Besides gaining information about the nature and scope of each abstracting service, the questionnaire will also determine the number of periodicals consulted during the year, the existence of a reproduction service and whether translations can be obtained On completion of this project, the Fédération plans to establish an international information centre, the first object being to give supplementary information on data revealed by the questionnaire It is hoped that all abstracting services will co-operate in the scheme

Wellcome Senior Research Fellowships in Clinical Science

To encourage medically qualified workers of exceptional ability to embark on careers of clinical research within the United Kingdom, the Wellcome Trustees are prepared to award one or two Wellcome Senior Research Fellowships in this field in 1962 The professors in charge of university departments of medicine, surgery and obstetrics in the United Kingdom are invited to put forward confidential nominations for these awards Direct personal applications by candidates for these Fellowships will not be considered The Fellowships will be awarded for one year in the first instance and will be renewable over four more years It will be a condition of the appointment that the Fellow should be granted the status and responsibilities of a lecturer in the university department in which he is employed On the assumption that the age of the Fellow at appointment will be about thirty, the stipend offered over the five-year period will be £1,900 a year, rising by increments of £150 to £2,500 Further information can be obtained from the Scientific Secretary, The Wellcome Trust, 52 Queen Anne Street, London, W 1

Christmas Lectures for Young People

Among the lectures organized for young people during Christmas and the following weeks are The one hundred and thirty-second course of Christmas lectures at the Royal Institution, to be delivered by Sir Lawrence Bragg on "Electricity", including "What is Electricity?" (December 28), "How Electricity Travels" (December 30), "Motors and Dynamos" (January 2), "Electrical Oscillations and Electromagnetic Waves" (January 4), "Our Electrical Supply" (January 6), and "Conveying Information by Electricity" (January 9) Further information can be obtained from the Royal Institution, 21 Albemarle Street, London, W 1 Five children's Christmas holiday lectures on "The Painter, the Anatomist and the Surgeon" (Mr H Thompson, January 1), "Science in Crime Detection" (Dr K Simpson, January 2), "Family Life in Russia To-day" (Dr J. Hunt, January 3), "The Romance of Huntington's Chorea" (Dr. Macdonald Critchley, January 4) and "How and Why We Hear" (Mr T Cawthorne, January 5) at the Royal Society of Medicine at 2 30 p m. Further information can be obtained from the Assistant Secretary, Royal Society of Medicine, 1 Wimpole Street, London, W.1 A young people's lecture, to be delivered by Prof M Stacey on "Nature's Plastics" at the Plastics Institute on January 5, at 2 30 p m Further information can be obtained from the Plastics Institute, 6 Mandeville Place, London, W.1

Announcements

Prof N R Dhar, recently head of the Chemistry Department and director of the Sheila Dhar Institute of Soil Science, University of Allahabad, has been elected as a corresponding member of the Paris Academy of Sciences in the Chemistry Section

Mr P S Briggs, of T N and F H Briggs (Tanners), Ltd, Leicester, has been appointed chairman of the Council of the British Leather Manufacturers' Research Association in succession to Mr E. A Hutchings, who has left the leather industry

Dr Olivier Heroux has been appointed scientist-in-residence at the U S Naval Radiological Defense Laboratory, San Francisco, California. This is the first appointment under a new programme at the Laboratory for visiting distinguished scientists from other scientific communities Dr Heroux is a member of the Canadian National Research Council and will be at the Laboratory during leave of absence of one year from his post in Canada

The annual meeting of the American Mathematical Society will be held at the Sheraton-Gibson Hotel, Cincinnati. during January 22–25 Further information can be obtained from J W T Youngs, American Mathematical Society, Bloomington, Indiana

Erratum Dr G B Robertson writes In "Crystal and Molecular Structure of Diphenyl" (*Nature*, 191, 593, 1961) the value of the constant in the asymmetric-unit plane equation was reported incorrectly due to a computational error The correct value of this constant is 0 00864, the equation of the plane is

$$-0\ 30584\,X + 0\ 55575\,Y + 0\ 77305\,Z' = 0\ 00864$$

and the perpendicular separation between the ring planes is ~ 0 017 Å (not ~ 0 2 Å) This separation is not statistically significant and the two rings are, therefore, coplanar within the limits of the experimental error

NEMATOLOGY AT THE IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

ON October 23, at Silwood Park, Sunninghill, the new Shell Parasitology Building was formally handed over to the Imperial College of Science and Technology. A plaque inside the door explains that "These laboratories were erected with the aid of a generous benefaction from Shell International Chemical Co., Ltd., in support of research into parasitology".

The new building consists of two floors, each of 2,400 sq. ft., and a small basement of 400 sq. ft. On the ground-floor are a lecture room and well-equipped teaching laboratory for the one-year postgraduate course in nematology, a preparation room for extracting nematodes from soils or from plant materials, and a radio-tracer room. On the first floor are three staff laboratories, a large laboratory for higher-degree students, a small teaching laboratory for undergraduates specializing in parasitology, and a photographic dark room. In the basement are three constant-temperature rooms separately controllable within the range 5°–30° C.

While research projects extend over the field of general parasitology (recent work has dealt with protozoal parasites of insects, and the bionomics of sheep trichostrongyle larvæ, for example), major prominence is given to nematology. The most pressing requirement was to house the postgraduate nematology course: it will now be possible to accommodate with comfort eight students, and at need up to twelve, instead of only four as hitherto.

This one-year course aims at giving a grounding in: (1) general nematology with an emphasis on plant-parasitic and soil-inhabiting species; (2) the principles of control by chemical and other means; (3) the art of experimentation. The course, the first of its kind in Great Britain, has a strong bias towards practical techniques and research methods. Of a total of fifteen students to date, ten have come from seven overseas countries, and it is hoped to maintain this overseas appeal, if only because plant eelworms are of great economic importance in tropical and subtropical lands.

The new laboratories are well equipped with such standard items as microscopes and calculating machines, and radiotracer equipment includes a versatile scaler with scintillation head, and monitor. Work is in progress with labelled chemical fumigants.

B. G. Peters

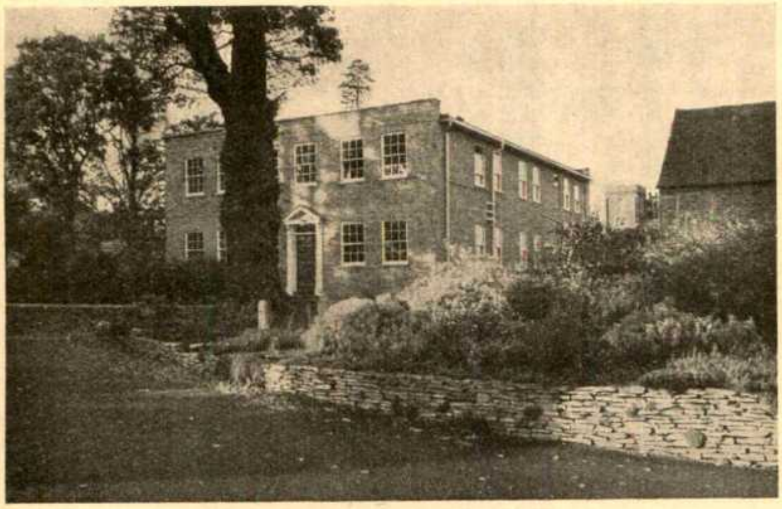

Fig. 1. Front view of the Shell Parasitology Building, Imperial College of Science and Technology

THE HUMBER LABORATORY

THE Humber Laboratory of the Department of Scientific and Industrial Research at Hull held two open days during November 9–10. This establishment is a branch laboratory of the Torry Research Station, Aberdeen, and like its parent Station is concerned with research on the handling and processing of fish from the time of catching to retail sale. Since the Humber Laboratory was opened in 1952 it has dealt with an ever-increasing volume of work, particularly in applied research and in giving scientific information to the fish trade. The buildings have been extended twice during the short life of the station, and the recent open days marked the putting into service of a new block containing laboratories, library, and a lecture theatre.

The exhibits at the open days were not limited to the activities of the Humber Laboratory itself, but also covered the much wider range of subjects dealt with at the Torry Research Station. Prominent among these is the matter of freezing fish at sea. Poorer catches from present fishing grounds and the loss of some traditional grounds by the extension of territorial limits are encouraging longer voyages by British distant-water trawlers. This in turn means that the use of ice is no longer an adequate method of preserving all the catch in an edible condition. Freezing and cold storage is one obvious answer to the problem of preservation, but the successful application of this technique has required the co-operation of scientists and engineers trained in widely different disciplines. This was illustrated by exhibits which range from techniques of showing the damage to muscle cells during freezing, and of estimating the extent of denaturation of proteins in cold store, to a model of freezing plant designed to work on small ships in rough weather. The work of the Torry

Research Station has been particularly concentrated on the design of trawlers which will freeze part of the catch rather than on factory ships to freeze and process all of it at sea, and a model of one of the first vessels of this type to operate commercially was exhibited.

When frozen fish is used on any large scale the problem of thawing for further processing becomes a matter of very considerable importance. The thermal diffusivity of wet fish is very much less than that of frozen fish, and hence the thawing of a block of frozen fish in warm air is a self-retarding process. Also the temperature gradient permissible in such a process is limited by the need to minimize the spoilage of the fish already thawed. This has led to examination of dielectric heating as a means of thawing, and a practicable technique has been worked out. A pilot plant of 6-kW. output operating at 36 Mc./s. was on show. In this, frozen fish was carried on a conveyor belt between the plates of a condenser and 2-st. blocks were thawed in about 15 min. Equipment of this type developed by the Torry Research Station has recently been put into commercial operation.

Despite the attention given to frozen fish, work on wet fish has been by no means neglected. A number of exhibits were concerned with the effect of temperature on spoilage by reference to the chemical and bacteriological changes involved. The Humber Laboratory has been particularly concerned with the temperature of fish throughout the chain of distribution and has conducted temperature surveys at all stages from dock side to retail shop. This work has entailed the development of a number of special instruments, notable among which is a spear-type resistance thermometer in which the resistance element is contained within a hypodermic needle. For making temperature measurements by thermocouple on board moving vehicles, the conventional electronic self-balancing potentiometer is not a very suitable instrument. It is too large and its power consumption is too great. The Humber Laboratory has developed a small, battery-operated potentiometer in which balance is indicated by a 'magic eye', and this has been used successfully for many measurements on lorries. Another simple and effective instrument is the 'temperature indicator' which shows a sharp change in colour when it exceeds a given temperature. This device consists of a tube of 1 ml. capacity containing two aqueous solutions separated by a frozen non-aqueous layer.

The Torry Research Station has long been concerned with the smoking of fish, and when the Humber Laboratory was opened most of the applied work on this subject was transferred to Hull. The 'Torry kiln' has made considerable strides towards becoming the conventional piece of apparatus for smoking fish. Examples of the wide range of smoked products that can be made in a Torry kiln were on show together with sectioned models of both Torry and traditional kilns. The Humber Laboratory has devoted considerable effort to the question of the more efficient production of wood smoke, and has developed a continuous, automatically controlled smoke producer which uses a fluidized bed of sawdust. Tests have also been carried out to evaluate the performance of various commercial smoke producers, and a number of these machines was on show.

The work on smoking has also led to the development of various new instruments. A simple robust smoke density meter operating on the usual lamp and photocell principle was exhibited together with a modification of it, the 'smoke density integrator', which indicates directly smoke density multiplied by time—a quantity of great importance in smoke curing. Although more recent background work has shown that the invisible vapours in wood smoke are of more importance than the visible particles in giving smoked products their characteristic odour and flavour, smoke density meters are still of very considerable value. A technique for measuring the concentration of vapours was also exhibited.

Much of the work of the Humber Laboratory which has been of the most direct value to the fish industry has depended less on the discovery of new facts than on the practical application of well-established knowledge. For example, very considerable assistance has been given in setting up quality-control schemes which are based on taste panel techniques developed at the Torry Research Station in past years. Similarly, much-needed advice on the best methods of packing fish in ice for inland transport is merely the application to fish of the well-known laws governing the transient state conduction of heat. Work of this nature is extremely necessary in a station of the Department of Scientific and Industrial Research which serves the needs of an industry which as a whole is technically backward, and in which scientific staff are found only in the largest and most progressive companies.

D. L. Nicol

FOOD AND AGRICULTURE IN DEVELOPING COUNTRIES

On October 13 the Nutrition Society held a symposium, at the National Institute for Research in Dairying, Shinfield, Reading, under the chairmanship of the director, Prof. R. G. Baskett, on agricultural problems in developing countries. The latest Food and Agriculture Organization review of food and agriculture* shows that the developed areas still have an abundance, even a surplus of agricultural products, while malnutrition and hunger are the order of the day in many less-developed countries. The past decade has seen a very considerable expansion in the world output of food. For example, the average annual combined output of wheat, rice and maize (including the Food and Agriculture Organization estimates for the U.S.S.R. and mainland China) rose from about 470 million long tons in 1948–52, to about 720 million long tons in 1959. This remarkable technical achievement rests far more on increased efficiency of production than on increases in crop area. The overall food output per head has risen, but this covers dangerous decreases in Latin America and Oceania, and little change in Africa, as well as considerable increases in Europe (where population growth has been slower than elsewhere), North America and the Near East. In the Far East, where food production per head fell sharply during the War years, the pre-war standard, low as it was, has been restored by increases in food production of about 3 per cent per annum The very considerable increases in food production claimed in China since 1950 are perhaps no more than an intensification of this general trend

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* Food and Agriculture Organization of the United Nations, Rome. *The State of Food and Agriculture*, 1961. Pp. viii+177. (Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1961.) 10*s*.; 2 dollars.

In the past two or three years, however, food output has been more static, and in 1961 (including the disastrous harvests reported from China) total production was probably no greater than in 1960 International trade and co-operation are moving part of the Western surpluses into the needy areas, but there are not enough railway wagons, ships, elevators and sacks in the world to touch more than the fringes of the problem For the hungry countries, continued increase in their own output, and particularly in yield per acre, is the only answer Most of them have already done a good deal, as the Food and Agriculture Organization figures show, but the great disparity in yield per acre between the best and the worst indicates that far more could be done, particularly in India and Pakistan, which harbour one-quarter of the world's cattle and where the crop yields are among the lowest in the world

The Nutrition Society meeting devoted itself largely to this question, with particular reference to tropical areas Prof L Dudley Stamp spoke on the climatic limitations to development in the tropics He distinguished first between the monotonous climate of the humid equatorial regions and the alternately wet and dry climate of the very diverse regions which he included in the tropics proper In neither area does lack of heat limit plant growth, but in the latter variability of rainfall, in amount and in timing, is often pronounced North and south of these regions lies the tropical arid zone, taken by the United Nations Educational, Scientific and Cultural Organization to lie beyond the 20-in (500 mm) isohyets

At tropical temperatures soils weather deeply and organic matter is rapidly oxidized, while leaching removes plant nutrients beyond the reach of crops, though not of trees Prof Stamp felt that shifting cultivation, with hoes (and weeds) and without ploughs or tractors, might represent the best scientific answer to the land management problem in the humid tropics (Clearly the many successful examples of plantation production of perennial crops must represent at least an exception to this generalization, though certainly in high rainfall areas the removal of vegetation, even for a very short period, in land clearing, is bound to create difficult or even dangerous hydrographic situations) In the dry areas, the accumulation of salts and the formation of pans, together with erosion, can produce arid semi-deserts In all areas of seasonal rainfall the heaviest agricultural tasks of the year fall on man and beast when they are weakened by the privations of the hungry season, and during the rains there are risks of flooding Water control and irrigation, Prof Stamp believes, are essential to development in all three regions The indirect consequences of the tropical climate such as deterioration of stored food, and the heavy burden of insect and other pests and diseases of plants, animals and man, are also important

To Prof A H Bunting (University of Reading), who spoke next on the problems of improving arable output, the impression left, though perhaps unintentionally, by Prof Stamp seemed unduly pessimistic In many developing countries encouraging increases in food production have been reported, and impressive yields have been achieved in some There are two parts to the problem of improvement the intensification of production and the development of new agricultural land

Apart from water, the tropical environment is by no means inherently unfavourable for crop production The highest agricultural dry-matter yields so far recorded in the world are in sugar cane in Hawaii (around 30 tons dry matter/acre/year) The poor yields of peasant production must be compared, not with those of modern scientific farming, but with peasant yields in medieval Europe Intensification is to be sought through improvements in protoplasm, production, and protection There are many examples of improvement of yield, quality and disease resistance in tropical crops by selection and breeding Better production often requires better land use, and the abandonment of shifting cultivation, but this can only come as part of a general process of improvement, usually on a regional scale On a smaller scale, improved rotations, fertilizers, timely and sufficiently dense sowing, and the use of mechanization (particularly at times of peak labour input) have all been shown to pay dividends Crop protection, including weed control, with the help of agricultural chemicals is well established for export crops in many tropical areas Seed schemes, in which dressed seed of improved varieties is supplied at sowing time by an official organization, already exist in cotton and other crops the general extension of such schemes would be a powerful aid to progress

The development of new agricultural areas was illustrated by photographs from the Sudan rainlands, where many millions of acres of land suitable for arable production lie unused for lack of perennial water supplies

Dr J C Shaw (Food and Agriculture Organization), speaking on animal production problems, estimated that the annual deficit of animal protein in human diets (which is especially marked in the Far East) is equivalent to 1 8 million metric tons of non-fat milk solids A Food and Agriculture Organization panel assessing livestock problems has given the highest priority to nutrition, followed by disease and management, with breeding last In cattle, better nutrition means better management of better grazing plus the conservation of cereal and legume feeds and of forage (Clearly this means that animal production, especially in drier areas, depends entirely on successful arable farming, but the converse is not necessarily true, as those who have advocated "ley farming in the tropics" seem to believe) Much work is needed on the improvement of forage species, particularly legumes, on the intake of food and its nutritional value, and on the nutritional requirements of cattle in the tropics Since much of the feed in the dry season comes from nutritionally poor materials, means of conserving or increasing their feed value must be sought For small animals, farming waste products are important, and formula feeds will have to be devised Breeding, in rigorous climates, should be based first on investigations of the productivity and improvement of the existing livestock, and should not necessarily be based on crossing programmes using improved stock from temperate countries Generally, in developing countries, the shortage is not of animal agronomists but of scientific information more modern veterinary schools are needed.

Dr Shaw's assessment of the priorities in animal production was closely supported by Mr T J Lansbury, recently of the University College of Ghana He, too, gave the highest priority to nutrition, which appears far more important in limiting the output of West African cattle (and also of goats, sheep and poultry) at present than their inherent characteristics Drought leads to shortage of feed and of water, and may lead to losses of 25 per cent of live weight in a single season The improvement of water supplies by conservation requires planned use of grazing, while the problem of dry-season feed supplements has scarcely been tackled, partly because there is little information on the composition of animal feeds in the savannah regions The West African ox does not seem to possess the superior digestive powers with which it is credited, and indeed digestibility is reduced when animals are fed on a low plane of nutrition, particularly in respect of phosphorus and protein Basic work on all these matters, which would no doubt include studies of urea and other non-protein nitrogen sources, is needed on the spot

Introduced varieties of pigs and poultry can do well in West Africa Pigmeat is not in demand, but great expansion of egg production is possible if the birds are properly fed and cared for and not regarded as mere scavengers An industry based on imported chicks is developing, but serious nutritional problems (protein, vitamins B and others, calcium and other minerals) remain to be overcome An industry for feeding-stuffs could be based on local materials which are at present exported for the formulation of feeds in Europe

The last speaker, Mr R S Rack, based his contribution on his remarkable work in the practical development of native fishing industries in Northern Rhodesia His address was notable for its active concern with the human problems of agricultural development He pointed out that the people of undeveloped countries have their own technical and social adaptations to their environment, and that the technological basis of their economy cannot be altered in isolation from its personal and social context This difficulty shows itself in attitudes to organization and work discipline (whether self-imposed or not) and to the employment of time, and in the obstacles often presented by traditional habits and customs The simple poverty of the native fisherman, and his social and family obligations, may prohibit the accumulation of capital, the size of his undertaking may in any event be too small to allow a single technical step (such as the purchase of an outboard motor) unless his whole scale of operation can be altered to take full advantage of it Other problems include the supply of fish, the availability of technically competent and equipped fishermen, and the existence or possibility of markets and suitable transport These factors were illustrated by an account of the successful measures taken since 1951 to improve the supply of fish (whether fresh, as a staple food, or dried as a relish in a cereal diet) to the Rhodesian Copper Belt, which involved an ice plant, a boat-building school, a marketing advisory organization and the encouragement of African traders, and is to lead on to a fisheries school which will teach navigation, the handling of powered boats and book-keeping in addition to the techniques of catching and preserving fish Another example arose from the development of a fishing industry among the traditionally non-fishing Tongas when they were resettled because of the construction of the Kariba Dam, and a third from the improvement of a sardine-type fishery on Lake Tanganyika

In the discussion following the papers, an interesting food chain involving water hyacinth, pigs and fish was described by Prof Stamp from Hong Kong, and some disagreement was evident on the usefulness of imported stock in the tropics

The Food and Agriculture Organization report, already mentioned, also deals with human and organizational problems, on a regional scale, in two special chapters One, on land reform and institutional change, surveys the situation in the main regions of the world and then discusses particular problems such as tenancy, financial support, marketing, settlement and community development The second is on the organization of extension education and research in Africa, Asia and Latin America Taken as a whole, the report is, as usual, a valuable and informative survey.

The meeting, and the report, justify a degree of optimism not only about the technical possibilities but also about the chances that they will be in part realized during the next two or three generations The social and historical imponderables are too great to make any further prediction useful but it is impossible to avoid the conclusion that a general limitation of human numbers is ultimately desirable if a measure of dignity and worthiness of human life is ever to be achieved

A H Bunting

SCANNING TECHNIQUES IN BIOLOGICAL AND MEDICAL MICROSCOPY

A CONFERENCE on the principles and applications of scanning methods in the microscopy of biological and medical specimens, arranged by the New York Academy of Sciences, was held in the Henry Hudson Hotel, New York, during October 5–6 Fourteen papers were presented in the four sessions of the meeting, and all but one were concerned with optical microscopy, the exception being on electron and X-ray scanning microscopy

The main group of contributions ranged from the straight application of television in microscopical observation to the design of a complicated flying-spot system for sizing cells M J Kopac (Graduate School of Arts and Sciences, New York University) described the use of closed-circuit television in connexion with the micro-surgical technique he has developed for operating on single cells At present the micro-instruments, of which there may be as many as eight in use in a single operation, are manipulated by hand It is planned to adapt the electronic techniques used for tracking targets in the air so as to control the movement of microneedles and micropipettes with servo-motors

Scanning techniques are especially useful for cytochemical studies of transient processes in cells C N Loeser and S S West (School of Medicine, Western Reserve University, Cleveland, Ohio) have been using a highly sensitive 'Intensifier Image Orthicon' television camera to record fluorescence emission spectra from individual Ehrlich ascites cells, stained with acridine orange The average uptake of dye per cell has been determined and correlated with the spectral measurements For quantitative investigations in the ultra-violet, two approaches are being followed G Z Williams (National Institutes of Health, Bethesda) and R G. Neuhauser (Radio Corporation of America, Lancaster), described the use of a new 'Vidicon' television camera sensitive to the ultra-violet as an image storage detector and for time-lapse work They obtain oscilloscope tracings of the changing ultra-violet absorption of single living cells, allowing deductions about reaction-rates On the other hand, V K Zworykin and C Berkeley (Rockefeller Institute, New York) have been exploring applications of the colour-translating television microscope In this instrument, the specimen is illuminated by three selected ultra-violet wave-lengths and the three images produced are electronically converted into images in three different colours on a television screen It is being used for examining ultra-violet radiation damage, the streaming of protoplasm and the absorption of metabolites such as vitamin A

A still wider range of application is open to the flying-spot technique, in which the specimen is explored point by point sequentially The source of illumination is the flying spot on the screen of an oscilloscope, which is demagnified through the microscope to throw a moving probe on the specimen The image is built up on a display tube, or the signal may be used directly for quantitative absorption measurements With suitable phosphors any part of the spectrum can be used, or indeed soft X-rays T O Caspersson (Karolinska Institutet, Stockholm) gave a masterly survey of the methods which can be used, and the factors influencing instrumental design, in the three main areas of investigation analysis of single cells or cell structures with high precision, analysis at lower accuracy of large numbers of cells or cell structures, and investigations on living material, which are often only possible by semi-quantitative or even qualitative methods He described a number of instruments in use, or in development, in his own laboratory for these different types of research, and discussed the choice between mechanical, optical and electronic systems of scanning

Most of the remaining contributions were variations on these themes J G Hoffman (University of Buffalo) described measurements of the nucleic acid content and the dimensions of mammalian cells and their nuclei, both by ultra-violet absorption in fresh cells and by absorption in the visible range in unfixed cells and in Feulgen-stained cells He discussed the use of a digital computer to facilitate analysis of the absorption measurements as they are obtained Britton Chance (Johnson Foundation for Medical Physics, Philadelphia) described investigations of respiratory enzymes by analysis of the fluorescence spectra excited in single cells J J Freed and J L Engle (Institute for Cancer Research, Fox Chase, Philadelphia) discussed the advantage of using a vibrating mirror to produce the scan, since it allows monochromatic illumination at any desired wave-length They described the instrument they have built for making repeated absorption measurements on single living cells It was found that continuous exposure of ascites tumour cells for 30 min at wave-lengths above 2400 Å produced no observable radiation damage, whereas at 2400 Å , or below, the typical radiation damage syndrome was observed

The chairman of the conference, P O'B Montgomery, who has been mainly responsible for pioneering flying-spot microscopy in the United States, described recent work in his laboratory (University of Texas Southwestern Medical School, Dallas) In addition to the development of a flying-spot interference microscope, he and his colleagues (W A Bonner and L L Hundley) have worked out techniques for simultaneous illumination of the specimen with both visible and ultra-violet light In one version of the apparatus, the two images appear side by side on the display tube Alternatively, a very small part of the specimen is imaged by ultra-violet while a larger field around it is imaged by visible light In this way the progress of ultra-violet radiation damage on selected cells, or even on different parts of a single cell, can be followed

One of the important uses of the flying-spot microscope is for counting and sizing particles, and instruments are commercially available for routine measurements of this type Attempts are now being made to extend the technique to the 'recognition' and counting of specific types of cells in tissue sections or smears, by applying to microscopy some of the experience gained in the use of radar for target spotting An instrument named the 'cytoanalyser' has been built for the mass screening of cervical and vaginal smears, to aid in the early diagnosis of cancer It is best described as an analogue-digital computer connected with an electromechanical scanning system, and is the fruit of collaboration between the Sloan-Kettering Institute for Cancer Research, New York, the American Cancer Society, the National Cancer Institute, and Airborne Instruments Laboratories of Deer Park, New York Its general principles of construction were described by W. E Tolles and H. P Mansberg of the last-named, and its functions and preliminary results of its operation by Elaine Diacumakos and Emerson Day of the first-named institution. The biological situation is complex, and it seems to be too early to judge how successfully the instrument can replace the human eye in spotting aberrant cells, and so dispensing with the squads of observers now employed in checking smears under the microscope In any event, these two papers were another striking illustration of the feeding back of experience from the field of war to the problems of peace

The remaining contribution, by V E Cosslett (Cavendish Laboratory, Cambridge), described the extension of flying-spot methods to the X-ray end of the spectrum, with some reference to the related technique of scanning electron microscopy Both have so far been mainly applied to metallurgical and mineralogical problems Very recently A D G Stewart (Engineering Laboratory, Cambridge) has built an ion gun into an electron scanning microscope, to produce etching of a specimen surface during continuous electron imaging It seems likely to be useful in studying the structure of teeth and other mineralized tissues, as well as of metals and alloys At the Cavendish Laboratory, an exploration is being made (with aid from the Medical Research Council) of the possible biological and medical applications of the X-ray scanning microscope—more accurately described as a microanalyser Some preliminary results were reported to the Conference, from the detection of foreign particles in lungs and other tissues to work on the mineralization of bone and teeth An appreciable proportion of iron (up to 7 per cent) has been found in the surface enamel of the incisors of a number of rodents. The technique has obvious uses in identifying dust particles in atmospheric pollution and related problems. The recent extension of its scope to the lighter elements, beryllium and carbon, makes an investigation of the possibilities of organic analysis desirable. However, it is doubtful if it can be of value in cytochemistry until the spatial resolving power is improved appreciably beyond the present limit of 0.25μ and the detection limit to better than one part in ten thousand.

The conference was throughout stimulating, and provoked much lively discussion, which often led back to fundamentals. It was the first meeting of its kind, and very much justified the initiative of the organizers. V. E. COSSLETT

TIME-RESOLVED SPECTROSCOPY

A CONFERENCE on "Spectroscopic Studies using Time-Resolved Techniques" was held in Manchester on June 16, under the auspices of the Institute of Physics and the Physical Society.

A brief introductory address was given by Prof. H. S. Lipson, president of the Manchester Literary and Philosophical Society, who welcomed members of the conference to the new rooms of the Society. He pointed out that the meeting was the first to be held there by the Institute of Physics and the Physical Society since these new buildings were completed, and which indeed had only been opened in September 1960.

The joint secretaries of the conference were Dr. L. Bovey, Atomic Energy Research Establishment, Harwell, and Dr. A. H. Gabriel, Atomic Weapons Research Establishment, Aldermaston. On the programme seven communications were listed and an eighth was added by Dr. C. Breton, who described some recent French work on plasma. As in other recent international conferences the scope of time-resolved spectroscopic work on plasmas, or indeed on discharges in general, has been shown to have extended very widely since the end of the Second World War, at which time very little work in this field had been done. This new work has, of course, largely been prompted by the rapidly growing interest in high-temperature plasmas, partly because of the widespread interest in the possibility of obtaining thermonuclear reactions and, more recently, partly because of work on the so-called magnetohydrodynamic conversion of energy. In both these closely related fields spectroscopic techniques are pre-eminent. This is because such techniques do not normally interfere with the discharge being investigated and because of their great range and power. The need for time-resolved studies, often on a sub-microsecond scale, has also led to the development of advanced optical techniques, and the latter were very much in evidence at the present conference.

Although speakers from France, the United States and South Africa all made contributions, the majority were provided by various workers from the Atomic Energy Authority at Harwell and Aldermaston.

The first paper was given by Dr. A. H. Gabriel, who has been responsible at the Atomic Weapons Research Establishment for the development of much striking optical apparatus for plasma work. He spoke about a system incorporating a fast-rotating mirror camera and a medium quartz spectrograph, used, for example, in work on fast pinched discharges with electron densities varying in the region 10^{15}–10^{18} per c.c. and lasting a few μsec. and time resolutions of the order of 0.1 μsec. or less. Dr. Gabriel then proceeded to describe various other optical techniques available, namely, an image-rotating device in which three mirrors are used with a vertical slit, and an apparatus for work in the vacuum ultra-violet region using near normal incidence and covering the wave-length range 2000–5000 Å. In such an instrument it was pointed out that astigmatism of the concave grating presents a difficulty. The spectrum is recorded near the base of the normal on to the Rowland circle and since only a limited wave-length range can be covered on one setting the position of the entrance slit assembly is made movable. A resolution of 3-mm. image displacement per μsec. is obtained. In connexion with all this work a precision capillary light source for calibration in the visible and quartz ultra-violet has been developed which gives virtually uniform illumination for about 12 μsec. and with an effective blackbody temperature of about 25,000°. Another source for vacuum ultra-violet work, based on that of W. R. S. Garton at the Imperial College of Science and Technology, was also described by Dr. Gabriel. Experiments at the Atomic Weapons Research Establishment that were mentioned include measurements of excitation temperatures, that is, state populations, etc., for various discharges, and studies of free-free continua in the temperature-range 10^5–2×10^6 degrees, and finally, Dr. Gabriel mentioned experiments involving observation of emission spectra of rapidly pinched discharges, for example, the influence of impurity lines at different times, and work on absorption spectroscopy using axial viewing from a hollow pinch in which electron densities could be obtained from a study of line widths.

The next speaker, Prof. J. D. Craggs of the University of Liverpool, described recent experiments on the measurement of line broadening due to the inhomogeneous field Stark effect in spark discharges (transient arcs). He briefly summarized the historical development of this technique, which is now widely used in plasma investigations, from the days when the statistical theory was considered adequate to the present time when a more accurate realization of the complexities of the effect, particularly with regard to the contribution made by electrons in the impact broadening process, has been reached. Recent experiments in Liverpool, notably by Dr. E. E. L. Mitchell, strongly support the recent theoretical work of Griem and Kolb in the United States. This is for the Balmer lines of hydrogen, but in view of the present importance of the impact broadening contribution of electrons, experiments have been carried out over the past two years in Liverpool by Mr. Lim, who has simultaneously studied sodium and argon lines (notably the former) broadened by the impact effect and the statistical-impact broadening of the Balmer lines. These results again support the Griem–Kolb theory and direct attention to the possibility of making electron density measurements with the use of impact-broadened lines The use of sodium–hydrogen discharges has led to a number of interesting excitation effects which are being studied and Prof Craggs briefly referred also to measurements of de-ionization of plasmas, with electron densities varying from about 10^{16} to 10^{18} per c c , in relation to the recent work of Prof D R Bates and Dr A E Kingston in Belfast and Dr R W P McWhirter in Harwell

Dr F D Harrington of the United States Naval Research Laboratory proceeded to describe a series of time-resolved spectrographs developed and used in recent years at that Laboratory. In particular the applications included investigations of exploding wires and high-velocity luminescent plasmas The instruments broadly fall into three general groups (*a*) the moving film type, (*b*) the rotating drum type, (*c*) the rotating mirror type Instruments in the first category are somewhat unusual in this respect, at least in European studies, and the technical excellence of the design and optical work was very apparent For example, Dr Harrington described an *f*6 6 ciné high-speed prismatic spectrograph for use in the near ultra-violet and visible region with film speeds of 9 ft /sec and 90 ft /sec giving respectively 460 and 4,600 frames/sec The resolving power was about 5 Å at a wave-length of 4000 Å with a dispersion of 125 Å /mm Another striking instrument described was a rotating drum continuous writing *f*/2 0 ultra-violet spectrograph Experiments were then described on exploding wire spectra for a time-range 0–10 µsec and using a 300-joule condenser discharge

Dr R Wilson of Harwell spoke on time-resolved intensity investigation of spectral lines emitted by impurity ions in *Zeta* hydrogen plasmas and concentrated on recent work on interpretation of these spectra rather than on the instruments involved The impurity lines were examined with monochromators covering the wave-length range 100–6000 Å and most of the work fell in the vacuum ultra-violet region where the strong lines of highly ionized ions occur Dr Wilson stressed that in a plasma of this type in which the electron density is of the order of 10^{14} per c c at an equivalent temperature of 20 eV , the dominant processes are ionization by electron collision and radiative recombination, as in the solar corona, and this meant a strong departure from thermal equilibrium Dr Wilson described recent experiments on the transient lifetime for spectral lines corresponding to successive studies of ionization and showed how these varied in a partially contained plasma Data were presented for the intensity transients of lines due to Ar I–Ar VIII The time duration of the optical transients for these different states increased up to Ar VI but thereafter remained constant, and similar results were described for O IV, O V and O VI Many new spectral lines, for example, due to Ne VII and Ne VIII, Kr V, Kr VI, Kr VII and Kr VIII and Xe V to Xe IX have been identified The containment times for many of these highly ionized states have been studied Thus, for example, in certain experiments Ar VIII was contained for about 450 µsec

Dr R V Williams of the Associated Electrical Industries Research Laboratories, Aldermaston, also described time-resolved spectroscopic work on toroidal plasma discharges such as those in the apparatus *Sceptre* 4 Dr Williams described the range of techniques available at the A E I Laboratories, including multi-channel recording with photomultipliers Experiments have recently been done on *Sceptre* 4 on radial variations of electron temperatures. for example, by studying the 2822 Å line of B IV which is concentrated near the centre of the discharge, and the 2066 Å line of B III was observed farther out, that is, nearer the tube wall These lines were excited when methane was added to the discharge in very small amounts, which are shown to have negligible effect on the overall properties of the discharge. Further radial work on the 2271 Å line of C V (C V is helium-like) were mentioned Various investigations of this kind indicated, for example, electron temperatures kT_e of the order of 20 eV Dr Williams also mentioned in this connexion new work in progress on F V and F VI lines which is being carried out again to obtain electron temperatures Plasma instabilities are being detected spectroscopically by examining high-frequency intensity fluctuations in spectral lines, the frequencies involved lie in the range 10–100 kc /sec and instabilities occurring in different parts of the discharge could be separately detected by observing lines from ions having varying ionization potentials For example, the 2781 Å line of O V was used for examining instabilities near the centre of the discharge and the second Balmer line (D_β) for instabilities occurring near the wall of the containing torus Further experiments involving the use of two monochromators so placed that each gave one of a pair of oscillograms of a single line, have given some support to recent theoretical treatments of plasma instabilities which indicate that the latter should be propagated in a torus with velocities depending on the magnetic field and the plasma pressure, the measured velocities of such instabilities agreeing quite well Predicted values were about 10^6 cm /sec

Dr N J Peacock of the Atomic Weapons Research Establishment Plasma Group at Aldermaston described spectroscopic work on the fast pinch discharges at that establishment The experiments involve rapidly rising axial magnetic fields, electron densities of the order 10^{16} or 10^{17} per c c and electron temperatures estimated, for example, from measurements of the continuum established in the wave-length region below 20 Å as 100 eV or higher Time-resolved spectrograms provide evidence in support of other work with streak cameras and with X-ray and neutron emission studies when maximum temperatures are reached during the second half-cycle of the driving field Time-resolved spectrograms have enabled temporal variations of electron density, ion and electron temperatures and plasma distribution to be made on helium discharges and other discharges including a few per cent of added impurities Studies of Stark-broadened profiles of the He II line 4686 Å agree well with Griem's theoretical work Time-resolved spectroscopic work on electron temperature and electron density taken for various radial positions in the tube enable diffusion-rates of the plasma through the containing field to be estimated Dr Peacock also described emission work on deuterium–oxygen discharges, for example, in which energy balance relations have been studied In this work electron temperatures have been estimated from the ratios of intensities of He I and He II lines and in various other ways Dr Peacock showed interesting space-resolved spectra for helium and temporal variations of ion density across the discharge showing a shell structure

Mr W W. Schroeder of the National Physical Research Laboratory, Council for Scientific and Industrial Research, Pretoria, described recent developments in photo-electric recording of spectra from transient sparks The techniques have been developed specifically to improve time-resolved spectroscopic analysis of solids, for example, the quantitative detection of copper in aluminium alloys The controlled gating of the photo-multiplier proved to be so good that special controlled spark sources had to be developed and a description of such a source was given based on the work of A Bardocz in Hungary This source used an air-blasted control gap in series with the test gap The jitter proved to be not more than 15 mµsec over 10^7 discharges, and the importance of ultra-violet illumination of the test gap by the control gap was shown to be important

It was possible to analyse a spectral line with a resolving time of 0 1 µsec The speaker stressed that in spectroscopic analysis of this kind time-resolved observations might well be of importance in the investigation of analysis and internal standard lines, etc

Dr. C Breton (Fontenay-aux-Roses) gave an account of spectroscopic observations made in that establishment, including the observation of impurity lines in the far ultra-violet in a toroidal discharge and a new technique for the study of line profiles The former work was carried out with a vacuum grating spectrograph using seven exit slits, each with a separate photomultiplier tube Impurity lines from carbon, oxygen and nitrogen ions were observed with a 20 kV. 200 microfarad capacity bank feeding toroidal discharges at a pressure of 10^{-3} mm of mercury Dr Breton described a new and interesting technique in which a broadened line profile is divided into thin slices by the use of successive layers of glass fibres some 50µ in diameter to centres of spaces approximately 55µ apart The assembly is used in a 1-m. focal distance monochromator giving an average dispersion of about 3 Å /mm in the first order and in the visible region Each fibre corresponds to an interval of about 0 15 Å and Dr Breton showed curves giving temporal variations of the profile of H_β, finally, he mentioned experiments on plasma radiation and the drift velocity of ions in a magnetic mirror machine.

J D Craggs

ELECTRON SPIN RESONANCE

On November 2 the Chemical Society held a symposium at Queen Mary College, London, on electron spin resonance In the first part of the symposium there were three papers In an introductory paper, Prof H C Longuet-Higgins outlined the principles of electron spin resonance measurements Only paramagnetic substances show this phenomenon, which arises from their unpaired electrons In a magnetic field the two spin states of a single electron are split apart in energy by virtue of the magnetic moment of the electron An oscillating magnetic field, applied at right angles to the permanent field, causes the electron to jump from one of these levels to the other if the frequency ν of the oscillating field satisfies the relationship

$$h\nu = g\,\beta H, \quad \beta = eh/4\pi mc$$

Here H is the strength of the permanent field and g is a number which equals 2·0023 for a free electron Electron spin resonance measurements acquire their interest from two subsidiary physical effects The first of these is the so-called hyperfine interaction, which arises when the paramagnetic molecule includes magnetic nuclei The value of g then depends on the orientation of all the magnetic nuclei, and a series of lines is observed rather than a single resonance As an example, n equivalent protons will give rise to $n + 1$ hyperfine lines with a binomial distribution of intensity, a single nucleus of spin I (which may be $\frac{1}{2}$, 1, $\frac{3}{2}$, etc) will give a set of $2I + 1$ equally spaced lines of equal intensity The foregoing remarks apply to a paramagnetic molecule tumbling over and over in the liquid phase But if the molecule is fixed in the external magnetic field, the spectrum may be more complicated because of direction-dependent interactions between the electron and the magnetic nuclei The directional variation of the spectrum may then enable one to identify the orbital in which the odd electron is moving

The other physical effect which provides useful information about the nature of the unpaired electrons is spin-orbit coupling An electron with orbital angular momentum is easier to orientate in some directions than in others, and this means that g must be regarded not as a pure number but as a tensor The axes of the g tensor must coincide with the symmetry axes of the molecule. and certain applications of this fact would be discussed later in the symposium

The next paper, by Prof S I Weissman, was concerned with line-widths in electron resonance spectra The width of a line is inversely proportional to the mean life-time of the spin states involved in the transition Measurements of line-broadening can therefore throw light on the rate at which electrons are transferred from one molecule to another (assuming this to be the process which determines the life-time) Prof Weissman gave some examples, including the reaction of the naphthalene molecule with its negative molecule ion and the exchange of a sodium ion between two naphthalene molecule ions As he pointed out, an electron resonance spectrum always supplies kinetic information, whether one is interested in it or not

The third paper, by Prof D J E Ingram, was concerned with the structural information which is provided by electron resonance measurements on single crystals Prof Ingram classified this information under three headings measurements on the g tensor, studies of the hyperfine structure and measurements of electronic splittings (sometimes called zero-field splittings) At Southampton he and his colleagues had determined the orientation of the hæm in groups in myoglobin and hæmoglobin by examining the way in which g varies when the crystal is rotated in the externally applied magnetic field In this particular case there is a very large variation in g, from 2 to 6, so that the orientation of the hæms can be very accurately determined In myoglobin there are two hæms per unit cell and their orientation as determined in this way was afterwards confirmed by the X-ray work of J C Kendrew An interesting molecule is hæmoglobin azide, where the g

tensor is completely anisotropic. J. S. Griffith had been able to determine from this tensor the order and spacing of the 3*d* orbitals on the iron atom; this order indicates very strongly that the histidine group attached to the iron atom is in a plane parallel to one of the diagonals of the hæm plane.

Information can also be obtained from the hyperfine splittings produced by protons or other magnetic nuclei. These splittings can be correlated with the types of group that are present and the angles which they make with one another. In order to extract such information from the data, one must, however, make use of molecular orbital theory, and the same applies to the sort of information which can be obtained from measurements of zero-field splittings.

In the second section of the symposium, four papers were presented.

The first paper, by J. R. Bolton and A. Carrington, was concerned with two topics. The first was the measurement and interpretation of the electron spin resonance spectra of the negative ions of toluene, *p*-xylene and *m*-xylene. These are of special interest because of the degeneracy of the lowest antibonding orbitals of benzene. This degeneracy is removed by introducing methyl groups, and one can predict from the molecular orbital theory which of the two orbitals is likely to be occupied in the ground-state of each ion. The spectra of these xylenes, and of their partially deuterated isotopes, completely confirm the theoretical predictions, and in particular the hypothesis that the methyl groups repel the additional electron.

The other topic discussed by Dr. Carrington was certain remarkable line-width alternation effects which occur in the electron spin resonance spectra of the durosemiquinone positive ion and in the positive ion of 5,8-dihydroxy-1,4-naphthasemiquinone. This effect is almost certainly due to *cis-trans* isomerization proceeding at a rate comparable with the separation (in cycles/sec.) between the hyperfine lines in the resonance spectra. Dr. Carrington also directed attention to the possibility of determining the signs of hyperfine interaction constants by studying the variation of line width as one proceeds from one end of a spectrum to the other.

In the next paper Dr. E. A. C. Lucken described experiments on various substituted *p*-benzosemiquinones and polynuclear aromatic quinones. He used these measurements in attempting to evaluate coulomb integrals and resonance integrals for the various types of atoms and bonds. These attempts were not altogether successful, but Dr. Lucken claimed that his experiments could be interpreted by supposing that the methyl group produced an inductive effect, but did not enter into hyperconjugation. In the subsequent discussion, Dr. Carrington said that he, J. R. Bolton and A. D. McLachlan had obtained strong evidence for hyperconjugation—at least in hydrocarbon cations—from experiments on the positive and negative ions of anthracene and its 9-methyl and 9,10-dimethyl derivatives.

Prof. M. C. R. Symons then gave a lecture on the use of electron resonance in identifying the products of radiation damage in some inorganic oxy-salts. As Prof. Symons pointed out, such work can provide interesting information about the closed-shell species from which the paramagnetic radicals or radical ions are derived. He illustrated this remark with reference to the manganate and hypomanganate ions which result from the addition of one or two electrons to MnO_4^-. Work on damaged crystals is, however, often complicated by the difficulty of identifying the products, especially if the common isotopes of the nuclei have no magnetic moment. Nevertheless, in some cases it may be possible to argue by analogy, as in the series ClO_3, SO_3^-, PO_3^{--}. Prof. Symons described some preliminary work on the oxyions of chlorine, nitrogen and sulphur, and offered an explanation for some apparent discrepancies between the results of various authors who had studied the NO_2 molecule in different environments.

The final paper in the symposium was given by J. R. Morton. When a crystal of L-alanine is irradiated with γ-rays the radical CH_3CHCO_2H is produced. The electron spin resonance spectrum of this radical shows hyperfine structure which varies with the orientation of the crystal and also changes markedly when the crystal is cooled to 77° K. It is possible to analyse this spectrum fully and to obtain the hyperfine coupling tensors for the CH proton and the three protons of the CH_3 group. The radical is found to be planar, with a CH_3—C—H angle of 121 ± 3°. The spectra at 77° K. indicate that the methyl group has ceased to rotate at that temperature, and an estimate of the height of the rotation barrier can be obtained from the spectra at intermediate temperatures.

At the end of the meeting Dr. D. H. Whiffen proposed a vote of thanks to Prof. K. W. Sykes for arranging the symposium and to Queen Mary College, London, for generous hospitality.

H. C. LONGUET-HIGGINS

THE HEALTH OF SEAFARERS

THE total number of seafarers on ships other than those engaged in coastal trading is in the region of 750,000. They form a definite community the members of which are linked together by mutual interest—and health hazards—at least as closely linked as they are to their parent countries. Although much has already been done to improve their working conditions and health standards, much remains to be done. In 1959 the World Health Organization undertook a study of the whole problem of the health of seafarers. As a preliminary fact-finding step, the World Health Organization Regional Office for Europe convened a conference at which it became clear that not enough was known of the health problems of seafarers or of the health services available to them. Following this conference, a questionnaire was drawn up by the Organization in consultation with the International Labour Organization and circulated to member States. The next step was to appoint a consultant to visit some of the major ports to analyse the problem in those ports where services were already in being and to supplement, where necessary, the information given in replies to the questionnaire. The ports visited were Liverpool, London, Marseilles, Athens, Alexandria, Bombay, Singapore, Manila, Hong Kong, Tokyo, San Francisco, Montreal, Helsinki, Gothenburg and Hamburg The findings of this investigation were reported to a Joint Committee on the Hygiene of Seafarers, set up by the two Organizations, that met in Geneva during May 1–4, and are reported in a recent issue of the *WHO Chronicle* (**15**, No 10, October 1961).

There seems to be general agreement that certain complaints occur more often than others, such as diseases of the gastro-intestinal tract, cardiovascular diseases, skin diseases, mental disorders, venereal diseases, tuberculosis and dental complaints The accident-rate is high. Owing to the lack of comparative morbidity statistics it is difficult to ascertain whether accidents and these diseases occur more frequently among seamen than among male groups with the same age distribution in the general population. There is also some national bias in disease incidence Venereal disease and tuberculosis remain common causes of incapacity in some of the developing countries, whereas mental disorders are more common in some of the developed countries There is little information about the number of deaths occurring among seafarers, but accidents seem to be the commonest cause, accounting for more than 50 per cent of all deaths in the merchant service in some countries

In certain countries there are no special hospital or clinic facilities at all for seafarers ashore In these countries seafarers are referred to the general hospital or private medical care facilities available to the public In the United Kingdom, Norway and Sweden, there are a few special clinics and hospitals based on some major ports and organized by the shipping companies and local authorities acting separately or in conjunction Finally, there are a few countries which provide free medical care for seafarers as a special class, in the United States and Canada through the public health service, in Israel by a private company, in Japan by the Seamen's Insurance Association In all cases, continuity of treatment from port to port or port to ship is poor

Some Governments have no definite regulations regarding the provision of a ship's doctor Most stipulate a physician if there are more than 100 persons aboard, and many a nurse or nurses also if passengers and crew exceed a given number A few countries require a doctor for less than 100 persons When, for any reason, no physician is available, medical care is provided by the master or chief officer, occasionally by another designated officer or by a nurse, in many countries the responsible individual is given special training for the work, though such training is not always of a high standard The ships of most countries must carry a medical guide and a medical chest, but the standards of equipment and inspection are sometimes inadequate Some States have no form of health insurance, either for the general public or the seafarer, in others, health insurance is included in a comprehensive scheme In some cases there are special compulsory arrangements for seafarers

Despite the defects reported and the apparent chaos of national regulations and facilities, much has been done to ensure certain minimum standards A great deal of useful work is done by the Red Cross and other voluntary bodies and by the shipping industry itself

Several conventions and recommendations concerning the health of seafarers have been established by the International Labour Organization These deal with medical examinations, medicine chests, medical advice by radio at sea, social security and sickness insurance, and can be extremely helpful to countries developing their health services for seafarers Not all these conventions and recommendations have been universally adopted, or fully implemented where they have been adopted

A good deal can be done towards the prevention of injuries and disease on board ship Attention to design and to lighting at strategic points will help to limit accidents, instruction in the composition and preparation of food is important to prevent gastro-intestinal disease, and periodic medical reviews have their place in the prophylaxis of mental illness, dental disease, venereal disease and tuberculosis The development of a system to produce reliable statistical data to fill existing gaps in the morbidity and mortality statistics on seafarers would be helpful in deciding what type of medical organization should be set up for them on an international basis

There is also the question of hazards on nuclear-powered ships Few of these vessels are in commission at present, but there will be many more The point at issue is not the danger to the public from a major accident, but the specific risks to the crew It is essential to have a skilled man acting as radioactivity monitor, and protective clothing should be available for all crews Periodic examinations for signs of radiation sickness are as necessary as at nuclear plants ashore All nuclear-powered ships should be clearly marked so as to be easily identifiable in case of collision or other accident

EFFECT OF MECHANICAL STRESS ON SOME SOLID-STATE POLYMERIZATIONS

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FOR some time workers in the field of free-radical reactions in the solid state have suspected that an important part of any mechanism will involve crystal imperfections and annealing processes[1-4] In this case it might be expected that mechanical stresses would exert a noticeable effect on the course of the reaction According to Adler's views[1,2], based on different considerations, a compressive stress would lead to an increase in the rate of reaction, since it would tend to reduce the strain-energy at the monomer–polymer interface[1], polymerization usually being associated with a decrease in volume

We have been examining the polymerization of crystalline acrylic and methacrylic acids (m p 12 5° C and 16° C respectively), initiated by ultra-violet light, and hope to report on recent work shortly. The purpose of this communication is to record some novel observations.

The specimens used were in the form of thin layers of non-overlapping crystals, approximately 10μ thick, formed by allowing degassed monomer to freeze in the narrow gap between a flat silica disk and a glass microscope coverslip. The monomer samples had a water content of less than 0·02 per cent. The crystals were formed and contained in a cell fitted with a quartz window and with provision for evacuation and cooling. By the compression of springs suitably located within the cell an even mechanical pressure of about 50 lb./sq. in. could be applied normal to the layer of crystals, at any stage of the reaction, without disturbing the sealing of the cell. The specimens were irradiated by ultra-violet light from a high-pressure mercury lamp, the cell being situated in a thermostat tank at 4° C.

The course of the reaction was followed by observing the crystal birefringence, which decreased during irradiation. A plot of birefringence against time was sigmoid in shape, showing marked autocatalysis for both monomers. No effects other than the decrease in birefringence were observed, at least in the early stages of reaction. The occurrence of polymerization was confirmed by raising the temperature above the melting point of the monomer, when polymeric pseudomorphs of the original crystals could be seen.

In the absence of pressure extensive polymerization of crystalline acrylic acid, indicated by a complete loss of birefringence and absence of liquid formation on warming, was achieved after 10 hr. irradiation. In otherwise identical experiments in which the compressive stress was applied throughout, only a trace of polymer was formed after 20 hr. irradiation, further, observation of the birefringence during this time showed a small decrease in the initial stage with no subsequent change throughout the time of irradiation (Fig. 1, curve *a*).

Fig. 1, curve *b*, shows the effect of application of stress during irradiation. The crystals of acrylic acid had been irradiated without stress for 5 hr., application of compressive stress then caused complete cessation in the decay of birefringence. When this stress was afterwards removed the reaction started again, being preceded by an induction period similar to that observed initially.

Methacrylic acid crystals behaved in an analogous way, with differences in detail. When pressure was applied at the beginning of irradiation the course of the reaction followed that without pressure for about 4 hr., but the rate of decay of birefringence then declined and complete loss of birefringence was only achieved after a period of irradiation exceeding 24 hr. In the absence of pressure complete conversion is attained in a much shorter time (Fig. 2, curve *a*).

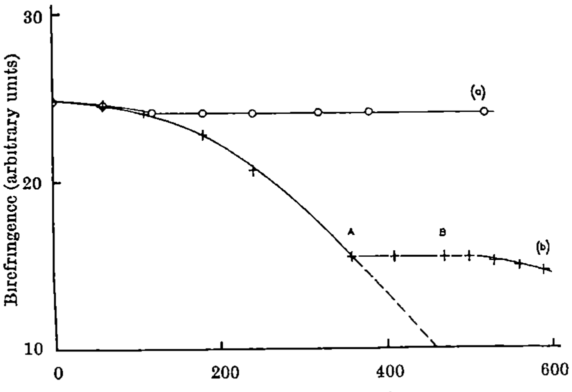

Fig. 1. Polymerization of acrylic acid crystals at 4° C. in ultra-violet radiation. (*a*) Stress applied throughout period of irradiation, (*b*) stress applied at *A*, released at *B*, ---, normal course of reaction without stress

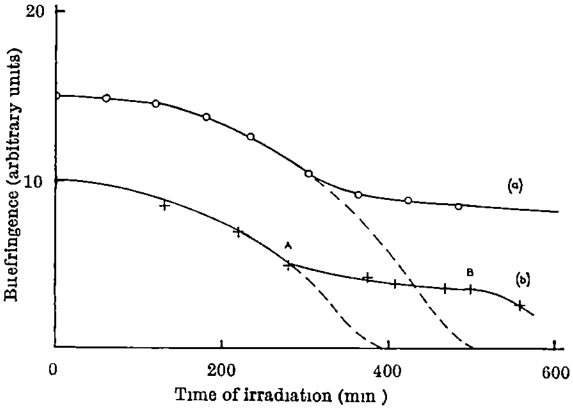

Fig. 2. Polymerization of methacrylic acid crystals at 4° C. in ultra-violet radiation. (*a*) Stress applied throughout period of irradiation, (*b*) stress applied at *A*, released at *B*, ---, normal course of reaction without stress

Application of stress to methacrylic acid crystals during irradiation, at a conversion of about 50 per cent, caused a marked and progressive reduction in the rate of loss of birefringence (Fig. 2, curve *b*), which eventually fell to an extremely small value. Removal of the stress produced an immediate acceleration. There was some indication that the effect of pressure was anisotropic, different crystal aspects being affected to different extents.

These results show that mechanical stress can have a large effect on the photopolymerization of crystals of these acids. The pressures used in this work are relatively very small—about two orders of magnitude less than those required to affect some solid-state phenomena[5]. In this connexion it is relevant to note that the reaction temperature was not far removed from the melting points, acrylic acid, which shows the greatest effect of pressure, was 8·5 deg. C. below the melting point, compared with 12 deg. C. for methacrylic acid. Further, it is apparently necessary for some polymer to be formed before the sensitivity to pressure is developed.

There appear to be three main types of explanation which can be advanced to account for these results. First, a macroscopic second phase—liquid or vapour—may be necessary for polymerization, for example, situated initially between the crystal and the boundary surface, this could be removed by the application of pressure, which might 'squeeze out' the second phase, or cause it to freeze. When the specimens were prepared the liquid was seen to freeze completely, the crystals growing in contact with both boundary surfaces, and no evidence for the existence of such a second phase was ever observed. Further, the effect of pressure on the freezing points of these acids is small, of the order of 1 deg. C. for 100 atm. Secondly, the pressure might remove imperfections, for example dislocations, which are the sites of initiation and propagation. This process might be considered as a sub-microscopic analogue of that described here and would reduce the mobility of the molecules below the limiting value required for polymerization. Either of the above explanations would imply that polymerization occurs in regions where there are discontinuities in preference to the perfect lattice Thirdly, one might suppose that applied stress produces (rather than destroys) imperfections[5], which trap propagating radicals and reduce the rate of polymerization However, the polymer produced itself constitutes an imperfection in the crystal, and since the reaction is autocatalytic one might argue that, in fact, imperfections facilitate the reaction

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SPECIFIC PARTIAL HYDROLYSIS OF NUCLEIC ACIDS IN NUCLEOTIDE SEQUENCE STUDIES

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THE specific partial hydrolysis of the nucleic acid chain is one of the main problems confronting the investigator working on the sequential analysis of the nucleotides in nucleic acids A method for the stepwise degradation of the chain, proceeding from one end and combining enzymatic and chemical procedures, has been developed by Whitfeld[1] and was recently improved by Zamecnik[2] and Cohn[3] Though it can be applied successfully to end-group analysis or to the analysis of short-chain fragments, the method has the same disadvantages as purely enzymatic procedures, namely, insufficient specificity and non-quantitative yields These factors impose a limit of a few sequences

Up to the present, only methods of enzymatic nature have been available for the specific partial hydrolysis of the nucleic acid chain, such as treatment with pancreatic ribonuclease (RNase) and with the ribonucleases specific for adenylic and guanylic acids isolated by Egami[4] We therefore investigated the possibility of modifying nucleic acids by chemical means in order either to achieve a chemical hydrolysis or to modify the known enzymatic degradations Chemical hydrolysis would be made possible if the β-elimination principle described by Linstead[5] could be applied Modification of the mode of action of pancreatic RNase would result if either of the two main pyrimidine bases in ribonucleic acid (RNA) could be removed or changed in such a way that RNase would no longer attack the adjacent 3″-phosphodiester bonds

By this approach one would expect to obtain optimally three or four different hydrolytic procedures yielding fragments of rather small length with overlapping sequences Since nucleic acid chains, often consisting of thousands of nucleotides, contain virtually only four different bases, it would, however, still be impossible to reconstitute the full sequence of a long chain, unless additional methods are devised for obtaining larger fragments

With this in mind we have investigated the reaction of hydrazine and hydroxylamine with RNA and its constituents

Levine and Bass[6] first described the reaction of hydrazine with pyrimidine nucleotides They demonstrated a splitting of the ring in uridylic acid with the formation of free pyrazolone and a urea residue attached to the ribose Witzel[7] used this reaction to prepare nucleoside 5′-phosphates from dinucleoside monophosphates containing uridine 3′-phosphate by degrading the uracil, removing the urea residue by weak acid hydrolysis and cleaving the resulting β-carbonyl phosphoric ester by alkaline hydrolysis

In a kinetic examination of the reaction of hydrazine with the four common nucleotides of RNA at different *p*H values and temperatures, we found, however, that it attacks both uridylic and cytidylic acids to such an extent that it is impossible to obtain a sufficiently specific degradation of either of them.

The work of H Schuster on inactivation by hydroxylamine of biologically active nucleic acids turned our attention to this reagent, which has recently been used extensively as a mutagenic agent[8,9] Preliminary investigations of the reaction kinetics in our laboratory soon proved the superiority of hydroxylamine to hydrazine for our purpose

A salt-free aqueous solution of hydroxylamine in concentrations of 3–10 molar was incubated at different temperatures and *p*H values with the common nucleotides, nucleosides and corresponding bases Reaction-rates were followed spectrophotometrically after electrophoretic separation of the reaction products No reaction was found in the case of adenine and its derivatives, whereas those of guanine and thymine reacted only very slowly under our experimental conditions

Both uracil and cytosine nucleotides and nucleosides are attacked, but the free bases react much more slowly The dependence on *p*H of the reaction-rates of the groups differ appreciably The *p*H optimum for the reaction with uridylic acid lies at *p*H 10 1, whereas that for cytidylic acid is 6 1 Fig 1 gives the reaction-rates, as measured by percentage of

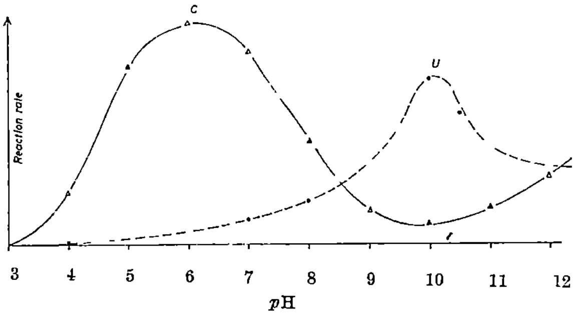

Fig 1 Dependance of rate of reaction of 6 *M* aqueous hydroxylamine with 2′,3′-uridylic acid (U) and 2′,3′-cytidylic acid (C) Reaction-rate measured as percentage of decrease of extinction in 10 min during the initial reaction phase

decrease of extinction during a 10-min interval in the linear phase of the reaction, as a function of the *p*H.

This curve clearly indicates a possibility of degrading cytidylic acid or uridylic acid selectively by controlling the *p*H. This possibility is, however, limited at *p*H 10·0, where cytidylic acid still reacts to some extent. Furthermore, when applied to nucleic acids, this *p*H leads to a slow alkaline hydrolysis of the chain of about 1 per cent per hr. In order to investigate the possibility of increasing the specificity of the reaction, and to evaluate the extent to which RNase action would be modified, an investigation of the chemical nature of the reaction and the reaction products was conducted.

Reaction of Uridylic acid and Uridine with Hydroxylamine

The rate of the reaction is directly proportional to the concentration of hydroxylamine in the range of concentrations investigated. Under our conditions, the reaction kinetics corresponded to those of a pseudo first-order reaction. Products of the reaction were separated electrophoretically and consisted mainly of urea, ribose oxime or its phosphate and an unknown substance with strong ultra-violet absorption. Urea and ribose oxime were identified by comparing the chromatographic and electrophoretic behaviour with standards. The other product was identified as isoxazolone-5, a substance of which only derivatives were known hitherto. Its structure is based on the following data. The electrophoretic mobility and potentiometric titration curve indicate an acid with *pK* near 3·4. The substance is stable only in the anionic form and decomposes rapidly in acid to form a variety of products, among others a hydroxamic acid. The ultra-violet spectrum is in agreement with the proposed structure, with an absorption peak at 247 mμ and a specific extinction of 7·9 × 10^{-3} at *p*H 13·0. Catalytic hydrogenation with Raney nickel or platinum ($Pt[H_2]$) yields β-alanine, ruling out the possible alternative configuration of isoxazolone-3, which would have given β-hydroxypropionic acid amide. Finally, the substance was synthesized from both[1] the sodium salt and the oxime of formyl acetic ester and from propiolic acid ethyl ester by reaction with hydroxylamine (Fig. 2).

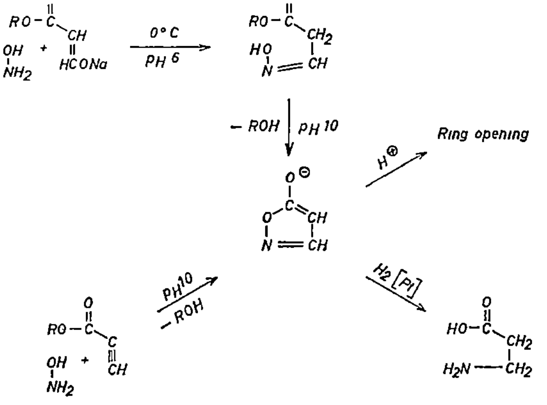

Fig. 2. Synthesis and reactions of isoxazolone-5

The synthetic product was identical in electrophoretic behaviour to the uridylic acid derivative; it also yielded β-alanine on hydrogenation and the acid breakdown products from both were chromatographically identical in four different solvent systems.

The reaction can be formulated in terms of a nucleophylic attack by the hydroxylamine nitrogen on the electrophylic C(4), or by the oxygen on C(6), followed by a cyclization and the formation of isoxazolone-5 and elimination of urea riboside or its phosphate from which the urea is displaced by another molecule of hydroxylamine (Fig. 3).

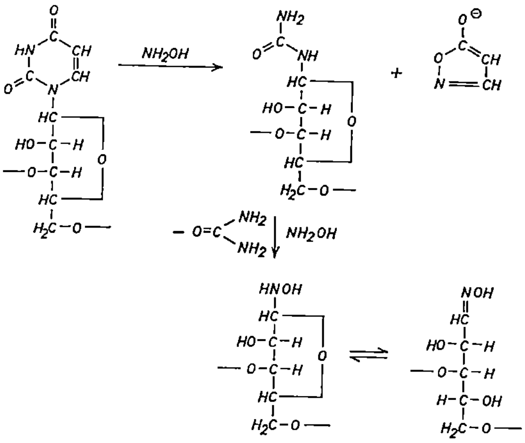

Fig. 3. Reaction of hydroxylamine with uridine and uridylic acid

The substitution of the N(3) atom in nucleosides and nucleotides would weaken its basic character, thereby increasing the susceptibility of C(4) or (6) to nucleophilic attack. This would explain why uracil reacts only very slowly. The slow-down of the reaction above *p*H 10·0 is probably due to the dissociation of the first hydroxyl group of uracil at *p*H 9·5. In the case of isocytosine, where the basicity of the ring is decreased, the rate of reaction with hydroxylamine is accelerated to such an extent that the free base reacts comparably to uridylic acid, also yielding isoxazolone-5.

Reaction of Cytidylic acid and Cytidine with Hydroxylamine

The ultra-violet extinction of cytidylic acid is obliterated within 90 min by treatment with hydroxylamine at the optimum *p*H of 6·0. Electrophoretic separation of this mixture at *p*H 3·5, however, yields mainly a strong ultra-violet absorbing band with a mobility between that of guanylic and uridylic acids. This substance was isolated by preparative electrophoresis on a 'Sephadex' block, purified on a 'Dowex-50' column, crystallized and identified as uridylic acid-6-oxime. The ultra-violet spectrum corresponds to that obtained by Fox and Bendich[10], being similar to that of cytidylic acid in the acid region with a peak at 278 mμ, which disappears at neutral *p*H. Electrophoretic mobility indicates a compound with a negative charge between that of uridylic and cytidylic acids at *p*H 3·5 and containing ribose and phosphate. On hydrolysis in 1 *N* hydrochloric acid for 1 hr at 100° C., the substance yields uridylic acid, which was identified by electrophoretic and chromatographic comparison with a reference, as well as hydroxylamine. The uridylic acid 6-oxime was found to be relatively stable, being hydrolysed only very slowly in acid or alkali at 37° C.

The initial disappearance of the ultra-violet extinction at *p*H 6·0 indicated that the uridylic acid 6-oxime was a secondary product and that some intermediate is involved This intermediate was partially purified by electrophoresis at *p*H 6 0 and could be converted quantitatively into uridylic acid 6-oxime by incubating in *N*/10 hydrochloric acid in a boiling water-bath for a few minutes Bautz and Freese[11] also recently observed this reappearance of the ultra-violet extinction after hydroxylamine treatment of cytidylic acid, but thought it was due to reconversion to cytidylic acid itself

In the presence of an excess of hydroxylamine the intermediate is degraded at *p*H values above neutral, possibly by a similar mechanism to that of uridylic acid breakdown, because two identical end products giving a positive hydroxamic acid reaction with ferric chloride reagent are found No equivalent heterocyclic intermediate could, however, be isolated An analysis of the intermediate yielded a nitrogen/phosphorus ratio of 3 93 1 An assumption that hydroxylamine was added to the 1,6 double bond of cytidylic acid yielding 6-hydroximino-1,6-dihydrocytidylic acid, could explain the spectrophotometric observations and was apparently supported by the demonstration of ammonia, later found to be a contaminant, in the acid degradation product It was not, however, in accordance with the Pullmans' calculation that the C(4) would be the most reactive centre in this reaction[12], and neither could it explain the anomaly between the reaction of hydroxylamine with cytidine monophosphate and with RNA (see below)

Evidence was obtained by Brown[13] that two molecules of hydroxylamine take part in the reaction and that the 4,5 double bond disappears This indicated a two-stage reaction with an initial addition of hydroxylamine to the double bond followed by substitution of the amino group by a second molecule of hydroxylamine Further supporting evidence for this hypothesis was obtained in our laboratory by demonstrating electrophoretically the formation of ammonia in the reaction yielding the intermediate, and of free hydroxylamine during the conversion of intermediate to oxime The reactions described are summarized in Fig 4

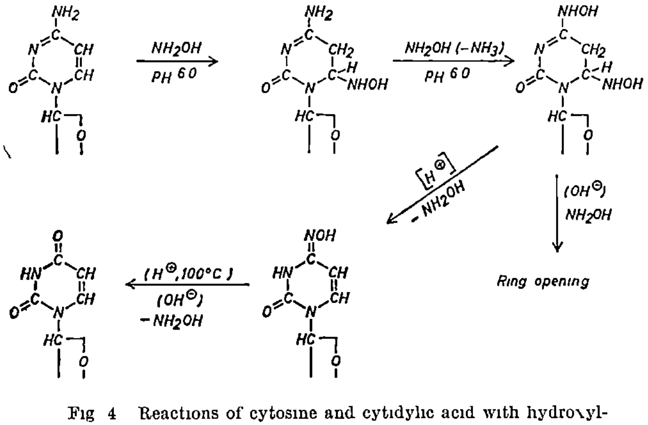

Fig 4 Reactions of cytosine and cytidylic acid with hydroxylamine

Application of the Hydroxylamine Reaction to RNA

Preliminary results indicating the applicability of the reaction to nucleic acids can be reported Experiments were performed on ribosomal ribonucleic acid from *Escherichia coli*, under strict *p*H control at *p*H 10 and 6 with 6 *M* hydroxylamine at 37° C After completion of the reaction, the RNA was precipitated with perchloric acid and parallel samples hydrolysed in alkali and pancreatic ribonuclease respectively Mono- and oligo-nucleotides were separated electrophoretically on 'Sephadex *G* 75'

At both *p*H values the overall reaction-rate was slower with RNA than with mononucleotides, probably due to the secondary structure of the RNA The specificity remains the same, mainly the uridylic acid residue being degraded at *p*H 10, although a certain amount of cytidylic acid breakdown also occurs In the RNase hydrolysate after *p*H 10 treatment, virtually all the uracil monophosphate disappears That RNase does not hydrolyse the chain at the sites where uracil has been removed is more conclusively shown by the marked decrease of cytidine monophosphate as well as of oligonucleotides with electrophoretic mobilities near those with uridylic acid end-groups in untreated RNA hydrolysates During incubation at *p*H 10 for 3 hr , about 5 per cent of the RNA becomes soluble in acid, indicating non-specific alkaline hydrolysis and possibly some hydrolysis caused by a β-elimination reaction

At *p*H 6 0 essentially comparable results were obtained, oligonucleotides with cytidine monophosphate end-groups diminishing markedly and cytidine monophosphate disappearing from the hydrolysate The alkaline hydrolysate of the same material showed no decrease in cytidine monophosphate content, however This can be explained by assuming that secondary structure bonding permits only the addition of the first hydroxylamine molecule to the 4,5 double bond of the uracil residue, thereby changing its aromatic character and inhibiting the RNase action The first-stage intermediate thus formed is then reconverted to cytidylic acid during the alkaline incubation This reconversion could not be obtained with mononucleotides, indicating that the second stage of the reaction, the substitution at C(6), is irreversible

The results of this investigation indicate that it is possible to modify nucleic acids by chemical means in such a way that a defined enzymic partial hydrolysis different from, and of higher specificity than, that obtained without the treatment becomes feasible

The foundation for the development of a chemical partial hydrolysis procedure on the β-elimination principle has also been laid This could be achieved, for example, by adding a suitable amine during the reaction of nucleic acid and hydroxylamine at *p*H 10

The specificity of the reaction, especially at *p*H 10 0, must still be improved The investigation of the reaction of hydroxylamine with nucleic acid, and the possible ways of effecting partial hydrolysis of the product, has only started and is being continued

We acknowledge the support and interest of Prof A Butenandt, in whose laboratory this work was performed Thanks are due to Dr H Schuster for stimulating discussions

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FERROMAGNETIC INCLUSIONS IN NUCLEIC ACID SAMPLES

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BROAD, occasionally very intense, magnetic resonance absorption signals have been observed in a wide variety of preparations of nucleic acids[1-4] The origin of these signals has not been specified, though it has been inferred that large numbers of unpaired electron spins (as many as 10^{21} spins/gm in some cases) are inherently present in these and certain other organic structures of high molecular weight A critical point in these arguments has been the purported absence of inorganic, magnetic impurities in quantities sufficient to explain the observed resonance intensities

Recently it has been found that samples yielding electron-spin resonance spectra similar to certain of those reported earlier exhibit *ferromagnetic* rather than *paramagnetic* behaviour[5] The possibility that the resonance depends critically on the existence of a polymeric organic structure has been disproved since denaturation of deoxyribonucleic acid (DNA)[4] or even heating well above the charring point does not destroy the electron-spin resonance signal[6] Furthermore, trace quantities of iron, the dominant iron-group impurity, have been found in amounts sufficient to explain the observed magnetism if it is assumed that the magnetic ions are present as concentrated ferromagnetic aggregates rather than as disperse paramagnetic centres[5,6] We now wish to summarize a series of subsequent experiments which confirm this hypothesis and which permit a good guess as to the specific iron compound involved as well as its origin

The generality of the phenomenon has been established by examination of a large number of nucleic acid samples as indicated by Table 1 Though a variety of signal shapes have been reported we are here concerned only with the (in our experience) dominant and well-defined absorption line characterized at room temperature by a maximum at g = 0 71444 f/H = 2 15–2 25 (f is the frequency of observation in Mc /s , H, the field for maximum absorption in gauss) and a width between points of largest slope of $\Delta H \simeq$ 1,000–1,500 gauss Though both g and ΔH increase appreciably in magnitude as the temperature is decreased the intensity of the resonance remains constant within experimental error from 300° K down to 20° K at least

The ferromagnetic nature of the phenomenon has been confirmed in the case of the three most strongly magnetic samples by demonstration of remanent moments and coercive forces[5] The static magnetic moments of these samples also proved independent of applied field and ambient temperature over wide ranges as expected Quantitative comparisons between the electron-spin resonance intensities and the static moments gave agreement within the experimental errors Such comparisons for two DNA samples are shown in Table 2

The number of unpaired spins responsible for an observed electron-spin resonance signal depends on whether the spins are paramagnetic or ferromagnetic The ratio of these spin concentrations is

$$\frac{N_{\text{para}}}{N_{\text{ferro}}} = \frac{3kT}{g\beta H_0} \, \frac{S'}{S(S+1)}$$

in which k is the Boltzmann constant, T the temperature, g the observed g-factor, β the Bohr magneton, H_0 the magnetic field, S' the electron spin in the ferromagnetic state and S the spin in the paramagnetic state For $S' = 2$ and $S = 1/2$ this ratio is slightly larger than 4,000 for experiments performed at 300° K with 11 7-kMc /s microwaves The intensity of the nucleic acid absorption signal has been compared with that due to a known concentration of paramagnetic ions in a diamagnetic host (1 per cent Ni^{2+} in $KMgF_3$) in order to calculate the number of paramagnetic spins ($S = 1/2$) required to produce an equivalent electron-spin resonance absorption The comparison can, of course, only be made at a particular temperature, 300° K being chosen in the present work A rough indication of these equivalent spin densities is given in Table 1 More exact numbers are shown for the two strongly magnetic samples in Table 2, as are ferromagnetic spin densities ($S' = 2$) calculated on the basis of the formula given above

Identification of iron as the most likely source of the observed ferromagnetism had been established by X-ray fluorescence analysis[5] (see also ref 4) In Table 2 we present the results of a quantitative analysis of the amount of iron in these samples which was not removed by treatment with versene The concentration of iron is much larger than N_{ferro} Lack of numerical agreement between the two sets of numbers is not an argument against inorganic iron compounds as the source of the ferromagnetism since the excess iron can be accounted for in a number of ways, some of which are mentioned later

Table 1. SAMPLES EXAMINED WITH ELECTRON-SPIN RESONANCE TECHNIQUES

The intensity refers to a specific absorption line described in the text. The criteria, strong, medium, weak, and very weak, correspond crudely to more than 10^{20}, 10^{20}–10^{19}, 10^{19}–10^{18}, less than 10^{18} equivalent paramagnetic electron spins ($S = 1/2$)/gm at room temperature

Sample	Source	ESR intensity
Herring sperm DNA	Nutritional Biochemical	strong
Salmon sperm DNA	California Corp for Bio Res.	medium
Calf thymus DNA *ΣI*	Sigma Chemical Co	weak
,, ,, ,, *ΣII*	,, ,, ,,	strong
,, ,, ,, *ΣII′*	,, ,, ,,	strong
,, ,, ,, *W*593	Worthington Biochem	weak
,, ,, ,, *W*594	,, ,,	weak
,, ,, ,, *W*595	,, ,,	very weak
,, ,, ,, *SK*	Dr L Cavalieri, Sloan-Kettering Institute	weak
Tetranucleotide DNA	Prof A Rich, M I T	medium
Yeast RNA M	Mann Biochemical	weak
,, ,, S	Schwartz Biochemical	weak
Aerobacter aerogenes RNA	Prof A Rich, M I T	weak
Bacteriophage (*T*2)	Prof E Lennox, N Y U Bellevue	weak*
Salmon sperm heads	Dr N Simmons, U C L A	weak*

* See text for qualifications

Table 2 COMPARISON OF SPIN RESONANCE, STATIC MAGNETIC MOMENT AND IRON ASSAYS OF TWO STRONGLY FERROMAGNETIC SAMPLES OF DNA

All listed quantities are per gm of nominally dry material

Sample method	Herring sperm DNA	Calf thymus DNA *ΣII′*
Electron-spin resonance	9 × 10^{20} para $S = 1/2$ 2 2 × 10^{17} ferro $S' = 2$	16 × 10^{20} para $S = 1/2$ 3 8 × 10^{17} ferro $S = 2$
Static magnetic moment	14 × 10^{20} para $S = 1/2$ 3 4 × 10^{17} ferro $S = 2$	6 × 10^{20} para $S = 1/2$ 1 4 × 10^{17} ferro $S = 2$
Bound iron	0 048 weight per cent 5 × 10^{18} atoms	0 049 weight per cent 5 × 10^{18} atoms

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Since the existence of the iron in a separate, probably inorganic phase was suspected, several samples were examined by electron microscopy. Dense platelets were found rather randomly embedded in groups of DNA molecules (Fig. 1). (I. Isenberg has also observed such inorganic inclusions in protamine nucleate by electron microscopy—private communication.) The diffraction pattern of the platelets definitely identifies them as single crystals of inorganic compounds (Fig. 2) though no chemical identification was possible from these discrete Laue patterns taken in arbitrary crystal directions. The relative number densities and average sizes of these inclusions (~ 1000 Å maximum linear dimension) correlates quite well with the relative levels of magnetism found. It has not been possible to remove the platelets by simple filtration of water solutions, filtration after ultrasonic agitation or centrifugation. A variety of quite large inorganic crystallites were brought down by centrifugation and filtration, some of these even proving quite strongly magnetic, however, the intensity of the electron-spin resonance signal in the supernatant DNA and the occluded platelet count were not appreciably changed. It appears that the embedded inorganic particles are tightly bound to the surrounding nucleic acid molecules which serve to protect the particles from chemical attack and drag the nucleic acid 'shroud' with them when mechanical separation is attempted.

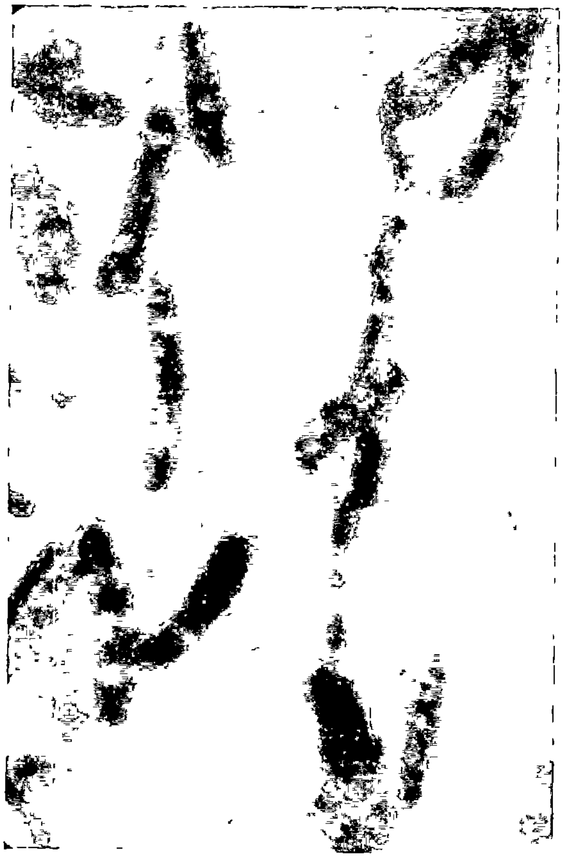

Fig. 1. Electron micrograph (80 keV.) of a nucleic acid sample (calf thymus ΣII) showing crystalline inorganic inclusions in groups of DNA molecules

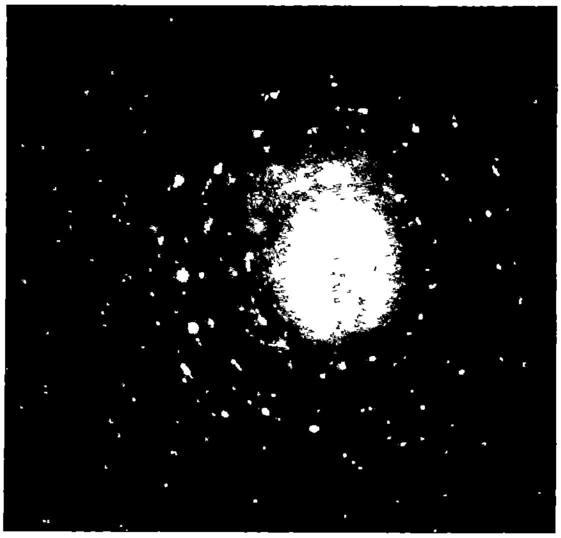

Fig. 2. Selected area electron diffraction pattern (80 keV.) from a group of crystalline inclusions similar to those shown in Fig. 1

Failure to identify the chemical composition of the occluded platelets by electron diffraction and to separate it from its host DNA for chemical analysis led to attempts at magnetic identification. The electron-spin resonance signal of a DNA sample (calf thymus ΣII′) has been observed as the microwave cavity was heated with a nitrogen gas atmosphere present. The spectrum found at 900° K. is quite similar to that seen at 300° K., but the line intensity decreases and the line shape becomes complex at somewhat higher temperatures though it returns to its usual shape and intensity on lowering the temperature. At these high temperatures the DNA is completely charred, once again emphasizing the lack of correlation between the ferromagnetic resonance and the molecular structure of DNA. (A sharp paramagnetic free-radical resonance is observed at $g = 2.00$ after the sample has charred appreciably.) Since the ferromagnetic resonance is still visible at 900° K. we can eliminate Fe_3O_4 as its origin since its Curie temperature is 855° K. While αFe_2O_3 with a Curie temperature of 950° K. appears possible, it must be eliminated since it is a parasitic ferromagnetic material the magnetization of which is too small to account for the observed signal. Also the resonance of αFe_2O_3 disappears suddenly near 260° K. whereas that of our nucleic acid samples does not[7]. This might, however, account for the disappearance of resonance reported by Blumenfeld[1]. (Another alternative in this regard would be Fe_3O_4 since its resonance is reported to broaden quite rapidly below 115° K. (ref. 8).) At present we are unable to suggest a specific iron compound the properties of which exactly parallel the behaviour of our nucleic acid samples. However, we do find a close parallelism in a preparation of colloidal magnetic particles produced by coprecipitation of Fe^{3+} and Fe^{2+} ions from solution[9]. These particles are of nominal composition Fe_3O_4, but evidently differ significantly from macroscopic crystals of that material. It would appear that this oxide-hydroxide of iron is the form responsible for the greater part of the resonances observed in nucleic acid preparations.

The rather low moment per iron atom found in the nucleic acid samples may be due to a number of causes: partial compensation of one magnetic sub-lattice by another (ferrimagnetism), lack of alignment of magnetic moments near the surface of particles due to strong local anistropy, formation of additional weakly magnetic phases, for example, αFe_2O_3, iron hydroxide, isolated ions

The next question is whether or not the iron ions which are commonly found as trace impurities in nucleic acid samples exist in a ferromagnetic form *in vivo*. We examined the resonance spectrum of salmon sperm heads which had been prepared, dialysed extensively and treated with versene by Dr N Simmons When they were first observed no broad electron-spin resonance was observed, in spite of the fact that Simmons's analysis showed a large concentration of iron However, after storage at ~4° C for several months, the electron-spin resonance spectrum was examined again and a weak ferromagnetic resonance was observed In a similar fashion we observed the electron-spin resonance spectrum of a freshly prepared sample of bacteriophage *E. coli T*-2 which was kindly given to us by Prof E. Lennox At first the dried bacteriophage showed several sharp resonance lines, characteristic of paramagnetic impurities However, after several months it too showed a weak ferromagnetic resonance which was not discernible at first.

In our opinion these observations are best explained by the following hypothesis the iron found in nucleoproteins is present in the living cells though not in the concentrated ferromagnetic form The latter, an oxide-hydroxide perhaps approximating Fe_3O_4 in composition, may be precipitated during extraction of nucleic acids or may even form quite slowly in intact but dried organisms The observed clumping of nucleic acid molecules about the inorganic platelets may be evidence for nucleation of the colloidal precipitation at charged sites, for example, the phosphate side groups. That the iron and other trace metals found in nucleic acid samples are indeed present in the biological source is assured by the work of Wacker and Vallee[10]

We wish to acknowledge assistance and advice received from numerous colleagues, in particular Dr. E Lennox, Dr. N. Simmons, H J Williams, R. C. Sherwood, Dr J. P. Wright, Dr J. K Galt, Dr J F Dillon, jun , and Dr. F Vratny

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VARIATIONS IN THE NUCLEAR DEOXYRIBONUCLEIC ACID CONTENT IN THE ADRENAL CORTEX OF THE FEMALE WHITE RAT DURING THE ŒSTRUAL CYCLE

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USING the cytophotometric technique for determination of the mean nuclear deoxyribonucleic acid (DNA) content as a test of cellular activity, one of us[1] investigated the function of the different layers of the adrenal cortex Arguments in favour of the opinion of Deane and Greep[2] were provided According to these authors the zona fasciculata is the glucocorticoid-producing layer, which function is dependent on the adrenocorticotrophic hormone secretion of the adenohypophysis, the zona glomerulosa is the mineralo-corticoid-producing layer and independent of the hypophysis The zona fasciculata interna and the zona reticularis are considered to be reserve layers. In the present investigations we have investigated the nuclear DNA content in the adrenal cortex of the female white rat during the different stages of the œstrual cycle It has already been shown by several authors[3-6] that the adrenal weight increases during œstrus. Hunt[7] has observed variations of the mitotic activity in the zona glomerulosa during the œstrual cycle

In a series of female white rats the œstrual cycle was determined by observing vaginal smears during two weeks After this period 5 animals in diœstrus, 4 in proœstrus and 5 in œstrus were selected and killed The adrenals were then fixed, dehydrated, embedded, sectioned and stained by the Feulgen reaction. The DNA content of about 50 individual nuclei in every layer of the cortex was determined by means of the cytophotometric technique of Lison[8]. Table 1 shows a survey of the pooled results

The distribution of the nuclear DNA in the different layers during diœstrus is very similar to that observed in the male cortex. This content is indeed significantly lower in the zona fasciculata interna and reticularis than in the zona fasciculata externa ($P < 0$ 001) In the zona glomerulosa, however, this content is 6 per cent lower than in the fasciculata externa ($P < 0$ 001) In the male rate the DNA content is generally the same in these two layers. In a few instances only, a lower content was observed in the zona glomerulosa[1]. In proœstrus the general distribution is preserved, but in every layer the DNA content is decreased This decrease is not,

Table 1

		$\bar{x}$	$\log \bar{x}$	s	$s_{\log \bar{x}}$	n
Diœstrus	*G*	1,093	3 0387	0 0841	0 0053	246
	F E	1,159	3 0643	0 0622	0 0039	245
	F I	1 048	3 0207	0 0614	0 0039	247
	R	1,082	3 0344	0 0684	0 0043	246
Proœstrus	*G*	982	2,9920	0 0584	0 0041	195
	F E	1,079	3 0331	0 0687	0 0048	198
	F I	1,006	3 0027	0 0572	0 0040	197
	R	978	2 9904	0 0631	0 0044	200
Œstrus	*G*	1,272	3 1045	0 0681	0 0043	243
	F E	1,159	3 0641	0 0618	0 0039	244
	F I	1,197	3 0781	0 0503	0 0032	247
	R	1,226	3 0955	0 0542	0 0034	247

G , zona glomerulosa, *F E* , zona fasciculata externa, *F I* , zona fasciculata interna, *R* , zona reticularis

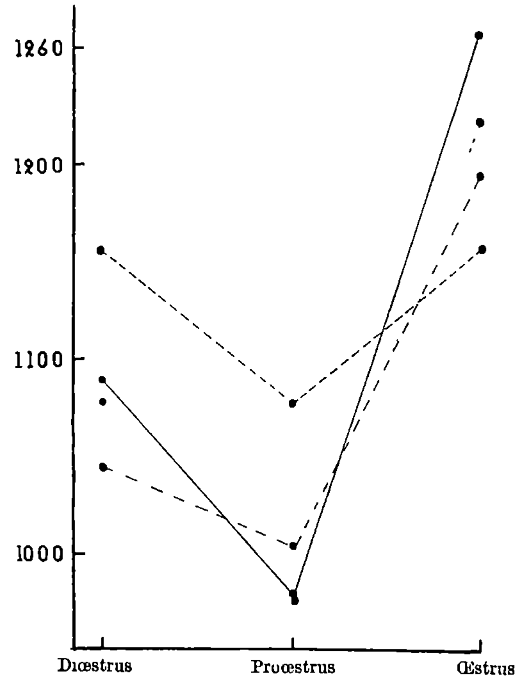

Fig 1 Variations in the nuclear DNA content in the different layers of the adrenal cortex during the œstrual cycle ---, zona fasciculata externa, ——, zona glomerulosa, , zona reticularis, —·—, zona fasciculata interna

however, significant in the zona fasciculata interna In œstrus the nuclear DNA distribution is strikingly changed. The differences between the zona fasciculata externa and the other layers are reversed In comparison with the proœstrus the DNA content in the zona glomerulosa is increased by 29 per cent and exceeds the zona fasciculata by 9 per cent The DNA content of the zona fasciculata externa returns to the value observed during diœstrus The DNA content of the zona fasciculata interna is increased by 19 per cent and by 27 per cent in the zona reticularis Fig 1 summarizes these variations during the œstrual cycle

The decreased DNA content in the four layers during proœstrus suggests a general inhibition of cell activity during this period. We have not found any biochemical and physiological observation in the literature until now which could support this conclusion The most prominent variations are found, however, during œstrus The increased DNA content in the zona fasciculata interna and reticularis suggests a stimulation of the corticosterone secretion. This result fits in with the already observed increase in adrenal weight[3-5] during œstrus and after œstrogen treatment[6] and with the increase in nuclear DNA in the zona reticularis of the male rat after œstrogen administration[1], it is also in agreement with the biochemical analysis of ketosteroid excretion during the œstral cycle[9] The changes of the nuclear DNA content develop within the very short time of one day. Very striking, also, is the increase of 29 per cent observed in the zona glomerulosa during œstrus This would indicate a stimulation of the aldosterone secretion Hunt[7] has already observed an increased mitotic activity in this layer Only a few biochemical data are available at present to explain this observation Gormall, Robertus and Laidlow[10] found a rise of aldosterone excretion after œstrogen treatment in one patient Our results fit in with earlier observations on the influence of stilbœstrol on the cortex of guinea pig and hamster[5]

These observations are once more in contradiction to the general agreement of the constancy of nuclear DNA and show that the variations of the DNA content may develop within a very short time in correlation with cell-activity

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EFFECT OF PLANT GROWTH COMPOUNDS ON UPTAKE OF AMINO-ACIDS BY PLANT ROOTS

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DURING an examination of the uptake of amino-acids by plant roots[1], it was noted that the herbicide 2,4-dichlorophenoxyacetic acid affected the amount of amino-acid taken up Since other workers[2-4] have reported effects by indole-3-acetic acid or 2,4-dichlorophenoxyacetic acid on uptake of solute it was decided to examine this effect further

Seeds sterilized with a dilute hypochlorite solution were germinated and grown in the dark at 25° under sterile conditions in Petri dishes containing filter paper moistened with a dilute salt solution After 5 days the roots were excised, washed, and blotted dry for weighing into 50-ml Erlenmeyer flasks or in Warburg vessels The flasks contained 0·02 *M* sodium phosphate solution, *p*H 5 2, 2 m*M* radioactive amino-acid and any additions as required The flasks were incubated in air for 2 hr at 25°, the roots washed free of medium and extracted with 80 per cent ethanol The radioactivity released from the roots was determined by drying an aliquot on an aluminium planchet and counting under a thin-window gas-flow counter Full details of the methods used are reported elsewhere[1]

The effect on glycine transport into mustard roots by several plant growth substances is shown in Table 1 In this experiment the roots were incubated for 45 min in 5×10^{-5} *M* solutions of the hormones before adding the labelled glycine.

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Table 1 EFFECT OF PLANT GROWTH SUBSTANCES ON UPTAKE OF GLYCINE BY MUSTARD ROOTS

Growth substance	Relative glycine uptake (percentage of control)
Indole-3-acetic acid	84
Indole-3-butyric acid	56
Indole-3-propionic acid	57
Ethylindoleacetate	94
Gibberellic acid	99
Maleic hydrazide	88
Furyl acrylic acid	8
2,4-Dichlorophenoxyacetic acid	39
2,4,5-Trichlorophenoxypropionic acid	16
2,4,5-Trichlorophenoxyacetic acid	29
Naphthalene acetic acid	55
β-Naphthoxyacetic acid	55
Methylnaphthaleneacetate	61
Naphthalene acetamide	104

Roots were incubated in 0 02 *M* sodium phosphate solution, *p*H 5 2 with 2 m*M* glycine-1-^{14}C Growth substances were added to give a final concentration of 5×10^{-5} *M*, 45 min prior to adding the radioactive glycine

The most effective inhibitor was furyl acrylic acid followed by the commonly used herbicides 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid and 2,4,5-trichlorophenoxypropionic acid Indolebutyric, indolepropionic, naphthaleneacetic and β-naphthoxyacetic acids, and methylnaphthalene acetate all reduced uptake by 40–50 per cent. Indoleacetic acid, ethyl indoleacetate, gibberellic acid, maleic hydrazide and naphthalene acetamide had little or no effect.

The inhibition by 2,4-dichlorophenoxyacetic acid was not confined to the uptake of glycine by mustard roots Under the same conditions as in Table 1, this herbicide reduced transport of methyl labelled L-methionine into mustard by 50 per cent and glycine transport into wheat roots by 40 per cent

Different concentrations of 2,4-dichlorophenoxyacetic acid were tested for inhibitory action (Table 2) At concentrations of 10^{-5} *M* and less, there was either no effect or a slight stimulation of glycine transport and respiration At 10^{-4} *M*, oxygen consumption was unaltered while glycine uptake was reduced by 26 per cent At 2×10^{-4} *M*, oxygen uptake was lowered by 18 per cent and glycine transport by 52 per cent At higher concentrations, both glycine and oxygen uptake were markedly reduced

Table 2. EFFECT OF DIFFERENT LEVELS OF 2,4-DICHLOROPHENOXYACETIC ACID AND 2,4-DINITROPHENOL ON UPTAKE OF GLYCINE BY MUSTARD ROOTS

Addition to medium	Concentration of addition (*M*)	Relative uptake (percentage of control)	
		Glycine	Oxygen
	—	100	100
2,4-Dichlorophenoxyacetic acid	10^{-3}	18	18
	5×10^{-4}	27	38
	2×10^{-4}	48	82
	10^{-4}	74	100
	5×10^{-5}	109	109
	10^{-6}	97	118
	10^{-7}	99	110
2,4-Dinitrophenol	10^{-4}	10	20
	10^{-5}	43	124
	10^{-6}	97	94

Roots were incubated in Warburg flasks in 0 02 *M* sodium phosphate *p*H 5 2, 2 m*M* glycine-1-^{14}C and 2,4-dichlorophenoxyacetic acid or 2,4-dinitrophenol at the concentrations given above The flasks were shaken for 2 hr at 25°, the gas phase being air

These results can be compared with those found using 2,4-dinitrophenol, where glycine transport was lowered despite a stimulation in respiration This suggests the possibility that the actions of 2,4-dichlorophenoxyacetic acid and 2,4-dinitrophenol are similar and perhaps due to effects on oxidative phosphorylation

Preliminary experiments with the incorporation of phosphate-32 into root nucleotides provide supporting evidence for an effect on oxidative phosphorylation by 2,4-dichlorophenoxyacetic acid as reported by Switzer[5] and Khubutiya[6]

It is reasonable to conclude that the reduction of amino-acid transport into plant roots caused by 2,4-dichlorophenoxyacetic acid is probably due to reduced levels of adenosine triphosphate, since uptake of amino-acid is known to be an energy-requiring process[1]

This work was supported by a grant from the National Science Foundation I wish to thank Prof J. H Quastel for suggesting the topic and for his guidance.

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TESTS FOR LIVER DYSFUNCTION IN DOGS

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THE reason for undertaking this investigation was to try to select a few simple biochemical techniques which would enable the veterinary surgeon to gain useful information about the state of the patient's liver without having to resort to laparotomy or biopsy

The pattern of the work had to be governed by the material available, which was provided, for the main part, by the Beaumont Animals Hospital It was not considered practical to undertake any test which necessitated even the temporary hospitalization of the patient, or the preliminary intravenous injection of any substance (for example, sulphobromphthalein) Therefore, tests had to be developed which could be carried out on a single blood sample

Serum Alkaline Phosphatase Estimations of serum alkaline phosphatase were carried out on 463 samples, and the normal value was found to be 8 King Armstrong (K.A) units In dogs, a species in which extrahepatic obstructive jaundice would appear to be a less common clinical entity than in humans, very high values were given by hepatocellular jaundice, for example, a value of 120 K A units was found in a case of leptospiral jaundice, and it is an interesting speculation that the pathology of the hepatic cell of the dog may differ from that of man, possibly showing a greater tendency to undergo cloudy swelling and thus to produce intrahepatic obstruction.

Raised serum alkaline phosphatase values were also found in young animals undergoing active growth of bone tissue, and in conditions in which there was an interference with calcium and phosphorus metabolism, that is, bone lesions (9–90 K A. units), and some cases of nephritis (9–60 K.A. units), cystic calculi (10–40 K.A. units) and enteritis (9–100 K.A. units). My experience of this test showed that 95 per cent of dogs with definite liver involvement gave positive results, and so did 75 per cent of dogs with suspected liver involvement. It also indicated the main disadvantage of the test, which is that high values are given by conditions which do not involve the liver (*vide supra*).

Serum Transaminases. Serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase.

Normal values were calculated from estimations carried out on the sera of 11 young healthy dogs, and were taken as follows: serum glutamic pyruvic transaminase less than 20 Sigma Frankel units, serum glutamic oxaloacetic transaminase less than 30 Sigma Frankel units. These values were comparable with those of Cornelius, Bishop, Switzer and Rhode[1], and Malherbe[2]. The results of estimations on more than 70 different clinical cases indicated that increases of transaminase activity, especially serum glutamic pyruvic transaminase (Hoe[3]), are reliable indications of early liver cell damage, before it is indicated by other tests. For example, in four puppies which had been inoculated with the virus of hepatitis contagiosa canis, considerable increases in serum glutamic pyruvic transaminase could be seen 5 days after inoculation while other liver function tests remained normal. Serum glutamic oxaloacetic transminase was also raised, but not generally as significantly.

Total Serum Cholesterol. Total serum cholesterol values were calculated for two groups of normal dogs, household and kennel. Normal values were found to be: household dogs, 258·0 ± 36·3 mgm/100 ml.; kennel dogs, 186 ± 13·9 mgm/100 ml. It was thought that these different values were probably caused by dietary differences, the average domestic pet living in the house receiving a diet considerably higher in cholesterol than that fed to most kennel dogs. In view of these findings, cholesterol values greater than 299 mgm/100 ml. were taken as raised.

The estimation of total serum cholesterol was carried out on 250 sera. Although raised values were found in only 57 per cent of jaundice cases, this estimation would appear to be of considerable use in the diagnosis of other diseases, including nephritis, most cases of which gave values in the 300–499 mgm/100 ml range. Raised values were also encountered in all cases of diabetes mellitus (310–1,360 mgm/100 ml.), and also in two animals which afterwards developed the disease, but which were not showing glucosuria at the time the serum was first examined; presumably this indicates a general disturbance in fat and carbohydrate metabolism found in the pre-diabetic patient. Two cases of hypothyroidism were encountered, and one of these was particularly interesting. When presented at the clinic, this Alsatian puppy was 9 months old, measured 25 cm high and weighed 3·6 kgm. A diagnosis of hypothyroidism was made when the total serum cholesterol value was found to be 700 mgm/100 ml. This value decreased to the normal level when thyroid therapy was instituted.

Serum Uric Acid. About 70 per cent of the 240 cases examined during this survey gave uric acid values less than 0·5 mgm/100 ml., and only 11 per cent were higher than 1 mgm/100 ml. If one compares the relative merits of serum alkaline phosphatase and uric acid estimations as tests of liver function it can be seen that uric acid is not such a good index as serum alkaline phosphatase. While 95 per cent of the cases with the highest serum alkaline phosphatase levels showed some liver involvement, the corresponding figure for uric acid was only 75 per cent; also, while all cases of jaundice gave raised serum alkaline phosphatase values 86 per cent showed increased serum uric acid. 46 per cent of the cases of nephritis examined lay within the range 0·5–0·99 mgm/100 ml. It was noticed during this work that high uric acid figures frequently appeared to carry a poor prognosis for the patient, and hence were found in most moribund animals.

Serum Bilirubin. This estimation was carried out on some 300 sera, but much difficulty was experienced in evolving a technique which was sufficiently sensitive to measure the very low concentrations of bilirubin found in the sera of non-jaundiced dogs. The most suitable method seemed to be that of Powell[4]. No attempt was made during this investigation to differentiate between direct and indirect bilirubin. Accurate measurement of the small quantities involved presented so much difficulty that it was not deemed worth while attempting the more complicated techniques which would have been necessary, especially as hæmolytic jaundice does not seem to be of much practical importance in dogs in the United Kingdom.

It was also noted that dogs showing bilirubinuria did not in many instances show bilirubinæmia. It has been stated[5] that about 20 per cent of apparently normal dogs excrete bilirubin in their urine. This finding has been confirmed by Wilkinson (personal communication, 1960).

Serum Proteins. Relative percentages of serum proteins for normal dogs were calculated after electrophoresis had been carried out on the sera of 26 young healthy dogs living under similar conditions. The method of Jencks, Jetton, and Durrum[6] was used and results were as follows: albumin, 57·2 ± 0·95, α_1-globulin, 6·2 ± 0·37, α_2-globulin, 9·7 ± 0·46; β_1-globulin, 8·8 ± 0·54, β_2-globulin, 9·0 ± 0·37, γ-globulin, 10·4 ± 0·55, albumin globulin ratio, 0·76 ± 0·087. Electrophoresis was also carried out on the sera of 140 dogs suffering from various diseases. Albumin decreased in most of the diseases investigated and this change was reflected in the albumin globulin ratio. Changes in α_2-globulin appeared to be non-specific. β_1-Globulin showed a significant increase in most of the neoplasia cases, but was decreased in all cases of jaundice and the majority of diabetes mellitus cases. β_2-globulin was a variable factor in most of the conditions studied but was always raised in jaundice. γ-Globulin was raised in jaundice and cirrhosis, but decreased in more than 90 per cent of the cases of diabetes mellitus, and it was felt that this was of some significance in view of the fact that such poor resistance to infection is shown by most human diabetic patients. Cirrhosis gave a characteristic electrophoresis diagram with poor resolution in the β- and γ-bands.

During this part of the investigation an attempt was made to establish a relationship between the results of the Kunkel turbidity test and the relative percentage of the γ-globulin as determined by electrophoresis. There was a significant correlation between the two factors, but the scatter about the regression line was too great to permit an accurate evaluation of γ-globulin by this method. It seems, however, that the Kunkel test can be used semi-quantitatively and this is in general agreement with the findings of most workers in human medicine.

The Takata Ara test appeared to be less specific than the Kunkel, and gave positive turbidities and flocculations for increases in any of the globulin fractions From my work on these tests it appears that in the absence of a positive Kunkel, a positive reaction with the Takata Ara may indicate an increase in the globulin fractions other than the γ-globulin

The most useful tests investigated seem to be serum alkaline phosphatase, the transaminases and electrophoresis, all of which can be performed on a a total of 0 9 ml. of serum. When it is possible these tests should be supplemented by the estimation of serum cholesterol, which can give useful information in cases of nephritis, pre-diabetes and in hypothyroidism

The estimation of serum alkaline phosphatase has been shown to be a useful index of liver function, and when used with discrimination it will yield more reliable information than that given by serum bilirubin estimations, except in the case of hæmolytic jaundice The transaminase tests (especially serum glutamic pyruvic transaminase) play an important part in the diagnosis of active liver cell damage and necrosis before any abnormality is shown by any other liver function test. Electrophoresis is of considerable value, especially in the diagnosis of cirrhosis.

Practical details of this work are published elsewhere (Hoe and Harvey[7,8])

I wish to thank Dr D G Harvey for advice and help throughout this work, also Mr G C Knight and my other colleagues in the Departments of Medicine and Surgery for the provision of clinical material I would like to express the sincere gratitude I feel to the late Mr. A. B MacIntyre, whose kindness and inspiration did much to further the work I am indebted to the Trustees of the Clement Stephenson Fund for a scholarship

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CHANGES OF BLOOD VOLUME IN THE PRE-HIBERNATING AND DEEP-HIBERNATING HEDGEHOG

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IN hibernating animals the blood volume is decreased during the hibernation sleep Although everyone who has been working on the physiology of hibernation is familiar with this decrease in blood volume, by simple observations, only scanty data on the blood volume of these animals appear in the literature[1,2]

In this Laboratory we have measured the blood volume of the common European hedgehog, *Erinaceus europaeus*, L at normal body temperature (about 35° C) as well as at low temperatures (about 5° C) The measurements were made by way of the ordinary dilution method using Evans blue The injections were mostly given into the inferior vena cava, and the samples mostly taken from this vessel.

By this method we found a marked decrease in blood volume in the hibernating hedgehog In the 'summer animals' we found the blood volume to be about 8 per cent of the animals' weight, while in the sleeping 'winter animals' the blood volume was found to be about 2 –3 per cent of weight (Fig 1)

The question is raised as to how this decrease takes place To find the answer to this question several analyses are made on the normal blood constituents of the animals, in different stages of hibernation and in the awakened state Further, the weight of the spleen is measured in the same periods when looking for any connexion between blood volume and the well-known function of the spleen to accumulate erythrocytes Finally, the most common constituents of the urine are measured in order to see if dehydration plays any part in the regulation of blood volume

In this discussion it is important to know what we mean by the term 'different stages of hibernation' As the term is being used by me it signifies three different periods of hibernation the pre-hibernating period, the deep-hibernating period, and the period of arousal

In the pre-hibernating period the body temperature is altering from the normal level (about 35° C) to hypothermal levels (about 5° C.) and vice versa, in about 24 hr Such variations in body temperature may take place about five to ten times during the period. The length of this period is about two months, in our animal room taking place in October–November The same tendency of variation in body temperature in hibernating hedgehogs has been reported previously[3]

In the deep-hiberating period (from about December to about March) the body temperature is rather constant, at about that of the room. In the period of arousal the same variations in body temperature as those taking place in the pre-hibernating period, are never observed in this Laboratory The end of

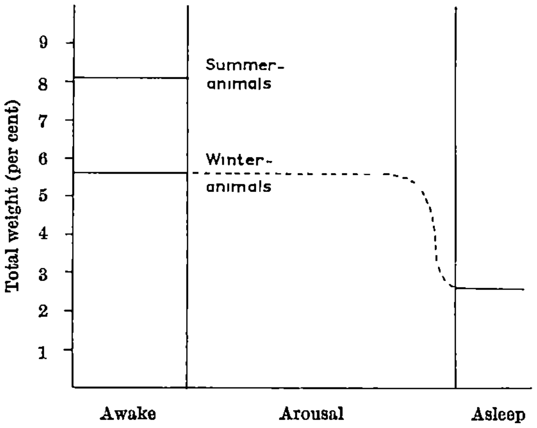

Fig 1 Blood volume—measured as 'Evans blue volume'—of hedgehogs awake, during induced arousal, and in deep hibernation

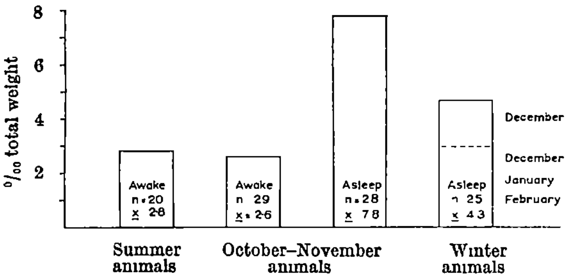

Fig 2 The weight of the spleens in pro mille of body-weight, from hedgehogs in the summer, autumn and winter months

n No of animals, *x*, mean value of weights The diagram representing the spleen weights of the winter animals is divided into two parts by a stippled line. The lower part gives the situation of the spleen weights from animals in deep hibernation, while the upper part gives the corresponding data from animals entering the deep hibernating period

this period is about April, and from that time onwards the animals are named 'summer animals'.

I have measured a great number of spleens from different animals throughout the greater part of a year (Fig. 2) Contrary to my expectations I found the weight of the spleen to be by far the greatest in the animals asleep in the pre-hibernating period, while the weight of the spleen in deep-hibernating animals was nearly the same as that found in the 'summer animals' I take this increase in weight of the spleen as indicating an accumulation of red cells in this organ, and this accumulation as being at its maximum in the sleeping, pre-hibernating animals

Effects of this accumulation of red cells in the spleen on the hæmatocrit and red cell number were measured, and here again the results were rather conspicuous, as both hæmatocrit and red cell number were decreased for 'October–November animals' as compared with the 'winter animals', being almost the same as those for 'summer animals' This change in red cell status during the pre-hibernating period is the same whether the animals are awake or asleep. It seems as if the course of events is not exactly the same in this period, comparing the status of red cells in the blood and the accumulation of red cells in the spleen, since in the latter case there is a great difference in the weight of the spleen when the animals are awake and asleep (Fig 2)

This apparent discrepancy provides the key to further considerations. The possibility exists that a destruction of red cells takes place in the pre-hibernating period, and that the spleen acts as an intermittent reservoir in this period, taking over parts of red cells from the circulation for further transport to the macrophage cells in the liver This would explain why the weight of the spleen is greatest in the pre-hibernating period and afterwards decreases Further, it would explain the decrease in the hæmatocrit and the red cell number in both the awake and sleeping 'October–November animals'. Finally, the decreased blood volume observed in awake 'winter animals' would be explained by the destruction of red cells

Now, the possibility exists that the decrease in hæmatocrit, red cell number, and blood volume could be explained in other ways than by simple destruction of red cells, for example, by accumulation of red cells in some parts of the circulatory system exclusive of the spleen

The following experimental observations provide an answer to these considerations A series of blood-volume measurements—combined with parallel hæmatocrit determinations—has been made on animals during induced arousal from the deep-hibernating state. The animals were aroused by taking them from their room (at about 5° C.) to the laboratory (at about 20° C.). Within 12–24 hr the animals were awake at about 35° C. body temperature, and blood-volume determinations were made according to the Evans blue method mentioned here. The results are given in Fig 1, where the fully-drawn lines give the blood volumes of sleeping and awake animals, and the stippled line the blood volume during different stages of arousal. In Fig 1 attention is directed to the following: (1) The blood volume of awake 'winter animals' does not reach the same level as that of the 'summer animals'. About one-third of the blood is lost (2) There is a rapid increase in the blood volume in the early stages of arousal, reaching its final level at the very beginning of this phase

From (1) the conclusion may be drawn that the decrease in blood volume in the awake 'winter animals' is due to simple destruction of red cells The suggestion that red cells might accumulate somewhere in the circulatory system of the awake animals does not seem to be very likely in the case referred to, where heart-rate and blood pressure are of the same order in the awake 'winter animals' as in the 'summer animals'[4]. The destruction theory is the more probable as we have observed a pronounced increase in the bile pigment in serum, urine and fæces in the animals in the pre-hibernating period (and to some extent also in the animals in the beginning of the deep-hibernating period). Further, in some of the 'October–November animals' we found the lowest hæmatocrit values ever observed during the course of the present experiments, and these values reveal a decrease in red cells (other things being equal) of about 30 per cent

However, the observed loss of blood by destruction of red cells does not tell the whole story about the decrease of blood volume observed in the sleeping 'winter animals' (Fig 1).

Most likely, the data obtained are a consequence of the method used At low heart-rates the blood flow is extremely slow and the injection of Evans blue in the circulatory blood, and more particularly the distribution of the dye in the plasma, takes a great deal of time Now, there is a limit to this distribution, due to the loss of Evans blue from the blood by diffusion In the experiments referred to in this article the limit was about 3 min in the deep-hibernating animals It seems rather likely that a full distribution does not take place all over the circulatory system during this time (and under this condition), and we should therefore like to name the volume calculated from the experiments on deep-hibernating animals 'the Evans blue volume', rather than the exact blood volume When increase in heart-rate during arousal and as a consequence a more rapid blood flow is obtained, the distribution of the dye should be more quantitative and 'the Evans blue volume' calculated should increase to the value of the true blood volume (Fig 1, 'arousal').

Now, red cells represent only half the blood What about the rest of the blood lost, or more particularly, the plasma ? Here again the results obtained by hæmatocrit determinations are of importance In the pre-hibernating period, we were much surprised to find a rather great scattering of the values, with variations from 36 as a minimum to 56 as a maximum However, as more results were obtained both from animals in this period and the following deep-hibernating period, the scattering in the hæmatocrit (as well as in the red cell number)

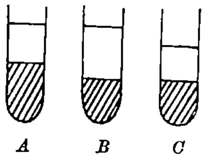

Fig 3 The relation between plasma and red cells *A*, in 'summer animals', *B*, in 'October–November animals', *C*, in 'winter animals'

became more easily explicable. The following hypothesis is based on these results as well as those on blood serum and urine obtained by experiments going on in this Laboratory at the time, but not referred to here

With regard to the blood status during the prehibernating period, two processes are going on with some lag in time. The first one is the destruction of red cells already discussed, and thereupon follows an excretion of plasma substances (water, salts, etc) In the very beginning of the period only destruction of red cells is going on, and the hæmatocrit decreases (Fig. 3). This should explain the lowest values obtained and referred to here. Afterwards the excretion of plasma begins, and the hæmatocrit increases until the normal values are reached, as in the deep-hibernating animals The net result is a decrease in total blood volume

My answer to the question raised at the beginning of this article should be then that the blood volume is decreased mainly by way of red cell destruction and ensuing plasma excretion The spleen is functioning as an intermittent reservoir regarding the accumulation—and perhaps also the destruction—of red cells 'The Evans blue volume' reported in this article is about one-third of the total blood volume in 'summer animals', but must be considered as a false datum More likely this volume reveals pronounced differences in blood-flow in different vascular areas

At this stage further questions arise, and more detailed determinations are called for, the most prominent ones being (1) the part played by the kidney in the regulation of blood volume observed, (2) the determination of the life-time of red cells both in 'summer animals' and 'winter animals'; (3) the proportion of new and old red cells in the same animals, (4) investigation on complement components during the whole cycle of hibernation, to look for any connexion between these components and the destruction of red cells observed

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ROLE OF WATER-REGULATING MECHANISMS IN AMPHIBIAN MORPHOGENESIS: A QUANTITATIVE HYPOTHESIS

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WATER is the only constituent of the amphibian embryo taken up in appreciable quantities from the environment during the course of development, and it accounts for almost three-quarters of the volume of the fully formed embryo Growth of the embryo and changes in its shape would therefore be expected to involve the uptake or redistribution of water. In the development of *Xenopus laevis*, for example, variations in water content account for almost all the changes in volume that occur, and moreover the water taken up is not uniformly distributed throughout the embryo It is taken up first into the blastocœl, then into the archenteron and, finally, after the latter has collapsed, into the cells themselves[1] This can be demonstrated by measurements of the density of the embryo and of its weight in water (reduced weight) The latter remains almost constant until the early tail bud stage, hence all but a small proportion of the volume changes which occur must be the result of water uptake. (Any change in density of the other embryonic constituents would produce a corresponding change in the reduced weight) A similar conclusion was reached by Rappaport in his work on the growth of explanted amphibian tail buds[2]

Fig 1 shows the volume of intact *Xenopus* embryos of different ages calculated from the density measured in a density gradient, and the reduced weight, measured on a Cartesian diver balance. It will be noticed that the rate of water uptake increases with age until the eighteenth hour, when there is a sudden decrease in volume resulting from the spontaneous emptying of the archenteron cavity. A similar plot of cell volume (calculated from the reduced weight and the density of opened embryos minus the contents of their intercellular cavities) illustrates the change in distribution of water between the cells and the intercellular cavities (Fig 1, open circles)

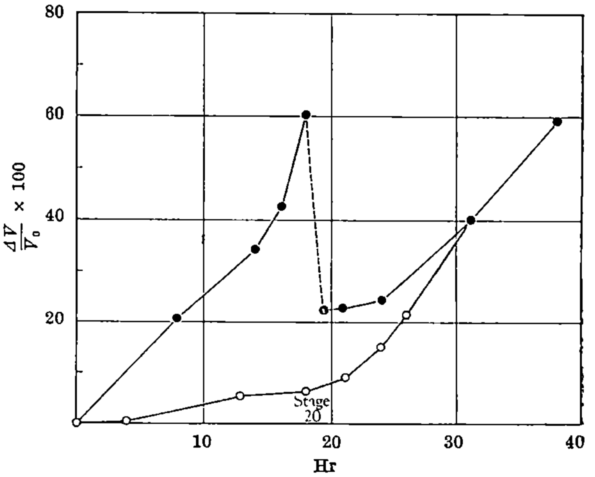

Fig 1 Changes in volume $\frac{(V_t - V_0)}{V_0}$ of *Xenopus laevis* embryos (open circles) and their cells (closed circles) during development at 25° C based on reduced weight and density measurements

$$\text{Volume intact} = \frac{\text{reduced weight}}{\rho_{\substack{\text{intact}\\ \text{embryo}}} - \rho_{\text{water}}} \quad \text{Volume cells} = \frac{\text{reduced weight}}{\rho_{\substack{\text{open}\\ \text{embryo}}} - \rho_{\text{water}}}$$

Each point represents the mean of 5 embryos

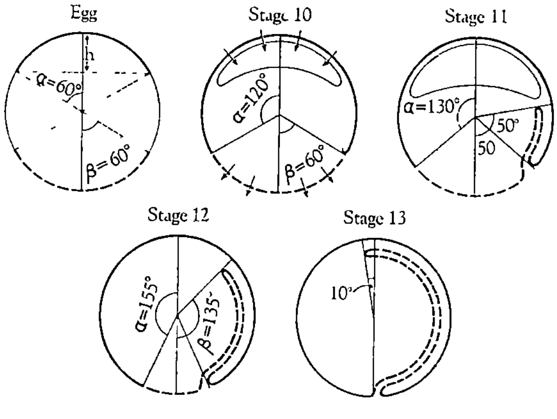

Fig. 2. Diagrammatic summary of morphological data from the *Normal Tables of Xenopus laevis*. Solid line represents the surface of the presumptive ectoderm and neural plate, and the broken line represents the surface of the presumptive endoderm

Measurement of the freezing-point depression of samples taken from the blastocœl and archenteron of single embryos has shown that the osmotic gradient between the environment and the intercellular cavities decreases with the age of the embryo, and therefore cannot by itself account for the observed water movements[2]. An alternative explanation has been suggested based on the notion that the water-regulating mechanisms of the cells give rise to a net influx of water through the roof of the blastocœl and a net efflux through the cells of the vegetative pole of the embryo[3]. If this notion were correct a difference in the magnitude of the two fluxes would account for the accumulation in the blastocœl; the invagination of the endodermal surface would itself account for the filling of the archenteron, and the increase in area of inwardly polarized ectoderm would account for the observed increase in the rate of water uptake.

This idea has been helpful in interpreting the morphogenetic effects of β-mercaptoethanol on the *Xenopus* embryo[4,5], and the chick blastoderm[6]. I have therefore attempted to present it as a quantitative hypothesis that can be tested experimentally.

If it is assumed that the accumulation of water in the embryo is due to the combined effect of two oppositely polarized areas of the embryonic surface, then the rate of water uptake by the embryo will be a function of the area of the inwardly polarized animal pole surface (A_a) and the area of the outwardly polarized vegetative pole surface (A_v). During the early stages of development, before the blastopore is completely closed, the rate of water uptake by the embryo may be written:

$$f'A_a - f''A_v \qquad (1)$$

where f' and f'' are appropriate rate coefficients. After the closing of the blastopore the rate is equal to $f'A_a$.

The areas A_a and A_v change absolutely and relatively to each other during the course of development (Fig. 2), and their sizes may be estimated from independent morphological data contained in the *Normal Tables of Xenopus laevis*[7] which give values for the radius of the embryo, and the angle subtended by a line joining the boundary of the relevant areas to the centre of the embryo and the animal vegetal axis. It is therefore convenient to express A_a and A_v in terms of these angles:

The surface of a spherical segment $= 2\pi rh$

For animal pole surface.

$$h = r(1 - \cos\alpha)$$

$$\text{and } A_a = 2\pi r^2 (1 - \cos\alpha)$$

Similarly $A_v = 2\pi r^2 (1 - \cos\beta)$

where α and β are the angles subtended by the boundary of the animal pole surface and the vegetative pole surface respectively.

Equation 1 then becomes:

Rate of water uptake =

$$2\pi r^2[f'(1 - \cos\alpha) - f''(1 - \cos\beta)] \qquad (2)$$

For the purpose of this argument only two parts of the egg surface need to be considered, first, the area from which the ectodermal surface of the neurula is derived—the pigmented area at the animal pole; and secondly, the corresponding area of the vegetative pole surface from which the lining of the archenteron is derived. The area around the equator is excluded from the argument, because it is the zone of transition between areas of opposite polarity and because there is no means of determining its rate coefficients. It is worth noting, however, that the presumptive mesoderm which forms intercellular cavities much later in development is derived from a region just below the equator.

The morphological data from the standard tables are summarized in Fig. 2. If the values of cos α are plotted against time, a straight line is obtained which shows that the area of the animal pole surface increases at a constant rate from cleavage onwards. A similar plot of values for cos β shows, on the other hand, that area of the vegetative pole surface does not change until stage 10, after which it too increases at a constant rate until stage 13, when it extends as a double hemisphere half-way round the embryo and forms the lining of the archenteron. As both these variables change in a linear fashion, intermediate values of cos α and cos β may be obtained by interpolation.

Although the value of the rate coefficients f' and f'' cannot be determined individually from the present data, it is known that, just prior to the collapse of the archenteron, the surface of the ectoderm and surface of the endoderm have approximately the same area, and since the volume of the cells is almost constant at this stage, all the water entering through the ectoderm must be passing through the endoderm. Therefore at this stage $f' = f''$.

If it is now assumed, as a first step, that f' and f'' are constant throughout development then f' and f'' = constant K and equation 2 becomes

$$\text{Rate of water uptake} = K2\pi r^2(\cos\beta - \cos\alpha) \qquad (3)$$

The value of K may be estimated by dividing the experimentally determined rate of volume change at any stage by the appropriate value of $2\pi r^2 (\cos\beta - \cos\alpha)$:

$$K \frac{\text{Slope of volume change curve}}{2\pi r^2(\cos\beta - \cos\alpha)} \qquad (4)$$

Alternatively it may be estimated from the surface area and the experimentally determined rate of volume change at a stage when the blastopore is closed and all the water entering the embryo accumulates in the archenteron, for example, at stage 20. The values obtained by the two methods are given in Table 1. The experimental values for the rate of water uptake of embryos developing at 25° C. were obtained by combining the results of a number of experiments similar to that illustrated in Fig. 1 and measuring the slope of the curve (Fig. 3) drawn by eye through the mean values of V_t/V_0.

The experimental values so obtained and the corresponding theoretical values calculated from equation (3) are shown in Fig 4. (The theoretical value for 18 hr. was derived from the surface area of the embryo calculated from the linear dimensions given in the *Normal Tables*, assuming that the embryo at this stage is a prolate spheroid) The absence of theoretical values for stages 13 and 20 stems from the fact that it is not known exactly when the blastopore becomes watertight. For comparison the corresponding theoretical and experimental values for the rate of water uptake by embryos developing at 20° C are also shown in Fig. 4

It will be seen that the experimental values, particularly for embryos developing at 25° C , are close to the theoretical values at all stages. Thus the assumptions on which this analysis is based appear to be consistent with the experimental observations. The hypothesis in its present form, therefore, seems to provide an adequate qualitative and quantitative description of some of the morphogenetic changes that occur during the early development of *Xenopus laevis* There seems to be no reason why it should not also be helpful in explaining the growth and maintenance of the fluid content of other intercellular cavities, for example, the neural tube, eye cup, cœlom and amniotic cavity, and perhaps in describing the changes in shape of cells and embryonic organs.

I am grateful to Prof Michael Swann in whose Department this work was carried out, and to my colleagues for their help, in particular Dr P. D. Mitchell, Dr H Kacser and Dr P. C Croghan. I am also very grateful to the Nuffield Foundation, which made the project possible

Table 1

(1)	Stage	Age (hr)	Experimental rate of water uptake (mm ^{3}/hr.)	Theoretical effective area $2\pi r^2(\cos\beta - \cos\alpha)$ (mm ^{2})	K (mm /hr)
		0	—	—	—
		2	0 000	0 65	—
		3	0 018	1 00	0 018
		4	0 028	1 43	0 020
		5	0 034	1 71	0 020
	8	6	0 044	2 02	0 022
		7	0 053	2 55	0 020
		8	0 065	2 98	0 022
	10	9	0 064	3 2	0 020
		10	0 049	2 6	0 019
	11	11	0 033	1 66	0 020
		12	0 026	1 42	0 018
				Mean	0 020
				Mean area of Experimental embryos	
(2)	20	18	0 16	7 3 mm ^{2}	0 022

Rate of water uptake per unit area K (1) calculated from equation (4) using morphological data from the *Normal Tables* (ref 6), and rates of volume change estimated from the slope of graph in Fig 3, (2) K calculated from the rate of volume change at 18 hr and the mean total area of the experimental embryos at this stage

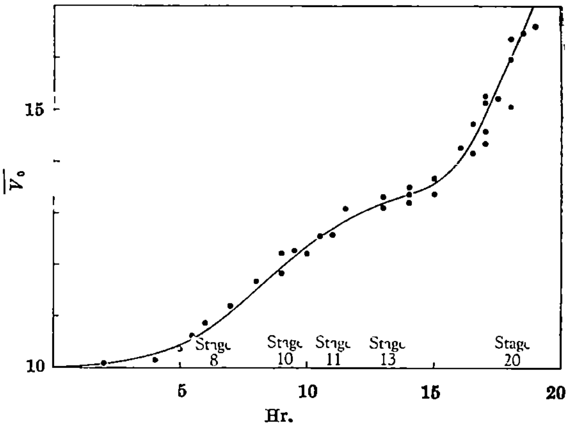

Fig 3. Changes in volume (V_t/V_0) of intact embryos from a series of experiments at 25° C used to obtain a measure of the rate of volume change at different stages (see text) Mean volume at T_0 = 1 25 mm ^{3}

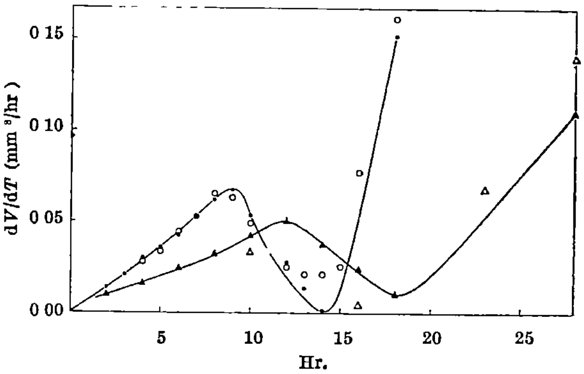

Fig 4 Theoretical values for the rate of volume change at 25° C. (circles) and 20° C (triangles) at various stages in development based on the hypothesis described in the text, and the experimentally determined rates derived from the volume changes in Fig 3 (open circles and open triangles)

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GROWTH HORMONE CONTENT OF SERUM IN INFANTS, CHILDREN, ADULTS AND HYPOPITUITARY DWARFS

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THE preparation of purified human growth hormone (HGH) from human pituitaries (Raben, Li) has made it possible to develop an immunological assay of human growth hormone by means of a human growth hormone rabbit antiserum

The hæmagglutination-inhibition method is sensitive enough to detect human growth hormone down to 0 2 μgm /100 ml [1]. The validity of the results depends chiefly on the specificity of the human growth hormone antiserum and the purity of the hormone preparation That human growth hormone has antigenetical properties has been repeatedly shown by antibody formation in the rabbit[1-10], and by anaphylactic reactions in the guinea pig[4]

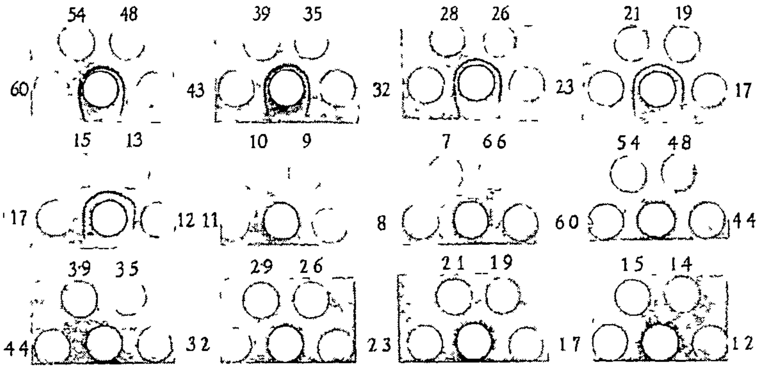

Fig. 1. Precipitin reaction between undiluted human growth hormone/rabbit antiserum (titre 1 : 800) and hormone dilutions (human growth hormone–Raben), as determined by the Ouchterlony technique. Dilutions of growth hormone, in μgm./ml., are arranged clockwise around the central well, which contains the antiserum

When human growth hormone and its specific rabbit antiserum are tested by the gel-diffusion method of Preer[3], the precipitin ring test[7,9], the Ouchterlony technique[7], immunoelectrophoresis[8,9] and by the hæmagglutination-inhibition test[1-3,10], they appear to be homogeneous.

Human growth hormone can be localized in sections of human anterior pituitaries and eosinophilic adenomas in the eosinophilic cells by means of fluorescent rabbit antibodies[6]. This can be regarded as further proof of specificity. Small amounts of contaminating antibodies cannot be entirely excluded by the methods mentioned, however, because the antiserum is diluted for use in the hæmagglutination–inhibition method these possible contaminants can be disregarded. Thyroid stimulating hormone, adrenocorticotrophic hormone and insulin do not interfere with the hæmagglutination-inhibition test of human growth hormone. Tests with the serum of hypophysectomized patients did not show inhibition in the hæmagglutination technique, indicating that only proteins of pituitary origin produce the specific inhibition[3].

Latex agglutination detects growth hormone in amounts down to 0·01 μgm. (ref. 5) and precipitin-ring-test down to 0·1 μgm./0·5 ml. sodium chloride[4]. So far only the hæmagglutination method has been applied to the estimation of concentration of growth hormone in human serum.

The method used for the hæmagglutination inhibition reaction was essentially the one described by Read[1]. Human growth hormone rabbit antiserum was produced by immunization with Raben's hormone preparation[11]. Titres ranged around 1 : 1,000. Sheep erythrocytes were washed in physiological saline, treated with diluted tannic acid and incubated with human growth hormone. With the cells so prepared, the titre of the human growth hormone rabbit antiserum was measured. For use the antiserum is diluted with physiological saline containing guinea pig serum (80 parts : 1 part, v/v). Dilutions of standard hormone solution were added to a constant amount of the diluted antiserum. Simultaneously serial dilutions of the serum to be tested were incubated with the same amounts of the antiserum. If all the antibodies are neutralized by the growth hormone of the serum or the standard solution, hæmagglutination is prevented. With decreasing hormone content of the dilutions, increasing hæmagglutination is observed. The end-point is that concentration of growth hormone at which complete hæmagglutination first occurs. Controls using washed and tanned erythrocytes without human growth hormone coating and hormone-coated cells without antiserum were always negative. The sensitivity of the method can be regulated by varying the antiserum dilution. If the dilution is not carried too far, that is, not quite to the titre-dilution, the end-point of the hæmagglutination-inhibition can be determined exactly. All samples were tested in quadruplicate and gave identical results, even in different assays.

Our own work using the Ouchterlony agar diffusion technique showed one single precipitation line between the undiluted human growth hormone rabbit antiserum and hormone concentrations ranging from 6,000 μgm./100 ml. down to 300 μgm./100 ml. (Fig. 1). No precipitation was seen when human growth hormone was tested against anti-human serum. In contrast a specific antiserum against human albumin with a high titre in one test produced a precipitation line, but only if growth hormone was used in a concentration of 100 mgm./100 ml., an amount far greater than that used in the hæmagglutination technique. On subsequent trials this observation could not be reproduced. This finding could be interpreted as indicating contamination of the growth hormone preparation with small amounts of albumin.

The growth hormone concentration in the serum of normal children and adults in our series using the hæmagglutination-inhibition technique varied between 24 μgm./100 ml. and 60 μgm./100 ml. for children and between 16 μgm./100 ml. and 36 μgm./100 ml. for adults (that is, 240 mμgm./ml.–600 mμgm./ml. and 160 mμgm./ml. and 360 mμgm./ml.). The mean values for children (42 children without cord-blood values: 39·6 μgm./100 ml., standard deviation 10·48) are significantly greater than the mean concentration in adults (18 adults: 26·0 μgm./100 ml., $S.E. = 5·53$), $P = 0·001$. The mean value of growth hormone in puberty (14 children from 11 to 17 years) is 43·43 μgm./100 ml. ($S.E. = 8·61$). This value does not differ significantly from the mean growth hormone concentration of 28 children under 11 years of age (without cord-blood values), which amounts to 37·75 μgm./100 ml. ($S.E. = 10·94$) with $P = 0·1–0·05$.

In nine specimens of cord-blood the values ranged from 24 to 36 μgm./100 ml. (that is, 240–360 mμgm./ml.).

Fig. 2 shows the results in relation to the age of the individuals tested. There was no clear relation between the growth hormone concentration at a certain age and the rate of gain in height of the corresponding period. There is a slight increase in the mean growth hormone concentration between the age of 11 and 17 years (puberty), which is not significant. In contrast, values of growth hormone in 18 adults showed an average which is significantly lower than the average during childhood.

In three hypopituitary dwarfs we found concentrations of growth hormone which are well below the lowest values encountered in normal children and adults. In this group a 6-year-old boy and a 13·5-year-old girl had a concentration of 6 μgm./100 ml., a 14·5-year-old male dwarf, who previously had

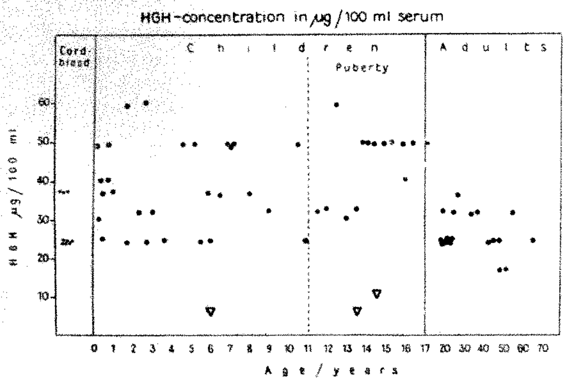

Fig. 2. ●, normal individuals; △, hypopituitary dwarfs

been treated with injections of growth hormone, showed a concentration of 10 µgm./100 ml. after treatment had been discontinued for 10 days.

Our findings for the growth hormone content in normal human serum correspond with the values given by Read (16–64 µgm./100 ml.). The content in the samples of cord-blood also falls in the range reported by him (24–48 µgm./100 ml.). In our three hypopituitary dwarfs the concentrations of growth hormone were a little higher than Read found in a similar group, but they still are clearly below the normal range.

Whereas Read believes that there is no difference in the content of serum between children and adults, our mean values for children are significantly greater than the mean concentration in adults. Investigations are under way to settle the question of the suggestive increase in the concentration of human growth hormone during puberty.

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SPERMATOCYTOGENESIS WITH POSSIBLE SPERMIOGENESIS OF GUINEA PIG TESTICULAR CELLS GROWN *IN VITRO*

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SPERMATOGENESIS has been described as having two phases. In the first phase, spermatocytogenesis, the germ cells undergo repeated mitoses and meiosis followed by structural changes which give rise to a new type of cell, the spermatid. In the second phase, spermiogenesis, the spermatids undergo a series of complex transformations which result in the formation of mature spermatozoa.

In general the three cell types of the testes considered of major importance in spermatogenesis are the spermatogenic or germ cells, the Sertoli or sustentacular cells, and the interstitial or endocrine cells. Recently, we have been able to propagate testicular cells continuously in culture and have identified these three cell types by morphological and histochemical methods. Moreover, it appears that spermatogenic cells in culture retain the maturation potential needed to form spermatids and possibly spermatozoa *in vitro*.

In experiments reported elsewhere[1] it was shown that testicular cells continuously propagated in cell culture contain antigen(s) required to induce experimental allergic aspermatogenesis in the guinea pig and that testicular cells carried through several fluid transfers continued to synthesize antigen(s) which react (Schultz-Dale) with sensitized ilea of guinea pigs in which allergic aspermatogenesis has been induced.

The purpose of this article is to describe the cytological characteristics of testicular cells grown in cell culture.

Testes were aseptically removed from mature albino guinea pigs, washed in warm (37° C.) *tris*-buffered saline and stripped of the tunica vaginalis. The testes were minced with scalpel blades and treated with 0·25 per cent trypsin in *tris*-buffered saline at 37° C. for 2–3 min. After trypsinization the cells were diluted with 10 vol. of cold *tris*-buffered saline to stop the action of trypsin, centrifuged at 2,500 r.p.m. for 3 min. and re-suspended in nutrient medium to a final concentration of approximately 2×10^6 cells/ml. 10 ml. of the cell suspension were used to seed each culture.

The culture medium contained Earle's saline, 'Millipore'-filtered horse serum, lactalbumin hydrolysate, chick embryo extract, a complex vitamin mixture and antimicrobial agents. Glutamine was added to the cultures immediately before cultivation.

Cells were transferred by treating the parent culture at 37° C. for 3–5 min. with 0·25 per cent trypsin in *tris*-buffered saline. After trypsinization cells were washed once with cold *tris*-buffered saline and re-suspended in warm medium so that each screw-capped milk dilution bottle received 10 ml. containing approximately $2 \cdot 0 \times 10^5$ cells. Cells were grown in a humidified incubator at 37° C. in an atmosphere of 10 per cent carbon dioxide and 90 per cent oxygen.

Single cells for photomicrographic and histochemical analysis were obtained by diluting cell suspensions to approximately 5 cells/ml. 1 ml. was placed in a Petri dish containing several coverslips. After

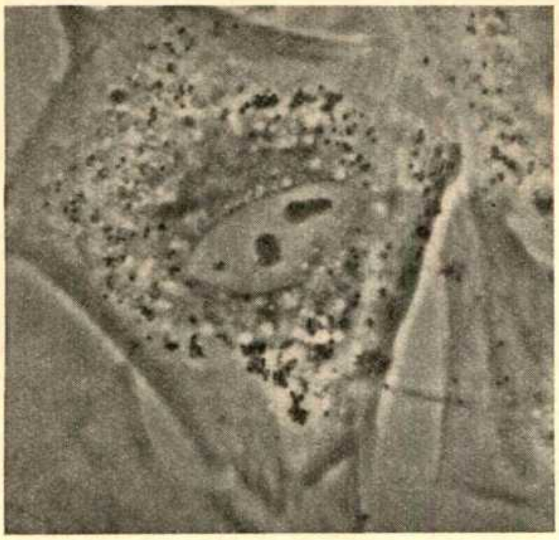

Fig. 1. Sertoli cell in culture 37 days. Phase contrast (× 1,188)

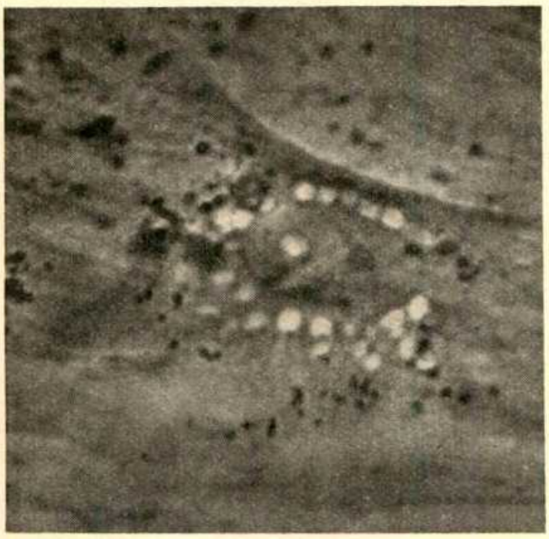

Fig. 2. Interstitial cell after 3 *in vitro* transfers: in culture 18 days. Phase contrast (× 250)

attachment to coverslips, cells were examined by phase microscopy or stained and photographed. Morphological and histochemical criteria used in identification of the different cell types were those already described and confirmed by others for the intact testis[2-6]. Coverslip preparations were fixed in 10 per cent neutral formalin for 8–10 hr. A triple stain for deoxyribonucleic acid, polysaccharides and proteins was used for differentiating between spermatogenic cells and other cellular components of the testes[7]. The nuclei stained blue to green, the mucus (polysaccharides) and structures in the Golgi zone, red; and the cytoplasm yellow. Thus the 'idiosomic' granules of the Golgi region in the primary and secondary spermatocytes, which later fused to form the 'acrosome' and 'head cap' of the spermatids, could be followed through various stages to the mature sperm by the bright red staining properties of the polysaccharide[3,5,6].

Fig. 1 is a coverslip preparation containing an epithelial-like cell with an ovoid nucleus and three nucleoli. The nuclear membrane is clearly outlined and the reticular cytoplasm contains fibrillar mitochondria, chromatin granules and lipoid droplets. This cell type is characteristic of the Sertoli cell and retains its morphological and histochemical properties during repeated passage in culture. Multiplication of the Sertoli cell *in vitro* results in a 'Sertolian syncytium' or a confluent growth connected by protoplasmic bridges.

Fig. 2 is a coverslip preparation of an epithelioid cell which contains a slightly elongated nucleus. The cytoplasm is highly granular and contains various inclusions. The perinuclear cytoplasm contains highly refractive, discrete, round globules, fairly uniform in size, which react positively when tested for neutral fats and lipoids. This cell type characteristic of the interstitial cell has been described in detail by Rasmussen[2].

The large spherical or oval cell type seen in Fig. 3 grew as a free-form, unattached, monodispersed cell suspended in the growth medium and contained a spherical mass close to the nucleus which stained red and is characteristic of primary and secondary spermatocytes. Primary spermatocytes (Fig. 3, *A*) are the largest of the developing germ cells. Secondary spermatocytes (Fig. 3, *B*, *C*), derived from the primary spermatocytes by the first reduction division, are somewhat smaller than the primary and generally the chromatin is less dense. Cells *B* and *C* have resulted from the division of what appears to have been a primary spermatocyte similar to *A*. Each of these secondary spermatocytes presumably will undergo complex structural changes to form spermatids.

Fig. 4 contains a group of cells, one of which (*A*) appears to be in early metaphase with tetrads typical of meiosis. The structures in this cell suggest the occurrence of parasynapsis with the organization of paired homologous chromosomes and the formation of quadripartite tetrads of chromosomal configurations. The presence of this cell distinctly separates meiosis *in vitro* from mitosis. *B* appears to be a spermatid flattening at the beginning of the maturation phase with the acrosomic granule transformed into a cone. The tadpole-like structure

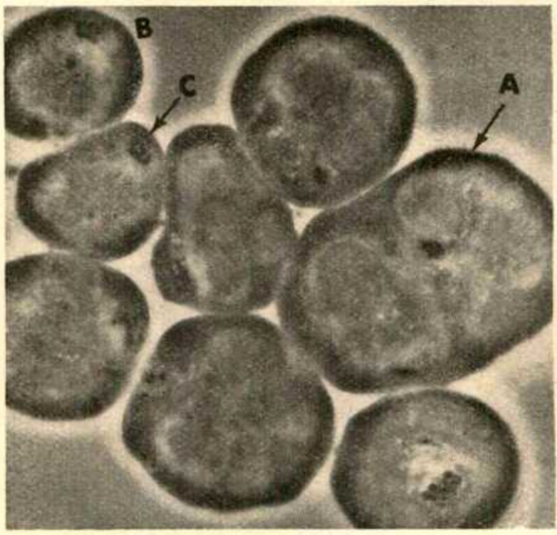

Fig. 3. Primary and secondary spermatocytes growing in supernatant fluid after 3 *in vitro* transfers; in culture 35 days. Phase contrast (× 1,188)

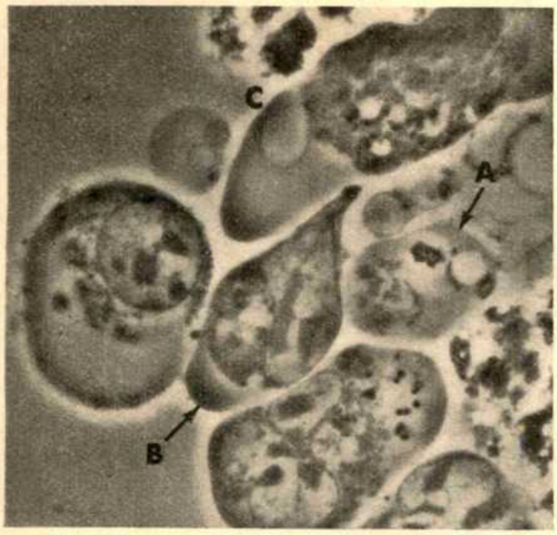

Fig. 4. A cell in meiosis (*A*) and spermatids in different stages of formation after 3 *in vitro* transfers: in culture 35 days. Phase contrast (× 1,188)

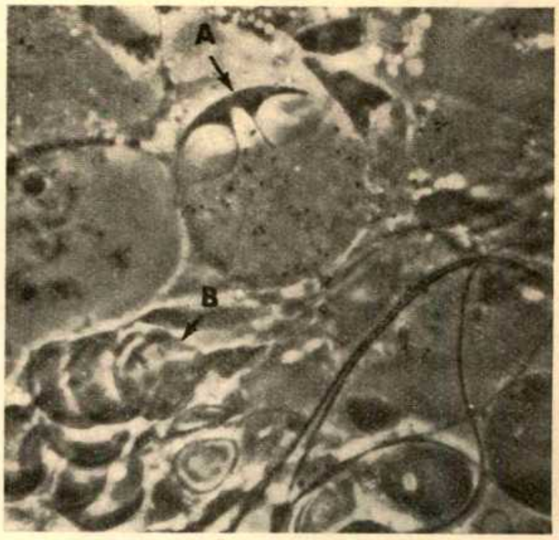

Fig. 5. Structures which appear to be mature and immature spermatozoa. Phase contrast (× 1,188)

(Fig. 4, *C*) is a spermatid with a profile view of the head cap.

In Fig. 5, *A* is a round cell which appears to contain the hollow heads of two sperms, while at the lower left portion of Fig. 5 are structures which appear to be mature sperm.

These findings show that spermatogenic, sustentacular and interstitial cells of the testes continuously propagated in culture retain their morphological and histochemical characteristics in addition to their immunological properties[1]. Moreover, it appears that spermatogenic cells carried in culture undergo metamorphosis to form mature sperm. It is important to know in such work as this whether spermatogenic cells taken from the testes were in an advanced stage of development and merely 'matured' into spermatozoa in culture or whether immature spermatogonia underwent all the necessary transformations to form mature sperm. This problem is now being investigated by time-lapse cinematography.

This work was supported by funds from grant *CY*–5188, National Institutes of Health and the Population Council, New York.

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FORMATION OF DIFFERENT HÆMOGLOBINS IN TISSUE CULTURE OF HUMAN BONE MARROW TREATED WITH HUMAN DEOXYRIBONUCLEIC ACID

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ALTHOUGH transformation of bacteria is an established fact, the transformation of mammalian cells is in a pioneering phase of research. In order to accomplish transformation, isolated deoxyribonucleic acid (DNA) must cross the cell membrane and become part of the genetical apparatus of the cell, thereby regulating the production of a substance within that cell which was absent before entry of DNA. It has been demonstrated[1] that intact DNA enters the mammalian cells and does become part of the nuclear material. The presence of a substance in the cell synthesized as a direct result of the new DNA is more difficult to demonstrate. In the human hæmoglobins each polypeptide chain synthesized is directly related to the presence of a gene or sequence of DNA in the chromosomes of the erythrocyte precursors. This was demonstrated by Itano and Robinson[2]. Incorporation of DNA from cells producing one type of hæmoglobin into cells which produce another kind of hæmoglobin should allow the detection of a new hæmoglobin in the cells into which the DNA has been incorporated. Production of hæmoglobins not previously present in tissue culture following incorporation of DNA into the erythrocytic series of cells has been accomplished here. These new hæmoglobins contain the polypeptide chains α^A, β^A, β^S, γ and probably an altered α-chain.

Bone containing red marrow obtained at surgery from an individual, homozygous for hæmoglobin *A*,

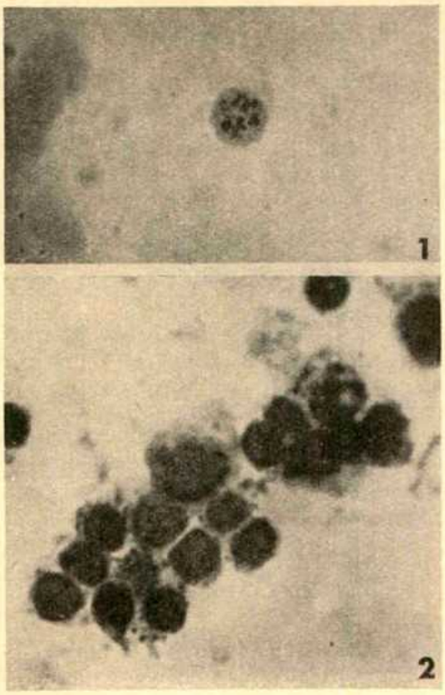

Fig. 1. Autoradiogram using Kodak '*AR* 10' stripping film. Exposure, 41 days. Stained with Wright-Giemsa. 1, mitotic figure showing incorporation of tritiated thymidine into the chromosomes of cells from bone from an individual with hæmoglobin *A* after 4 days of tissue culture. 2, group of prorubricytes and rubricytes showing incorporation of tritiated DNA into cytoplasm and nucleus of bone marrow from a sickle-cell anæmia patient after incubation for 24 hr. with tritiated DNA

was incubated in Tyrode solution containing 10 per cent of the patient's plasma. Methyl-labelled tritiated thymidine, 500 μc. in 0·02 mgm./0·5 ml. (New England Nuclear Corp.) was added to the tissue culture. After maintaining the tissue culture at 27° C. for four days, autoradiograms of the marrow cells were prepared using Kodak '*AR* 10' stripping film (Fig. 1, 1). DNA was extracted by the salt extraction method used by Spizizen[3]. The extracted DNA gave typical absorption spectra[4] and the concentration calculated by $E_{1\,\text{cm.}}^{1\%} = 192$ at 260 millimicra was 1 μgm./μl. Nine hundred μgm. of DNA were added to bone marrow obtained by sternal puncture from a patient with sickle-cell anæmia. The hæmoglobin present was homozygous hæmoglobin *S* with 8 per cent fœtal hæmoglobin. This bone marrow and the added DNA were maintained in tissue culture for 10 days at 27° C. in a sealed culture dish in Tyrode solution containing 10 per cent of the patient's plasma. A plasma clot formed during the course of the incubation. Cells were removed at 16, 24 and 48 hr. after the addition of DNA and slides were prepared with Kodak '*AR* 10' stripping film for autoradiographical examination. All autoradiograms showed incorporation of the tritium-labelled DNA into the cytoplasm or the nucleus of nucleated erythrocytes (Fig. 1, 2), and into some other marrow cells. After 10 days, an aliquot of patient's plasma labelled with iron-59 as ferrous chloride was added and the tissue culture was incubated under continuous shaking in a water-bath at 37° C. for 1·5 hr. The cells were washed with Tyrode solution and a hæmolysate prepared which was fractionated by electrophoresis in *p*H 8·6 veronal buffer. The total hæmoglobin component was found to be labelled with iron-59 and contained 45 per cent of the total radioactivity on the paper strip. The specific activity was 100 counts/min./mgm. of hæmoglobin. These results agree well with previous experiments reported in ref. 5. This demonstrates that at the end of 10 days of tissue culture the erythrocyte cells were capable of synthesizing labelled hæmoglobin.

Electrophoresis was used to compare the cell hæmolysate from the tissue culture of DNA-treated cells with the cell hæmolysates from peripheral blood of the individual homozygous for hæmoglobin *A* (source of the DNA) and the patient with homozygous hæmoglobin *S* with 8 per cent hæmoglobin *F* (the original tissue culture). The results are in Table 1.

Fig. 2 depicts the results of agar electrophoresis showing the different hæmoglobins formed in tissue culture treated with DNA.

Electrophoresis in 7 *M* urea–0·1 *M* veronal buffer at *p*H 8·2 separates the polypeptide chains of hæmoglobin[6]. This method was used to separate the polypeptide chains of the new hæmoglobins, hæmoglobin *A* and hæmoglobin *S–F* found in the cell hæmolysates (Fig. 3). The identification of the

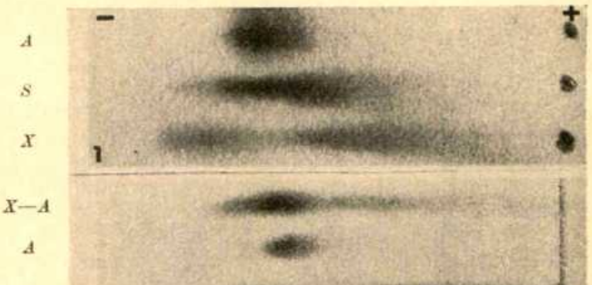

Fig. 2. Agar electrophoresis of cell hæmolysates from tissue culture treated with DNA, labelled *X*, from peripheral blood of the sickle-cell anæmia patient. labelled *S* (original bone marrow tissue culture) and of an individual with hæmoglobin *A*, labelled *A* (source of DNA). All material was in the form of cyanmethæmoglobin and was of equal concentration. 1, 2, 3, *tris*-EDTA-boric acid *p*H 7·1; 4, *tris*-citric acid *p*H 6·1; 5, *tris*-EDTA-boric acid *p*H 8·6. The origin is designated by a pencil mark. Stained with bromphenol blue

polypeptide chains present in the cell hæmolysates was accomplished by electrophoretic comparison with purified α^A, β^A, β^S and β^C polypeptide chains isolated by countercurrent distribution[9].

The absorption spectra in the range 200–700 mμ for all the above cell hæmolysates were superimposable and typical for oxyhæmoglobin containing a small amount of methæmoglobin.

From the results of these experiments it appears that cells which were genetically determined to synthesize only α^A, β^S and γ polypeptide chains of hæmoglobin, on incorporation of the genetical material (DNA) from cells which synthesized only α^A, β^A and γ polypeptide chains, now are able to synthesize the originally present α^A, β^S and γ polypeptide chains and in addition β^A and possibly an altered α polypeptide chain. These polypeptide chains in the cells altered by DNA combine to form two hæmoglobins differing electrophoretically from hæmoglobins *A*, *S* and *F*.

Table 1. ELECTROPHORESIS OF THE HÆMOLYSATES (HÆMOGLOBIN *X*) OF THE TISSUE CULTURE HUMAN BONE MARROW TREATED WITH DNA COMPARED WITH CONTROLS OF HÆMOGLOBIN *A* (SOURCE OF THE DNA) AND HÆMOGLOBIN *S*8–*F* PER CENT (BONE MARROW BEFORE TREATMENT WITH DNA)

Whatman 3 *MM* Filter Paper	−	Origin*	+
Veronal		.	*A*
*p*H 8·6, 0·05 *M*		.	*X*
		.	*S*
Tris-citric acid	*A*	.	
*p*H 6·1 (ref.6)	*X*	.	
	S	.	
	X	.	
Phosphate	*A*	.	
*p*H 6·8, 0·05 *M*	*X*	.	
	S	.	
	X	.	
Phosphate	*A*	.	
*p*H 6·5, 0·05 *M*	*X*	.	
	S	.	
	X	.	
Tris-ethylenediamine tetraacetic acid (EDTA)-boric acid		.	*A*
*p*H 8·9 (ref. 7)		. *X*	
		S	
		. *X*	
Agar (ref. 6)			
Tris-EDTA-boric acid	*A*	.	
*p*H 8·6	*X*	.	
	S	.	
	X	.	
Tris-EDTA-boric acid	*A*	.	
*p*H 7·1	*X* *X*	.	
	S	.	
	X *X*	.	
Tris-citric acid	*A*	.	
*p*H 6·1	*X*	.	
	S	.	
	X	.	

All cell hæmolysates are of equal concentration and in the form of cyanmethæmoglobin.

* Origin represents the point of application of the samples, + and − the electrodes and the positions *X*, *S*, *A* designate the relative mobilities of the hæmoglobins found in the previously described cell hæmolysates.

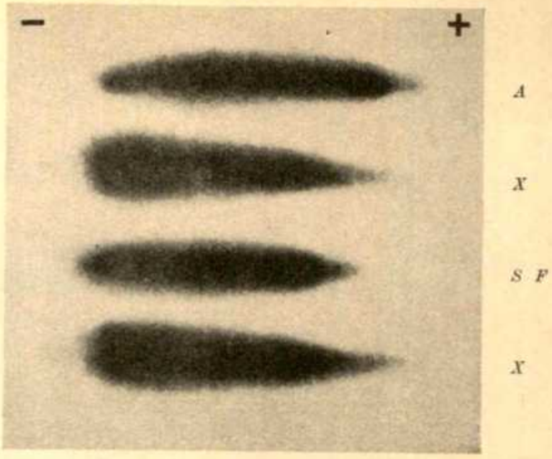

Fig. 3. Electrophoresis on Whatman 3 *MM* paper using 7 *M* urea-0·1 *M* veronal *p*H 8·2, 350 V., 5 m.amp. for 5 hr. Hæmolysates as designated in Fig. 2. The electrophoretic mobility of the polypeptide chains of hæmoglobins, in this method, is towards the anode $\beta^A > \gamma > \beta^S$. The α-chains migrate towards the cathode. Stained with bromphenol blue

Bone for the extraction of DNA was made available by Prof. H. Boyd, of the Department of Orthopedic Surgery, University of Tennessee, and Campbell Clinic, Memphis. Bone marrow was made available by Prof. A. P. Kraus, Department of Medical Laboratories, University of Tennessee College of Medicine. I thank them.

This investigation was supported in part by a grant from the United States Public Health Service *H*-3546(*C*3).

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HÆMOGLOBIN α^A

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NORMAL adult hæmoglobin (Hb-*A*) consists of two α^A-polypeptide chains joined to two β^A-polypeptide chains and has a molecular formula $\alpha_2^A\beta_2^A$ (ref. 1). Most human hæmoglobin variants, as well as fœtal hæmoglobin, $\alpha_2^A\gamma_2^F$ (refs. 2, 3), and hæmoglobin A_2, $\alpha_2^A\delta_2^{A_2}$ (refs. 4, 5), have the same type of structure. Two hæmoglobin variants have been described which do not contain α-chains, namely, hæmoglobin *H*, β_4^A (refs. 6–8) and hæmoglobin

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* Beit Memorial Fellow.

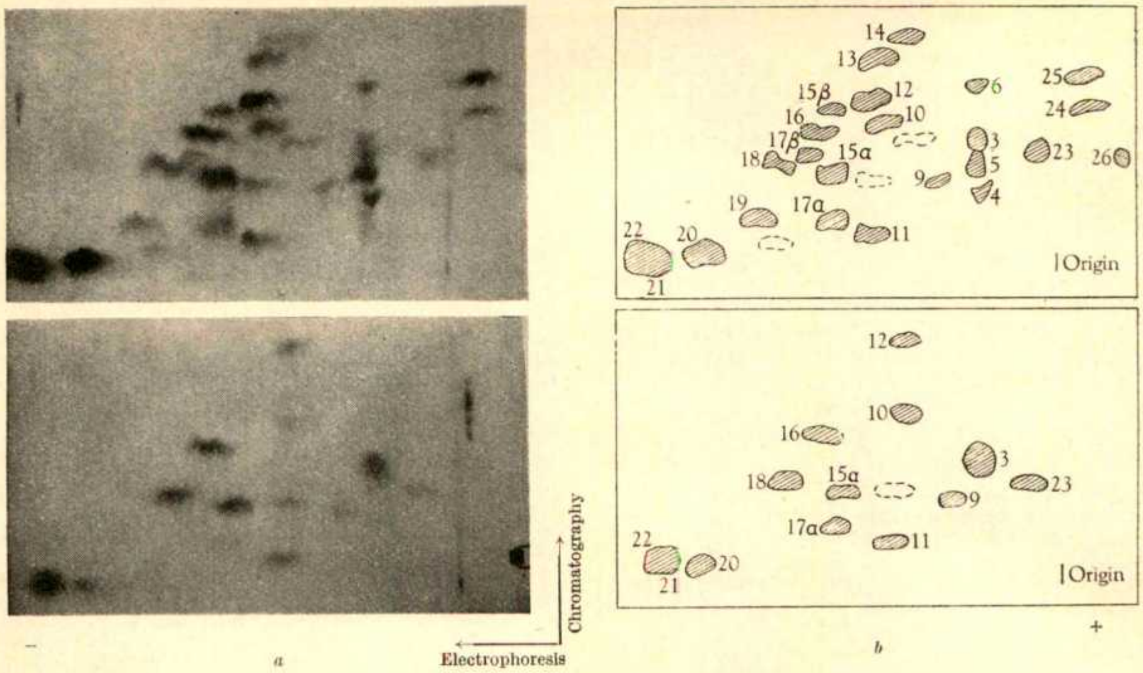

Fig. 1. Analysis of peptides obtained by tryptic hydrolysis of (top) Hb-*A* and (bottom) Hb-α^A; electrophoresis in pyridine, acetic acid, water (90 : 0·4 : 10), *p*H 6·4, followed by ascending chromatography in isoamyl alcohol, pyridine, water (35 : 35 : 27). (*a*) Photographs of peptide pattern stained with ninhydrin; (*b*) tracings of peptide pattern (Method and numbering system as ref. 28)

'Bart's', γ_4^F (refs. 9–11). In this report, the properties of a hæmoglobin, now called hæmoglobin α^A (Hb-α^A), consisting of a single α^A-chain, are described.

Hb-α^A was prepared by eluting purified Hb-*A* from a column of carboxymethyl cellulose using an 0·3 *M* sodium acetate buffer of *p*H 4·7, dialysing the eluted hæmoglobin solution back to a neutral *p*H and then separating the resulting hæmoglobin mixture by starch block electrophoresis[12,13]. In phosphate buffer of *p*H 7·0 and [Na⁺] 0·08 *M* Hb-α^A migrated more rapidly towards the cathode than Hb-*A*.

Fig. 1 compares the peptides obtained from the tryptic digestion of Hb-α^A with those obtained from Hb-*A*. It can be seen that those peptides, namely, 4, 5, 6, 12, 14, 15β, 17β, 19, 24, 25 and 26, arising solely from the β^A-chain of Hb-*A* are absent. In contrast, peptides 3, 9, 10, 11, 13, 15α, 17α, 18 and 23 which originate from the α^A-chain appear in the 'fingerprint'. Hb-α^A was also dissociated and recombined[14,15] with canine hæmoglobin, hæmoglobin *G*/*C* and hæmoglobin *I*. In the recombination experiment with canine hæmoglobin, only the slower of the two new species which form in the recombination of canine hæmoglobin with Hb-*A* was observed (Fig. 2). This species is known to have the composition $\alpha_2^A\beta_2^{Can.}$ (ref. 16). When Hb-α^A was recombined with hæmoglobin *G*/*C*, $\alpha_2^G\beta_2^C$ (ref. 17), the only new species formed was hæmoglobin *C*. On the other hand, it is known that when hæmoglobin *G*/*C* is recombined with Hb-*A*, both hæmoglobins *G* and *C* form[17] according to the equation:

$$\underset{\text{Hb-}G/C}{\alpha_2^G\beta_2^C} + \underset{\text{Hb-}A}{\alpha_2^A\beta_2^A} \rightarrow \underset{\text{Hb-}G/C}{\alpha_2^G\beta_2^C} + \underset{\text{Hb-}C}{\alpha_2^A\beta_2^C} + \underset{\text{Hb-}G}{\alpha_2^G\beta_2^A} + \underset{\text{Hb-}A}{\alpha_2^A\beta_2^A}$$

Thus, the appearance of hæmoglobin *C* but not hæmoglobin *G* in this experiment as well as the formation of the species $\alpha_2^A\beta_2^{Can.}$ but not the species $\alpha_2^{Can.}\beta_2^A$ in the recombination with canine hæmoglobin suggest that the new hæmoglobin consists solely of α^A-chains. Recombination of Hb-α^A with hæmoglobin *I* produced only Hb-*A*, confirming the presence of α^A-chains in Hb-α^A. This experiment is analogous to the recombination of hæmoglobin *H* with a hæmoglobin possessing abnormal β-chains[18,19]. In the latter, hæmoglobin *H* donates β^A-chains which combine with the α^A-chains of a β-chain variant to form Hb-*A*, while in the experiment reported here Hb-α^A donates α^A-chains for the formation of Hb-*A*, this time with the β^A-chains of an α-chain variant.

The sedimentation properties of Hb-α^A (ref. 20) resemble closely those of sperm whale myoglobin and the hæmoglobin of the brook lamprey, *Lampreta fluviatilis*. Thus, the value of 1·7 *S* for the sedimentation coefficient of Hb-α^A may be compared with the figure of 1·7 *S* for myoglobin determined under the same experimental conditions, or of 1·8 *S* quoted for lamprey hæmoglobin[21]. Moreover, the molecular weight of Hb-α^A, 17,000 ± 1,000, is similar to the value of about 18,000 for myoglobin and 17,200 for lamprey hæmoglobin[21] and is near the molecular

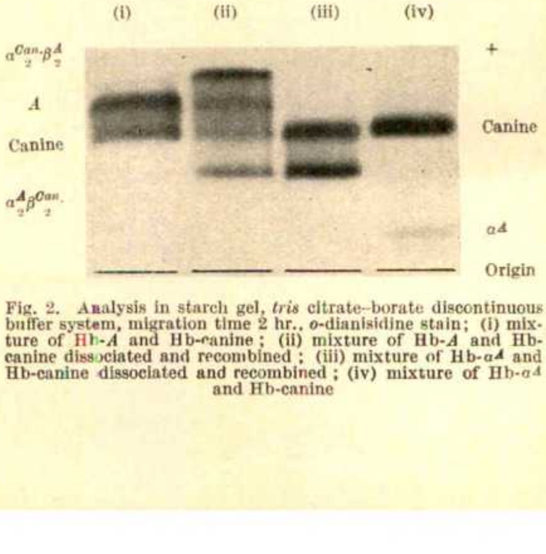

Fig. 2. Analysis in starch gel, *tris* citrate–borate discontinuous buffer system, migration time 2 hr., *o*-dianisidine stain; (i) mixture of Hb-*A* and Hb-canine; (ii) mixture of Hb-*A* and Hb-canine dissociated and recombined; (iii) mixture of Hb-α^A and Hb-canine dissociated and recombined; (iv) mixture of Hb-α^A and Hb-canine

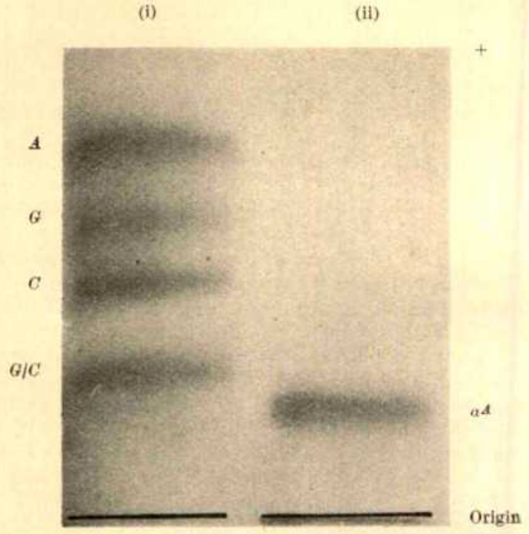

Fig. 3. Analysis in starch gel, *tris* citrate–borate buffer system, migration time 2 hr., *o*-dianisidine stain; (i) hæmolysate containing Hb-*A* + *G* + *C* + *G*/*C*; (ii) Hb-α^A

weight expected for the α^A-chain on the basis of its amino-acid composition[22,23]. Hb-α^A therefore consists of a single α^A-polypeptide chain.

The absorption characteristics of the cyanmet-derivative of Hb-α^A are, with one exception, identical to those of Hb-*A*. The one difference is found in the wave-lengths of the maximum of the 'protein' band which are at 274 mμ for Hb-*A* and at 270 mμ for Hb-α^A. This shift to lower wave-lengths would, however, be expected for a hæmoglobin consisting only of α^A-chains since the latter have a significantly higher tyrosine : tryptophan ratio than the globin of Hb-*A* itself.

Hb-α^A has a distinctive electrophoretic mobility, migrating nearer to the cathode than hæmoglobin *G*/*C* at *p*H's between 7·0 and 8·7 (Fig. 3). It is stable in solution, either as the carbonmonoxy or cyanmet-derivative, for a week at 4° C., but gradually precipitates on longer storage. When deep-frozen at − 15° C. it precipitates almost completely from solution in 48 hr.

These experiments have, therefore, shown that a hæmoglobin consisting of a single α^A-chain is capable of separate *in vitro* existence. Moreover, its electrophoretic mobility and stability in solution are such that if it occurred *in vivo*, even in small amounts, it would be detected by starch-gel electrophoresis either in the *tris* citrate–borate system at *p*H 8·7 (ref. 24) or in phosphate buffer[15,25,26]. Starch-gel analyses of fresh hæmolysates from a number of individuals, normal or with hæmoglobinopathies or thalassæmia, carried out by us have failed to reveal any such component. However, it is probable that small amounts of this hæmoglobin are formed in some recombination experiments, particularly if prolonged acid treatment is used. The only other hæmoglobin with an electrophoretic mobility slower than hæmoglobin *G*/*C* is hæmoglobin Gower 2, found in the red cells obtained from a small human fœtus[27]. However, the position of the tryptophan fine structure band of the latter hæmoglobin is at 2902 Å. which is significantly shorter than that found for Hb-α^A and these two hæmoglobins are therefore not the same. It would thus appear that Hb-α^A does not occur *in vivo* and this is of some importance in any consideration of the synthesis of the hæmoglobin molecule.

This investigation has been supported by a grant from the Colonial Medical Research Committee and also by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council.

We are indebted to Miss Jean Atwater for a sample of a hæmolysate containing hæmoglobin *I* and to Dr. A. Edmundson for a specimen of crystalline sperm whale myoglobin.

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TELEVISION MICROSCOPY

A Flying-spot Interference Television Microscope

AN all-electronic flying spot interference television microscope for the rapid determination of cellular dry mass has been developed in this laboratory. The work has stemmed from the suggestions of Barer[1] and from consultation with F. F. Roberts and F. Smith of C. Baker Instruments, Ltd.

The interferometric technique of dry-mass determination has been well documented[2] and mechanical systems of scanning have been developed[3].

The equipment developed in this laboratory uses the flying-spot technique of scanning. A low-speed system with 10-sec. sweep is used in order to improve the ratio of signal to noise and to ease the requirements on the computing circuitry. The system consists of the flying-spot scanner tube, interference microscope, multiplier phototube, and a photoformer for the continuous solution of the intensity-phase angle equation. A monitor presents a picture of the photoformer output, which represents the relative phase angle present in the cell and, therefore, the dry mass of the various portions of the cell on a grey scale. The photoformer output is fed to an integrator circuit which sums the phase angle in the entire field. The integration output at the end of each sweep, therefore, represents the total dry mass present in the optical field. The information from the integrator is continuously read out on a strip chart recorder.

Auxiliary pulse generating equipment is so arranged that the integrator may be made to sum only the information in a selected area of the field. The area being integrated is indicated on the monitor by brightening. This area may be a square or rectangle with a minimum size of about 2μ at the magnification at present used.

The grey scale dry-mass images of the living cell may be continuously recorded on 16-mm. motion picture film. When such a film is presented at a speed of 16 frames per sec. a time-lapse motion picture is obtained.

This system has several advantages over previously described techniques. (1) The data on the dry mass of whole cells or segments of cells are obtained and recorded by two methods every 10 sec. (2) The dry-mass images of the specimen are continuously displayed on the monitor tube during the course of the experiments. (3) The visual presentation of the dry-mass image makes the positioning of the brightened area of the raster quite easy and this permits continuous determination of the dry mass of selected areas of the cell.

This work was supported by grants from the Atomic Energy Commission, the Southwestern Medical Foundation, the Damon Runyon Memorial Fund, and Tobacco Research Industry Committee.

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Ultra-Violet Television Microscopy

SEVERAL attempts to use ultra-violet-sensitive television cameras in association with ultra-violet microscopes to give an immediate display of the image have been reported[1]. From published photographs the sensitivity and image quality do not appear to be very high and results obtained with other types of image converters have also been disappointing. A newly developed E.M.I. 'Vidicon' camera tube incorporating a quartz window and a specially modified arsenic triselenide target layer appears to be very promising. The spectral response curve has a peak at 4000 Å. with a half-intensity band-width of about 1500 Å. The sensitivity at 2537 Å. is approximately 0·10 μamp./μW./cm.[2]. This appears to be several times higher than previously reported values. The layer has unity gamma and reasonably low inertia.

This tube has been used in conjunction with quartz objectives and condenser, illuminated with monochromatic light of wave-length 2537 Å. from a low-pressure mercury lamp and simple monochromator. With a signal current of between 0·05 and 0·1 μamp., excellent images of living cells at magnifications of 3,000 times could be displayed on a television screen. The intensity of illumination was adequate even with the glycerine immersion objective of N.A. 1·25 and it was possible to take good-quality photographs of the screen using a 35-mm. camera with exposures as short as 1/25 sec. The great advantage of the system is that it enables microscopy to be carried out as easily with ultra-violet as with visible light. Focusing presents no problem and there are no special mechanical requirements. The lenses were mounted on a simple student-type microscope stand. Many fields containing large numbers of cells can be scanned in a short time and the very shallow depth of field makes it possible to focus the different levels through a relatively thick specimen, thus helping to build up a three-dimensional impression.

In this connexion the technique of immersion refractometry[2] has been adapted for ultra-violet work. The lens-like behaviour of an uncompressed spherical cell makes observation of internal structures difficult. This can be partly overcome by immersing such cells in a suitable protein medium having a refractive index approaching that of the cytoplasm. This method has enabled the structure of chromosomes to be seen very clearly in undamaged cells. Since, however, the cytoplasm of most cells shows some degree of absorption at 2537 Å. it is difficult to bring about actual reversal of contrast by immersion in protein alone. If a little nucleic acid is added to a less-concentrated protein solution it becomes possible to match the cytoplasm exactly against the surrounding medium or to make it appear bright instead of dark. This method opens up the possibility of measuring both the refractive index and absorption of living cells in the ultra-violet to obtain quantitative cytochemical information. It also makes it possible to vary at will the contrast of internal detail, a technique referred to elsewhere as "optical dissection"[2]. Finally, the method offers considerable promise for rapid microspectrophotometry. It is quite simple to scan any line across the image of a cell and display a light intensity curve on an oscilloscope screen. There is no difficulty in principle in converting such curves to an optical density scale and in feeding the results to an electronic integrator to measure the total mass of absorbing material.

We wish to thank E.M.I. Electronics, Ltd., for the loan of television equipment and Cooke, Troughton and Simms, Ltd., for the loan of optical equipment.

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LETTERS TO THE EDITORS

SPACE SCIENCE

Interaction of West Ford Needles with the Earth's Magnetosphere and their Life-time

THE life-time of the copper needles which were to be placed in orbit for the West Ford experiment is a matter of great concern. It is generally believed that the pressure of solar radiation (*not* drag) will bring the needles down in about seven years provided they are launched into a 'resonant' orbit (for example, a polar orbit at altitude 3,800 km.); for a good description of the resonance effect, see I. I. Shapiro and H. M. Jones[1]. These authors also show that if the semi-major axis of the orbit is changed by more than 150 km., the resonance would be destroyed and the life-time greatly increased.

My earlier article[2] discussed the problem of the drag of a charged body moving through a plasma and considered also just how a body acquires an electric charge in space. Although originally applied to micrometeors, this work can be carried over directly to the West Ford dipoles, which are copper needles of length 1·77 cm., diameter $2 \cdot 8 \times 10^{-3}$ cm., and mass 10^{-4} gm. I calculate a capacity C of $1 \cdot 5 \times 10^{-13}$ farad (my earlier article[2] adopts the wrong value of capacity, and, through force of circumstances, went to press before I could correct it; all further results there must be changed in proportion).

A more detailed examination of the charging effects for the particular conditions of the magnetosphere where the needles are put in orbit still leads to a potential of the needles close to the value adopted earlier. Its 'effective' value is $- 3 \cdot 6$ V. Hence the nominal charge Z is $\sim 3 \times 10^6$ electrons.

A further detailed examination shows that within a small error it is permissible to replace the needle by a point charge when calculating its total interaction force with the magnetospheric plasma. Then with the use of the relations given earlier[2], the drag force acting on the needles turns out to be $F \sim 10^{-23} Z^2 n$ dyne, where n is the ion density $\sim 3 \times 10^3$ H^+ ions per cm.^{3} at the West Ford orbit. The resultant rate of decrease of the orbit radius is $\sim 3 \times 10^{-10} Z^2$ km. per annum. Hence the orbit shrinks at the rate of $dr/dt \sim 3 \times 10^3$ km. per annum.

The greatest uncertainty enters into the determination of the electric potential of the needle. I estimate it to be within $- 7 \cdot 2$ to $- 1 \cdot 8$ V., with a probability of more than 90 per cent. With these extreme limits the orbit would shrink at a rate as high as $1 \cdot 2 \times 10^4$ km. per annum (33 km. a day) or as low as 750 km. per annum.

It is clear, therefore, that not only will the drag spoil the resonance but also it will in all likelihood provide an upper limit for the life-time which amounts to a few months, possibly as much as three years.

However, one cannot entirely exclude the possibility that the root-mean-square potential is less than 0·25 V., leading to $dr/dt < 15$ km. per annum (in which case the radiation pressure resonance would be preserved and the life-time would be ~ 7 years); or that the root-mean-square potential is about 0·36 V., leading to $dr/dt \sim 30$ km. per annum, in which case the resonance would be destroyed and the life-time *increased* to nearly a century.

A detailed report has been prepared and will be published later.

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PHYSICS

Lattice-type Vibrations in Associated Liquids and the Origin of Anomalous Rayleigh Scattering

WHEN a sample is irradiated with monochromatic light of frequency ν_0 and the scattered light at right angles to this incident beam examined by means of a spectroscope, a line spectrum is observed containing besides the Rayleigh component ν_0, the Raman lines $\nu_0 \pm \Delta E_i/h$, where ΔE_i are the energy differences between stationary states of the sample. Although an arc having a relatively isolated line is normally used as the source of monochromatic light, all arcs do give rise to some continuum and this also appears in the spectrum as Rayleigh scattering. This continuum can be reduced by a carefully designed arc (such as the Toronto type[1] mercury arc), while the Rayleigh scattering is minimized by preparation of the sample for investigation in an optically clean condition. However, even under such favourable conditions some continuous scattering does occur, but the fact that it appears to depend on the nature of the scattering medium, extending less than 100 cm.$^{-1}$ from the exciting line in the case of unassociated liquids[2–4] and their corresponding solids and many hundreds of cm.$^{-1}$ in the case of highly associated liquids[5] and solids, would suggest that it is not Rayleigh scattering as is often thought. Its origin is, therefore, of interest, and it is the purpose of the present communication to discuss the possible sources of these anomalous Rayleigh 'wings'.

Phonon scattering, giving rise to a Brillouin spectrum, can be quickly ruled out as the major cause of this broadening since the velocity of sound in liquids and solids is of the order of 10^5–10^6 cm.sec.$^{-1}$, corresponding to frequency shifts of only ~ 1 cm.$^{-1}$ at 4,000 Å.

In unassociated liquids and their corresponding solids, rotation of the molecules should give rise to broad wings on either side of the exciting line, the broadness of these wings depending on the rotational constant and the temperature, and in general being less than 100 cm.$^{-1}$ for all but very light molecules. This is normally believed to be the predominant cause of the Rayleigh wings in these systems[3,4,6]. Although in associated liquids and solids rotation of molecules

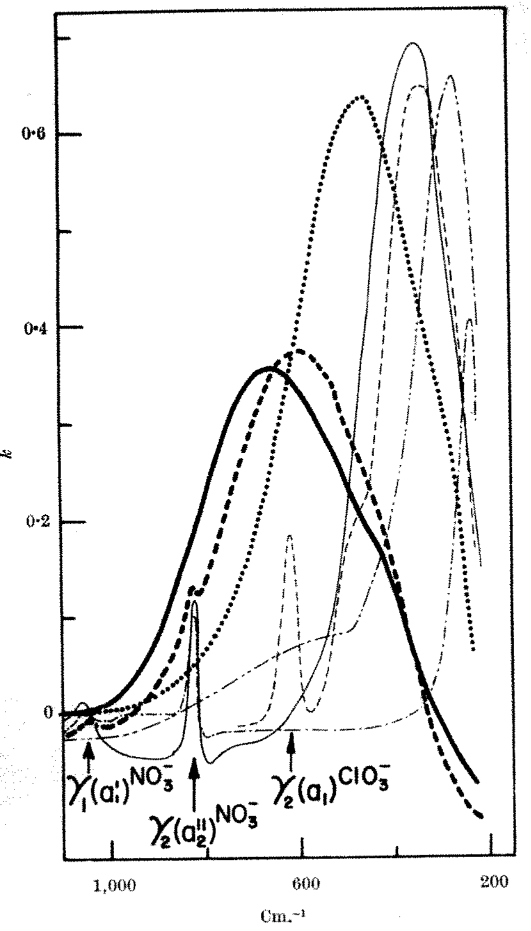

Fig. 1. The low-frequency infra-red spectra of some highly associated liquids. ▬▬, Water (20° C.); ■■■■, $LiNO_3$–H_2O (5·92 *M* at 20° C.); ————, $LiNO_3$ (313° C.); ·—·—·—, $NaNO_3$ (347° C.); – – – –, $LiClO_3$ (250° C.); ……, LiOH (490° C.); —··—··—, NaOH (350° C.). For both lithium and sodium hydroxides the ordinate scale should be multiplied by two

may still occur, the major cause of the Rayleigh wings probably arises from excitation of the optical modes of an associated or lattice-like structure. Such excitation of the optical modes may give rise to both a first- and a second-order Raman effect, the frequency maximum of the former corresponding roughly to that of the optical frequency observed in the infra-red, while the second-order Raman effect will extend out to approximately twice this frequency. Thus, solid sodium chloride, which has an infra-red absorption frequency[7] at 164 cm.⁻¹, exhibits a second-order Raman effect[8] extending out to 350–400 cm.⁻¹.

In the case of highly associated liquids, excitation of the optical branch of the quasi-lattice-like structure has not been reported previously. By using a one-angle reflexion technique[9] and an analysis due to Robinson and Price[10] the optical constants in the 200–1,000 cm.⁻¹ region have now been obtained for water, aqueous lithium nitrate, fused lithium nitrate, chlorate and hydroxide, and fused sodium nitrate and hydroxide, and the absorption spectra are shown in Fig. 1. The intense band in this low-frequency region can be assigned in every case to the excitation of the optical branch of a quasi-lattice, and the frequency of this band suggests that the Raman effect should show broad wings out to 400–900 cm.⁻¹ in fused salts and ~ 1,600 cm.⁻¹ in aqueous solutions, consistent with the known anomalous Rayleigh scattering in these systems.

It would appear of interest in the light of the above suggested origin of the anomalous Rayleigh wings to study this continuous scattering more fully, especially in the case of highly associated liquids. In this direction the 'laser', giving an intense monochromatic light source with no spurious arc continuum, should be ideal as a Raman source and should certainly clarify this question.

I wish to thank H. J. Bowlden for helpful discussion and criticism of the manuscript.

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The Balance of Energy in Nuclear Fission

EARLY experimental observations on the range of fission fragments in air[1] suggested that the kinetic energy of fragments of nearly equal mass formed in fission (that is, symmetrical fission) was less than that of fragments of different mass (that is, in unsymmetrical fission), and very recent measurements[2] have clearly confirmed this, and shown that the deficiency amounts to about 30 MeV. (32,210 micromass units, μM.U.). The conservation of energy in fission may be expressed by an equation of the form:

$$\left({}^{A}_{Z}M\right) = \left({}^{A}_{Z_1}{}^{1}M\right) + \left({}^{A}_{Z_2}{}^{2}M\right) + E_{12} \qquad (1)$$

where ${}^{A}_{Z}M$'s are the masses of the fissioning nucleus and of the fragments, 1 and 2, of mass number A and charge Z, and E_{12} is the energy released expressed in mass units. This last appears as the kinetic energy of the fragments together with the neutrons and γ-rays emitted in fission. It has been shown[3] that the neutrons and γ-rays associated with symmetrical fission are not markedly greater in energy than those associated with unsymmetrical fission, and it appears that the deficiency in the kinetic energy in question must be associated with an excess in the masses of the unstable fragments. The masses of the unstable fragments formed initially in fission are not known, however, and attempts to predict them from present empirical mass formulæ have not shown the expected mass excess[4].

Koenig, Mattauch and Wapstra have recently compiled a consistent set of all known stable and unstable masses[5]. In this communication it is shown that an extrapolation of these known masses suggests

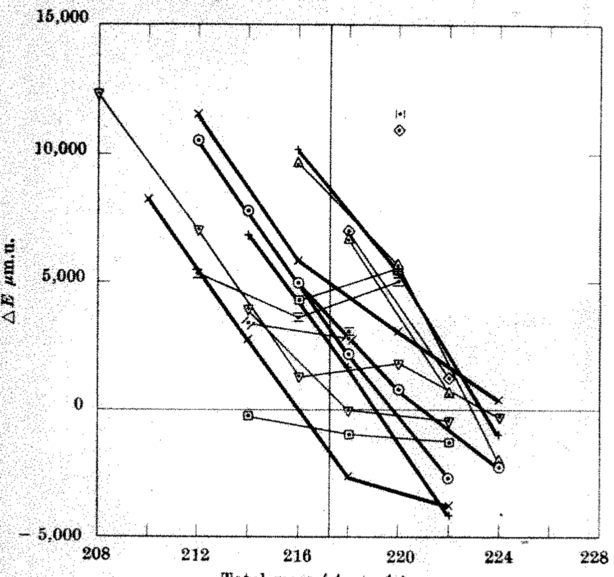

Fig. 1. Mass differences $({}_{54}Xe + {}_{38}Sr) - 2({}_{46}Pd)$

	$A_2 - A_1$	$(A_2^* - A_2) - (A_1^* - A_1)$
/·/	34	− 9·25
⌶	36	− 7·25
⊡	38	− 5·25
▽	40	− 3·25
×	42	− 1·25
⊙	44	+ 0·75
+	46	+ 2·75
△	48	+ 4·75
◇	50	+ 6·75
|·|	52	+ 8·75

that symmetrical fission fragments have greater mass than unsymmetrical fragments.

For symmetrical fission, (s), equation 1 may be written:

$$\left({}^{A}_{Z}M\right) = 2\left({}^{A_s}_{Z_s}M\right) + E_s \qquad (2)$$

From equations (1) and (2):

$$\left({}^{A_1}_{Z_1}M\right) + \left({}^{A_2}_{Z_2}M\right) - 2\left({}^{A_s}_{Z_s}M\right) = E_s - E_{12} = \Delta E \qquad (3)$$

No protons are lost in fission and, therefore:

$$Z_1 + Z_2 = 2Z_s \qquad (4)$$

and because the average number of neutrons in symmetrical fission is about equal to that from unsymmetrical fission:

$$A_1 + A_2 = 2A_s \qquad (5)$$

In Fig. 1, ΔE from equation 3 is plotted against the total mass with $Z_s = 46$ (for symmetrical fission of ${}^{236}_{92}U$), and $Z_1 = 38$, $Z_2 = 54$, for all the known masses (on the carbon-12 scale) subject to condition (5). Lines connect points for mass differences $(A_2 - A_1)$ for combinations of nuclides which differ by equal numbers of neutrons. Heavy lines show mass differences for combinations of nuclides which are nearly equally deficient in neutrons with respect to their most stable isotopes, that is, when:

$$(A_2^* - A_2) \simeq (A_1^* - A_1) \qquad (6)$$

where * indicates the most stable mass. The graph suggests that extrapolation of the heavy lines to a total mass of 236 (the mass of the compound nucleus before fission or neutron emission) would lead to combinations of nuclides with a high negative value of ΔE.

In Fig. 2 ΔE for the other combinations of Z_1 and Z_2 have been plotted for highly unsymmetrical to symmetrical combinations of nuclides. Values of A have here been chosen subject to conditions (5) and (6). Fig. 2 suggests that linear extrapolation to higher masses would lead to high negative values of ΔE only for the elements lanthanum to tellurium and their complements (atomic numbers 57–52 and 35–40). If such an extrapolation is valid, it may be associated with the observations that the symmetrical fragments and also the highly unsymmetrical fragments are formed in low yield and with low kinetic energy,

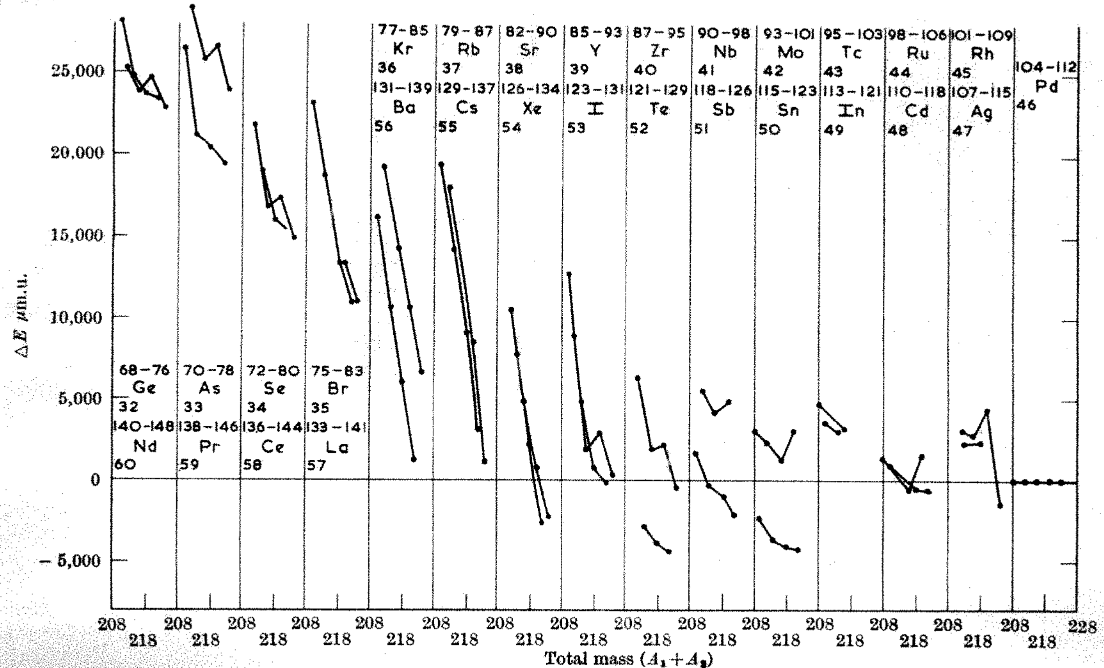

Fig. 2. Mass differences between unsymmetrical and symmetrical fragments

because there is less energy released in the formation of these fragments than in the formation of fragments with atomic numbers in the range shown. In addition, Fig. 1 gives a qualitative indication that the highest release of energy would occur in fission when nuclides are formed which are equally deficient in neutrons, and this is in accordance with the equal-charge displacement hypothesis for the distribution of charge in fission[6].

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ENGINEERING

Non-Linear Transmission Lines for Millimetre Wave Generation and Reception

THE properties of non-linear transmission lines, containing, for example, ferroelectric material of the barium titanate class, have been examined in connexion with a computing system[1]. Their possible use as a variable capacity parametric amplifier element is obvious, and it has been shown recently that an electromagnetic shock-wave can be propagated[2]. This effect occurs when a signal of large amplitude is injected into the line. The high-field part of the wave travels at a greater speed than the low-field part so that a sine wave becomes a saw-tooth. After a certain time this has a vertical leading edge analogous to a shock-wave in a gas. Such a wave is rich in harmonics and a number of lines, driven with power derived from the same microwave magnetron and with outputs recombined through phase shifters, could be an efficient harmonic generator.

A less obvious method of generating higher frequencies with a non-linear line is as follows: Suppose a low-level microwave signal is fed into a wave-guide filled with ferroelectric, and that this guide is constructed so that it is possible to apply a d.c. bias voltage to change the permittivity of the dielectric. Suppose the signal pulse duration is for a certain time and that during this time the bias is changed so that the velocity of wave propagation increases. The emergent signal will then occupy a shorter time, but as it contains an unchanged number of oscillations the frequency will be increased. Also as energy is conserved the power will increase, if the losses can be neglected.

The contrary case, where the wave is slowed down during the pulse so that the pulse is stretched in time and reduced in frequency, is also of interest. In principle this would permit millimetre waves to be converted to a more easily detected frequency. These effects are large if the switching time of the bias voltage is of the order of the time taken by a wave to pass along the transmission line, which imposes some technical limitations.

However, the chief difficulty at present is in the ferroelectric material. Measurements at 10-cm. wavelength of permittivity variation with bias have shown that the effect is still appreciable in a barium–strontium titanate ceramic[3]. Measurements at 3-cm. wavelength showed that relative permittivities of several thousand are still obtained[4]. There appears to be no published work on these materials at higher frequencies and with d.c. bias applied.

I hope to make some measurements along the lines indicated in this communication at shorter wavelengths than 3 cm. As I have no facilities at present for material manufacture, these measurements will be initially with barium–strontium titanate supplied by an industrial research laboratory. I would be interested to hear from any organization producing titanates or the newer ferroelectric materials which would care to collaborate in a programme of millimetre wave measurements.

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GEOLOGY

Carbonatitic Lava from Fort Portal Area in Western Uganda

IN an excellent paper Holmes[1] quotes chemical analyses of two spectacular carbonated lavas, one from the Katwe region, the other from Kalyango volcano near Fort Portal. In earlier work, Holmes and Harwood[2] described a similar carbonated lava from Lake Kyekora, also near Fort Portal. Present examination of a vesicular lava of high carbonate content poured out from Kalyango volcano shows close geochemical affinities with intrusive carbonatites. It must be emphasized that the lavas examined are fresh and unaltered and that the carbonated nature cannot be attributed to alteration after solidification. A proposed investigation of the numerous lavas in the Fort Portal field may elucidate the formation not only of the characteristic ultrabasic potassic volcanics of south-west Uganda and adjoining parts of the Congo, the petrogenesis of which Holmes has presented so admirably, but also the genesis of the carbonatites themselves.

The field relationships of the Kalyango and other similar 'carbonated' lavas have been described by Combe[3]. Extrusive equivalents of carbonatites are rare, one other occurrence being from Kerimasi volcano in Tanganyika[4].

A mineralogical, chemical and spectrographic investigation of the lava from Kalyango volcano shows the following characteristics: The rock is light-grey in colour, variably vesicular with phenocrysts of biotite up to 1 cm. across. It contains frequent inclusions of basement rocks and occasional rounded, possibly eclogitic fragments consisting of a dark-green clinopyroxene and pink garnet. The dense groundmass is powdered with magnetite and the vesicles are lined with a crust of small crystals of calcite and are often filled with spherules and rosettes of fibrous

Table 1. CHEMICAL ANALYSES OF CARBONATITIC LAVA FROM UGANDA

	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	FeO	MnO	MgO	CaO	Na_2O	K_2O	H_2O+	H_2O-	P_2O_5	CO_2	Total
1.	12·99	1·74	3·03	7·93	4·44	0·40	8·55	35·97	0·73	0·20	3·45	1·65	3·32	14·79	100·62
2.	13·81	1·96	3·20	8·05	4·33	0·44	8·65	35·42	1·03	0·17	3·76	2·89	3·57	11·27	100·05

1. Vesicular carbonatitic lava from a flow, Kalyango Volcano, Fort Portal. Total includes SrO = 0·63, BaO = 0·15, S = 0·35, F = 0·3.
2. Vesicular carbonatitic lava rich in zeolites, same locality. Total includes SrO = 0·60, BaO = 0·20, S = 0·40, F = 0·3.

(Analysis by O. von Knorring.)

zeolites. Under the microscope the ground-mass has a felt-like appearance containing minute grains of pyroxene, olivine, biotite, magnetite, some ilmenite, apatite and calcite. The larger clinopyroxene and biotite grains are seen to be corroded and the latter are often replaced by calcite along the cleavage. In polished sections magnetite and pyrite are the most conspicuous ore minerals. A partial analysis of magnetite showed some 11 per cent titanium dioxide; in reflected light, however, no exsolution lamellæ of ilmenite were seen. From partial analysis, the biotite is a magnesia-rich variety with a high titania content, and rather similar in composition to a biotite recorded by Combe and Holmes[5].

Among the major constituents of the lava (Table 1) silica and alumina are extremely low compared with other ultrabasic igneous rocks. Low silica contents of this kind are known in certain carbonate-rich alnöitic dyke rocks, for example in the so-called aillikite from Aillik Point in Labrador[6,7]. The calcium content is high, and most of the calcium is combined in silicates. The carbon dioxide content appears to be variable, and therefore lavas with even higher carbonate contents are possible, as shown by the almost pure carbonatite analysis by Holmes. Phosphorus, usually a minor constituent, is extremely high in the lava.

The pattern of the minor and trace elements is typical of carbonatite rocks. A feature common in some of these rocks is the dominance of strontium over barium, in addition to high lanthanum and niobium contents. In carbonatites the latter element often forms pyrochlore; in this case, however, a black, opaque perovskite in cubes 0·01–0·05 mm. across is believed to be the principal carrier of niobium.

The spectrographic results of the lava (Table 2) show, on one hand, similarities with rocks of the Toro-Ankole field, for example the katungites, and on the other close resemblance to carbonatites as determined by Higazy[8].

Table 2. SPECTROGRAPHIC ANALYSES OF CARBONATITIC LAVA FROM UGANDA (P.P.M.)

	Li	Sc	V	Cr	Co	Ni	Ga	Y	Zr	Nb	Mo	La	Pb
1.	19	13	200	35	45	45	14	65	550	550	7	600	40
2.	14	—	250	30	25	50	12	85	800	550	7	600	45

(Analysis by Miss J. M. Rooke.)

A carbonatite magma, containing water and other volatiles like phosphorus, fluorine and sulphur, can effectively react with silicate rocks during intrusion through the sialic crust to produce the characteristic suite of rocks encountered among carbonatites and associated alkaline complexes. As a result of this interaction a part of the alkalis are released and may migrate to induce metasomatism in the country rock, as often expressed in the well-known phenomenon of fenitization. Selective concentration of alkalis in addition to differentiation may, moreover, result in sodium- and potassium-rich rocks.

The present carbonatitic lava provides further evidence for the magmatic nature of carbonatites and supports the hypothesis advanced by Holmes, that a carbonatite (magma or fluid) is instrumental in producing the remarkable group of volcanic rocks in south-west Uganda and the Congo.

We are indebted to Prof. W. Q. Kennedy for his interest in the work, Mr. A. Cawley, director of the Uganda Geological Survey, for permission to publish this communication, and to Miss J. M. Rooke for the spectrographic analyses.

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CRYSTALLOGRAPHY

Structure of Molybdenum Sesquisulphide

THE existence of molybdenum sesquisulphide, Mo_2S_3, as a distinct phase has been in doubt for a considerable time. It was only in 1955 that McCabe[1] convincingly demonstrated the existence of the phase and obtained its X-ray powder pattern. An attempt to index the powder pattern has been made by Stubbles and Richardson[2]; they tentatively propose a tetragonal unit cell with $c = 10·9$ Å.; $c/a = 1·27$.

I have undertaken a new investigation of Mo_2S_3 by X-ray powder methods, using a Philips diffractometer. It was found that the true symmetry of the phase is monoclinic; the unit-cell dimensions are: $a = 8·633_5$ Å.; $b = 3·208$ Å.; $c = 6·092$ Å.; $\beta = 102·43°$. (Evidently, Stubbles and Richardson[2] were misled by the coincidence that a is almost exactly equal to $\sqrt{2} \times c$.) With a cell content of Mo_4S_6 the density is calculated as 5·806 gm./cm.^{3}.

The lines 010 and 030 are not observable in the powder diagrams, while 020 and 040 are very strong. Furthermore, the intensity sequence of the reflexions $h0l$ is found to be the same as that of $h2l$ and $h4l$; the same is true of $h1l$ and $h3l$. This indicates that Mo_2S_3 crystallizes in the space-group $P2_1/m$, with all atoms in the special position $2(e)$: $\pm (x, \frac{1}{4}, z)$. The intensity relations mentioned here made it possible to divide the intensities of the (not too numerous) pairs of coinciding powder lines. Thus, a complete set of experimental structure factors covering the copper-$K\alpha$ sphere was obtained and the structure could be determined by the usual methods.

A three-dimensional electron-density map is shown in Fig. 1. The sulphur atoms form a somewhat distorted close-packed lattice with layer sequence *chh*. The metal atoms are inserted in part of the octahedral holes of this lattice. The holes between neighbouring h-layers are completely occupied, while only half the

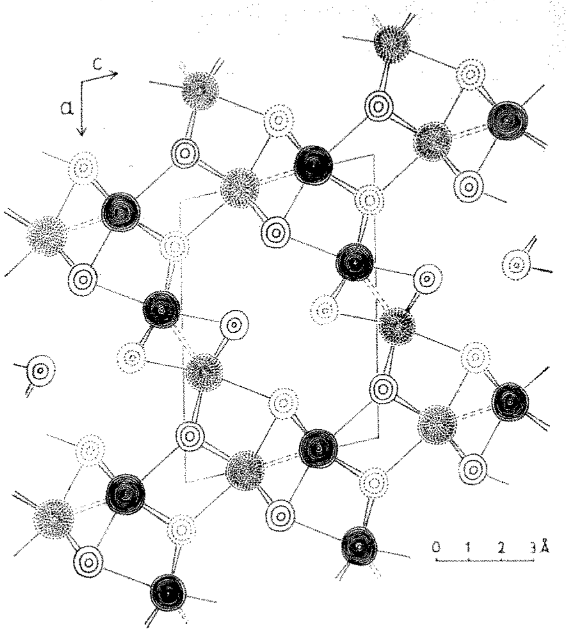

Fig. 1. Electron density of Mo_2S_3. Contours are at approximately 10 el./Å.^{3}. Full-line contours refer to atoms at $y = \frac{1}{4}$; broken contours to atoms at $y = \frac{3}{4}$. Mo—S bonds are indicated by full lines, the Mo—Mo chains by broken lines. The outlines of the unit cell are also indicated

holes between the other pairs of neighbouring sulphur layers are filled. Thus, the sequence of layers in the *a* direction is: . . S Mo S $Mo_{1/2}$ S $Mo_{1/2}$ S Mo S . . .

The molybdenum atoms are not in the centres of the sulphur octahedra; they are shifted by 0·32 Å. in the direction of one of the octahedron faces, in such a way that zig-zag Mo—Mo chains running in the *b* direction are formed. Within these chains the Mo—Mo distances are 2·85 Å., which is slightly longer than in the metal itself. Each molybdenum atom has three sulphur neighbours at distances of 2·36 Å. and three more at 2·57 Å. In MoS_2, where the metal is in trigonal-prismatic surroundings[3], the Mo—S distances are 2·35 Å.

I wish to thank the Climax Molybdenum Company of Michigan for a gift of pure Mo_2S_3. The assistance of Dr. C. F. van Bruggen and Mr. H. Schurer in this investigation is gratefully acknowledged.

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CHEMISTRY

Incorporation of Trivalent Cations in Crystalline Potassium Chloride

DIVALENT metal halides such as calcium chloride, dissolved in alkali halide crystals, increase the ionic conductivity[1] and give rise to additional bands (the *Z* bands) in the absorption spectra of coloured alkali halides[2]. For some time there has been speculation concerning the possibility of incorporating trivalent cations and the effect of such additions on electrical and optical properties.

Little experimental information is at hand. Hayes and others[3–5] suggested, on the basis of paramagnetic resonance studies, that sodium fluoride grown from a melt with added chromium chloride contained the chromium as Cr^{++}; on irradiation, reduction to Cr^{+} was thought to occur. Schnerb and Bloch[6] found that potassium chloride crystals grown from aqueous solution containing added ferric chloride were opaque. Schulze[7] considered the dependence of the ratio of Sm^{++} to Sm^{+++} in potassium chloride, grown from a melt containing samarium chloride, on the pressure of chlorine over the melt.

Lidiard[8] showed that if the ionization potential associated with the removal of an electron from an atom or ion to yield a given oxidation state were greater than about 22 V., the element would not exist as a substitutional impurity in solid potassium chloride in the given oxidation state but in the lower one. Of elements having the stable + 3 state the third ionization potentials of which are known, only yttrium, lanthanum and cerium have potentials somewhat less than 22 V. The third ionization potentials of other rare earths are unknown: however, these elements, being similar to cerium, may dissolve in potassium chloride in the trivalent state. The boiling points of the lanthanon trihalides are all above 1,475°, so that evaporation from molten potassium chloride is negligible.

We have attempted to prepare single crystals of potassium chloride containing lanthanum, yttrium, gadolinium, dysprosium, samarium and neodymium; we have performed chemical analyses, examined the ionic conductivity, and studied the absorption spectra after additive and radiative coloration of a number of samples. Fifteen crystals were doped with gadolinium, four each with dysprosium and samarium, three each with lanthanum and yttrium, and one with neodymium.

Each of the anhydrous trihalides was prepared by drying the hydrate in a stream of dry hydrochloric acid mixed with nitrogen; the product was stored in a sealed vial and introduced into molten potassium chloride by breaking the vial under the surface of the melt. The composition of the melt was usually about 1 per cent in the added trihalide. Single crystals were grown by Czochralski's method. In the presence of air, the melt gradually became turbid due to formation of oxyhalide of the added element; some crystals were grown in an atmosphere of dry hydrochloric acid, which prevented this occurrence. The crystals frequently contained milky regions due to precipitation of the trihalide as a separate colloidal phase on cooling from the temperature of growth. Viewed along the axis of withdrawal of the seed-crystal, the white portion usually formed an X. The reason for this interesting observation is as yet unknown.

The crystals were analysed for rare earth elements (we include here yttrium and lanthanum) spectrophotometrically by means of the alizarin red *S* method due to Rhinehart[9]. Ionic conductivity was determined by means of a vacuum-tube volt-ohm-meter (supplied by the Precision Apparatus Co. Inc.), using platinum electrodes pressed against the faces of the sample crystal. Crystals were coloured additively by a method previously described[10]; a copper-target tube operated at 35 kV. was used for X-ray coloration.

The results of the chemical analysis clearly separated the crystal samples into two quite distinct categories. Those containing precipitated material as evidenced by a milky appearance contained from 0·90 to 133 × 10^{17}. rare earth ions/cm.^{3}. The former was doped with yttrium, the latter with gadolinium. Clear sections of crystal in no case contained more than 0·70 × 10^{17} cm.$^{-3}$, and in most cases the rare earth content was less than 0·50 × 10^{17} cm.$^{-3}$, the lower limit of detection. Dr. W. Hayes kindly examined clear sections of the gadolinium- and dysprosium-doped crystals using paramagnetic resonance, and reported no observable resonances attributable to trivalent ions. In the case of gadolinium, the sensitivity was such that 0·01 × 10^{17} trivalent ions/cm.^{3} would have been observed.

The ionic conductivity, and its temperature dependence, of the clear sections of all crystals was indistinguishable from that of crystals grown from recrystallized reagent-grade potassium chloride without added trihalide. Extrinsic conductivity of crystals containing colloidal precipitate was higher; calculations based on the data of Kelting and Witt, for a sample containing 28·5 × 10^{17} gadolinium ions/cm.^{3}, indicated that the conductivity at 370° corresponded to that due to 2 × 10^{17} trivalent ions/cm.^{3} or 4 × 10^{17} divalent ions/cm.^{3}. However, some of the additional conductivity may arise from irregularities in the crystal caused by the strain in the neighbourhood of the colloidal particles, and in view of the conductivity measurements on clear sections and the measurements of Hayes, we must conclude that the solubility of the trivalent cations at room temperature is below the limits of detection used here. The solubility of the rare earth elements in the divalent state may be somewhat greater, but in any event not greater than about 4 × 10^{17} ions/cm.^{3} at 370°. Experiments with radioactive tracers would be useful.

The absorption spectrum of additivity-coloured crystals doped with the trivalent metal halides exhibited no new bands resolvable by observation at 90° K. Crystals containing colloidal precipitate could not be coloured by treatment with potassium vapour. We presume that under the stringent reducing conditions, the rare earth element was reduced to metal; in fact, metallic specks were formed which were soluble in concentrated hydrochloric acid to give solutions yielding a strongly positive test for rare earth element with alizarin red *S*.

On the other hand, such crystals were coloured by X-irradiation. A broad band with a maximum at 325 mμ was observed in several crystals containing dysprosium and samarium in precipitated form. This band has not been obtained at room temperature in the case of undoped crystals, although Dorendorf[11] observed two small bands in this region in nominally pure potassium chloride irradiated with X-rays at −180°, which he designated V_6 and V_7. Either the rare earth impurity causes the band or enhances the stability of Dorendorf's bands at room temperature.

We conclude that the solubility of these trivalent cations is too small for them to exert any pronounced effects on the properties of potassium chloride crystals of ordinary purity. Their incorporation in potassium chloride of exceptional purity would probably be observable through their effect on extrinsic conductivity.

The assistance of F. Block and G. Robidart in obtaining and preparing the rare earth halides and that of Dr. W. Hayes, who measured the paramagnetic resonance absorption of several samples, is gratefully acknowledged.

This work was supported by the U.S. Office of Naval Research.

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Purification of Hydrogen Isotopes by Self-Displacement Chromatography at Low Temperatures

Gluekauf[1] has shown that protium and deuterium can be separated by displacement gas chromatography using columns of palladium black dispersed on asbestos. The most effective method was stated to be that of self-displacement, whereby the columns were initially charged at room temperature with a mixture of isotopes and then heated from the inlet end, progressively desorbing the gases. Because protium was more strongly adsorbed than deuterium, it functioned as the displacing gas in a displacement chromatography system, and the gases were desorbed as a band of deuterium followed by a band of protium. We have discovered that a similar method, using a column containing Molecular Sieve 5*A* initially at liquid-nitrogen temperature, results in an excellent separation of hydrogen isotopes with the difference that in this case the leading band consists of protium.

A column was prepared from two lengths of copper tubing each 10-ft. long by 3/16 in. bore, filling them with Molecular Sieve 5*A* of particle size 30–44 B.S.S. mesh and then activating the sieve by heating to 350° C. *in vacuo*. Each section was coiled into a helix of 4 in. diameter and they were joined together in series at a valve, which, with valves at the inlet and outlet ends, permitted isolation of both sections. The sections could be immersed separately in liquid nitrogen contained in Dewar flasks. Initially, the column was either evacuated, or filled with helium or deuterium at room temperature and isolated. It was then cooled and a measured volume of a hydrogen isotope mixture admitted to the first section of the column. The valve between the two sections was opened, and the column was slowly and progressively warmed from the inlet to the outlet end by successive lowering of the two Dewar flasks. The mixture absorbed at the inlet was thus caused to move along the column, and two bands of the pure isotopes developed. When the pressure inside the columns equalled atmospheric, the outlet of the second column was opened to a monitoring katharometer and the bands of displaced gases collected. The order of displacement was helium (if initially present), protium, a mixed fraction of both isotopes and finally pure deuterium. The trace obtained from the monitoring katharometer during the separation of a 10 per cent protium in deuterium mixture is shown

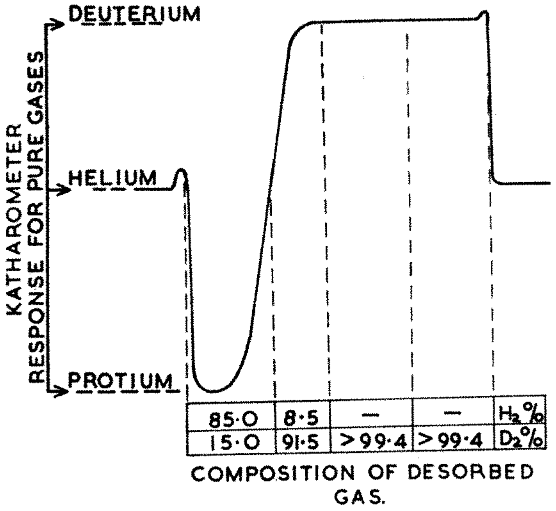

Fig. 1. Output of monitoring katharometer during self-displacement chromatography of a 10 per cent protium in deuterium mixture

in Fig. 1, the fractions collected being shown by the dotted lines. The fractions were analysed by gas elution chromatography[2].

If the original mixture is deficient in deuterium (the displacing gas) there may not be sufficient present to complete the displacement of the protium and in this case the purity of the deuterium band will not be as high. This was found to occur (Table 1), but even for a mixture containing only 25 per cent deuterium a considerable purification of deuterium was possible.

Table 1

Composition of original mixture	Fractions collected Composition H_2 (per cent)	D_2 (per cent)	Vol. (ml.)
90 per cent D_2, 10 per cent H_2	85	15	33·0
	8·5	91·5	40·2
	—	> 99·4	121
	—	> 99·4	101
25 per cent D_2, 75 per cent H_2	> 99·4	—	110
	99·4	0·6	79
	27·3	72·7	42
	10·6	89·4	50

The process is capable of operation on a larger scale and charges of 2·5 litres in volume have been processed using a first section of copper tubing 15 in. in length by 3/4 in. bore, packed with 1/16 in. pellets of Molecular Sieve 5*A* and the second of the same dimensions as used previously. The second section was effective as a front sharpener as well as contributing to band formation, by reducing the diffusion effects inherent in wider bore columns and hence the volume of the mixed band between the two fractions. Results with the larger columns were comparable to those with the small columns. For example, from 2·0 litres of mixture containing 8 per cent protium, 1·7 litres of deuterium containing 1 per cent or less of protium were obtained in a single run over a period of 1 hr.

The adsorptive capacity of the Sieve is 43·5 ml. of protium/gm. and 47·5 ml. of deuterium/gm. With such relatively high capacities, a laboratory-scale apparatus may readily be constructed which is capable of purifying several litres of hydrogen isotope mixture per hour. The process can be made repetitive, for after the first run the column requires no further preparation, the deuterium remaining being readsorbed on to the first section by cooling before the next charge is admitted.

With the limitation that the columns had to be filled initially with helium at atmospheric pressure, columns containing alumina impregnated with ferric oxide were found to operate in a similar manner, but their adsorptive capacity was less. Self-displacement chromatography using columns of pure alumina would appear to offer a method of separation of quantities of the spin isomers of either protium or deuterium as it has been shown they may be separated by elution chromatography on such a column[3].

We are grateful to the Managing Director of the Production Group of the United Kingdom Atomic Energy Authority for permission to publish this communication.

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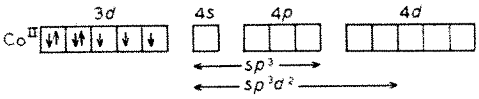
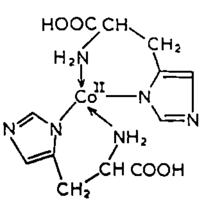
Structure of the Oxygen-carrying Cobalt (II) Histidine Complex

Bis-histidinato cobalt (II) is one of the few synthetic compounds which can combine reversibly with molecular oxygen. Hearon *et al.*[1] obtained a value of 4·44 Bohr magnetons (B.M.) for its effective magnetic moment at room temperature in aqueous solution, and showed that on oxygenation it became almost diamagnetic with the absorption of one oxygen molecule for two cobalt atoms. For three unpaired electrons the spin-only moment would be 3·88 B.M., and the considerably larger experimental value obtained in this case was ascribed to incomplete quenching of the orbital contribution to the moment. From the magnetic measurements Hearon assigned a tetrahedral structure (*sp*³ hybridization), I, to the complex:

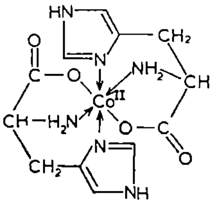


in which the cobalt atom was co-ordinated to the α-amino group and an imidazole nitrogen atom of each histidine molecule. However, histidine can behave as a tridentate ligand, and an octahedral configuration, II, involving co-ordination of ionized carboxyl groups is not unlikely. Hearon considered this to be only a secondary possibility.

Because of the differing orbital contributions, tetrahedral and octahedral cobalt (II) complexes generally have effective magnetic moments at room temperature in the ranges 4·3–4·7 and 4·8–5·6 B.M. respectively[2]. This would appear to confirm the tetrahedral structure of the complex, but Hearon has reported its aqueous solution to be pink, typical of octahedral rather than the commonly blue tetrahedral complexes.



(I)



(II)

We have prepared solid *bis*-histidinato cobalt (II) in oxygen-free conditions, using an apparatus already described[3], by concentrating the solution obtained by dissolving cobalt (II) hydroxide in an aqueous solution of histidine—the method used by Jaselskis[4] Our analyses agree with his formulation of the compound as the monohydrate The solid is pink with an effective moment of 4 81 B M at 291° K , it obeys the Curie–Weiss law between 80 and 300° K , and $\theta = 17°$, these values are comparable with those obtained for other spin-free, octahedral cobalt (II) complexes by Figgis and Nyholm[2b] The variation with temperature of molar susceptibility χ_M and effective moment μ_{eff} is shown in Table 1

Table 1

$T°$ K	86 2	116 3	147 8	176 2	204 7	232 1
$\chi_M \times 10^6$	29,750	22,850	18,630	15,900	13,890	12,320
μ_{eff}	4 55	4 63	4 71	4 75	4 79	4 80
$T°$ K	261 5	291 0	305 3	322 2		
$\chi_M \times 10^6$	10,910	9,833	9,453	8,956		
μ_{eff}	4 80	4 81	4 82	4 83		

The magnetic moment of the compound re-dissolved in water was 5 16 B M at 297 2° K , much higher than that recorded by Hearon The measurements were repeated on a solution of the complex prepared by mixing aqueous solutions of histidine and a cobalt salt, and a value of 5 13 B M was obtained This method was used by Hearon, but he allowed momentary exposure to the atmosphere during the filling of the susceptibility tube There is some inconsistency in the concentrations recorded by Hearon for his solutions and we believe that either crystallization or partial oxidation of the compound may account for the low moment obtained Our values of moment from the solution measurements are well within the range expected for octahedral spin-free cobalt (II) complexes, and agree with the value of 4 97 B M reported by Michaelis[5] for the complex in solution

The visible absorption spectrum of the complex in aqueous solution[1] is very similar to the spectra of octahedrally co-ordinated cobalt (II) complexes[6] and shows a weak band at 486 mμ ($\varepsilon = 18\ 4$) The reflectance spectrum of the solid has a peak at the same wave-length

In the infra-red spectrum of the cobalt histidine complex obtained in a 'Nujol' mull a broad, intense band appears at 1,587 cm $^{-1}$, which is unchanged on deuteration Since it is well below the 1,700 cm $^{-1}$ region where non-ionized carboxyl groups absorb[7], it must arise from the asymmetrical stretching vibration of ionized and probably co-ordinated carboxyl groups Consequently, structure I, which necessitates unco-ordinated non-ionized carboxyl groups, cannot be correct

These measurements show that the cobalt atom is in an octahedral environment and are in agreement with the recent work of Leberman and Rabin[8], who have deduced from potentiometric titration data that all three available donor groups of the histidine molecules are co-ordinated to the metal atom in complexes with cobalt (II), nickel (II), zinc (II), and probably cadmium (II)

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Some Tetrahydropyranyl Derivatives of Glycine, Serine and Urea

THE provision of model compounds containing amino-acids or their analogues attached to sugars is of importance in evaluating the stability of the numerous types of carbohydrate-peptide linkages theoretically possible in mucoproteins. The ability of 2,3-dihydropyran to condense with $-OH$, $-SH$, and $-NH_2$ groupings suggested a route to the glycosidically linked model compounds The tetrahydropyran-2-yl derivatives so formed had the added advantage that the lability of the glycosidic linkage could be investigated without attendant complications such as acyl migration under alkaline conditions or Amadori re-arrangement catalysed by acid which might ensue in the presence of a hydroxyl substituent at C–2 of the pyran ring

In the condensation between 2,3-dihydropyran and *N*-benzylsulphonyl-serine (DL-mixture) the tetrahydropyran-2-yl residue was introduced solely at the hydroxymethyl grouping, no evidence for reaction with the acidic hydroxyl was detected *N*-Benzylsulphonyl-serine (9 7 gm) in ether-ethyl acetate (1 1 v/v) was stirred at ambient temperature for 15 days with 2,3-dihydropyran (9 7 gm) in the presence of two drops of concentrated hydrochloric acid After neutralization (silver carbonate), the solution was evaporated and the residue extracted successively with water and ethyl acetate The latter extract yielded crystalline *O*-tetrahydropyran-2-yl-*N*-benzylsulphonyl-serine (10 4 gm), m p 130–132°, after five recrystallizations from ethyl acetate The optically inactive product contained C, 52 5, H, 5 8, S, 9 55 per cent and had an equivalent weight of 349 by titration, $C_{15}H_{21}NSO_6$ requires C, 52 4, H, 6 1, S, 9 3 per cent and an equivalent weight of 343 Addition of aqueous 0 5 *N* silver nitrate to a cooled (0°) methanolic solution of the serine compound precipitated the crystalline silver salt Found C, 40 8, H, 4 5, N, 3 1, Ag, 23 4 per cent, $C_{15}H_{20}O_6$-NSAg requires C, 40 0, H, 4 5, N, 3 1, Ag, 23 95 per cent Under similar conditions *S*-tetrahydropyran-2-yl-L-cysteine yields the silver mercaptide of cysteine[1]

Preparation of the *N*-glycoside adducts was rendered difficult by their instability An aqueous solution of glycine (10 gm), tetrahydropyran-2-ol (5-hydroxypentanal) (13 4 gm) and sodium bicarbonate (12 gm) was stirred at room temperature for 90 hr The residue obtained on evaporation (below

40° C.) was kept in a vacuum desiccator over phosphorus pentoxide for 3 days and further dried for 1 hr. at 63° C. Thereafter, the residue (4·7 gm.) was extracted with dry methanol and the crude sodium salt of *N*-tetrahydropyran-2-yl glycine precipitated with ether. After six such treatments a relatively pure but unstable amorphous product was obtained giving a positive Erhlich reaction[2] (without acetylacetone). On paper chromatograms irrigated with butan-1-ol/ethanol/water (4 : 1 : 5) it decomposed into a mixture of tetrahydropyran-2-ol (detected with a 2,4-dinitrophenylhydrazine spray[3]) and sodium glycinate (revealed by ninhydrin[4]). The corresponding ethyl and benzyl esters of *N*-tetrahydropyran-2-yl glycine were more stable and moved as chromatographically homogeneous components with R_F 0·89 and 0·90 respectively (cf. glycine ethyl ester R_F 0·4) as revealed by chlorine–potassium iodide treatment[5]. Kaverzneva and Kalis[6] have shown that the sodium salt of *N*-D-glucosylglycine is readily hydrolysed in aqueous solution at room temperature. Other workers[7] have demonstrated that *N*-D-glucosylglycine ethyl ester undergoes slow hydrolysis in aqueous solution at room temperature and is rapidly and quantitatively hydrolysed at 90° C. The pyranyl derivatives were prepared by reaction of the appropriate ester and dihydropyran for 17–20 days at room temperature in dimethylformamide (ethyl ester) or ether (benzyl ester, anhydrous magnesium sulphate added) containing a trace of mineral acid. A mixture of dihydropyran (2·1 ml.) and urea (0·6 gm.) in dimethylformamide (30 ml.) containing a trace of concentrated hydrochloric acid gave, after 96 hr. at 17° C., two products with R_F 0·70 and 0·93 (butan-1-ol–ethanol–water). The mixture was taken up in water (30 ml.), extracted with chloroform (6 × 50 ml.) which was back-washed with water (2 × 50 ml.). The dried chloroform solution contained the more mobile component which, after re-crystallization from acetone (twice), chloroform (twice) and ethanol (six times), yielded pure *NN'*-di-tetrahydropyran-2-yl-urea, m.p. 211–212° C., dropping to 201–202° C. after standing for 24 hr. The freshly isolated compound contained C, 57·9, H, 8·5, N, 12·3 per cent, $C_{11}H_{20}O_3N_2$ requires C, 57·9, H, 8·8, N, 12·3 per cent. The aqueous layer obtained after chloroform extraction of another urea-dihydropyran condensation was freeze-dried and separated on a cellulose column eluted with butan-1-ol/ethanol/water (4 : 1 : 5). The *N*-tetrahydropyran-2-yl-urea fraction (R_F 0·70) so obtained was repeatedly recrystallized from chloroform and ethanol to yield the pure compound, m.p. 187–188° C. (melting at 178–179° after 24 hr.). Found: C, 50·0; H, 8·3, N, 19·4 per cent, $C_6H_{12}O_2N_2$ requires C, 50·0, H, 8·3, N, 19·4 per cent. Hydrolysis of both pyranyl ethers at 100° with 'Zeo-Karb 225' (H^+) afforded urea and tetrahydro-pyran-2-ol (characterized as its 2,4-dinitrophenylhydrazone, m.p. 107–109° C.[8]). Benn and Jones[9] have characterized the products of the acid-catalysed condensation of D-glucose and urea as *N*-β-D-glucopyranosyl- and *NN'*-di-β-D-glucopyranosyl-urea.

The relative stability of the tetrahydropyranyl derivatives was determined by hydrolysis of an aliquot (8–10 mgm.) in 1 ml. of aqueous acid or alkali, neutralization and estimation of the tetrahydropyran-2-ol liberated using alkaline hypoiodite[10]. Control experiments showed that the original compound was stable under the conditions used in estimation. *N*-Tetrahydropyran-2-yl-urea was more stable under alkaline conditions [0·1 *N* sodium hydroxide for 15 min., percentage hydrolysis 30 (80° C.), 57 (100° C.)] than acid conditions [0·01 *N* hydrochloric acid for 15 min., percentage hydrolysis 11 (40° C.), 67 (60° C.), 87 (80° C.), 100 (100° C.)]. A similar stability was exhibited by *NN'*-di-tetrahydropyran-2-yl-urea [0·01 *N* hydrochloric acid for 15 min., percentage hydrolysis, 97 (100° C.). 0·1 *N* sodium hydroxide for 15 min., percentage hydrolysis, 61·5 (100° C.)]. *N*-Tetrahydropyran-2-yl-glycine decomposed in water within a few minutes, but was stable in ethereal suspension at room temperature. The stability of *O*-tetrahydropyran-2-yl-*N*-benzylsulphonylserine was not much different under corresponding acid [0·01 *N* hydrochloric acid for 15 min., percentage hydrolysis, 25 (40° C.), 71 (60° C.), 92 (80° C.), 100 (100° C.)] and alkaline conditions [0·01 *N* sodium hydroxide for 15 min., percentage hydrolysis, 37 (50° C.), 67 (60° C.), 100 (100° C.)].

We thank the Medical Research Council for a grant and one of us (S. M. A.) thanks the Council of Scientific and Industrial Research (Pakistan) for a scholarship. Our thanks are also due to Prof. M. Stacey for his interest in this work.

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Carcinogenicity of the Methyl-1,2-Benzanthracenes and their Derivatives

A number of theories have recently been put forward to account for the carcinogenic activity of the polycyclic aromatic hydrocarbons, in their present form, the majority of these theories do not provide a rationale for the fact that a number of chemically dissimilar carcinogens appear to operate through some common protein deletion principle[1]. Previous descriptions of a possible mechanism of carcinogenesis[2-4] have pointed out, however, that, in principle at least, the unifying mechanism by which such carcinogens act may be their formation of charge-transfer complexes with proteins, we are now able to discuss the activities of the methyl-1,2-benzanthracenes and the mono-fluoro-10-methyl-1,2-benzanthracenes (Fig. 1) in such terms.

The calculation of the electron distribution in these molecules has been performed within the framework of the usual linear combination of atomic orbitals–molecular orbital (L.C.A.O.–M.O.) method, where we describe the molecule by specifying which molecular orbitals are allowed for the π electrons and what are their energies. The orbital energies are given in the form

$$E_i = \alpha + k_{ij}\gamma$$

α and γ being the usual Coulomb and resonance integrals of the Wheland approximation The difference in energy between two levels i and j is quite simply

$$\Delta E_{ij} = (k_i - k_j)\gamma$$

and is therefore independent of α The values of the coefficients $(k_i - k_j)$ are listed in Table 1

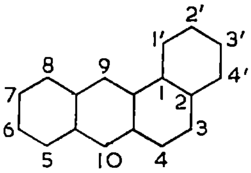

Fig 1 Numbering of the ring positions in 1,2-benzanthracene

In the case of the aromatic hydrocarbons, the criterion for activity was established[2,3] as

$$\Delta E_{1,\bar{2}} \approx 3\ 24 \pm 0\ 12\ \mathrm{eV}$$

This energetic criterion was taken as implying that for a π complex to describe the transition state of the hydrocarbon–protein interaction, matching of energy levels in the complex was necessary, the highest filled orbital of the hydrocarbon coincided with the lowest filled band of the protein while the second unfilled level acted as a resonating level for conversion of electrons from the valence band to the lowest unfilled level of the carcinogen An inspection of Table 1 now shows that the active range for the methyl-1,2-benzanthracenes and the fluoro-10-methyl-1,2-benzanthracenes is

$$1\ 3341\gamma < \Delta E_{1,\bar{3}} < 1\ 4462\,\gamma$$

There are two exceptions only in the case of the methyl benzanthracenes to activity as defined by this criterion both 5,8-dimethyl and 2′6-dimethyl 1,2-benzanthracene are allowed to be active whereas they are in fact inactive The experimental data for the mono-fluoro derivatives are less complete, their most interesting feature is the dependence of the activity on fluoro-substitution in the '*K*' region, for the 4 isomer is strongly carcinogenic while the 3 derivative is inactive[5,6] This is now consistent with theory which shows 3-fluoro-10-methyl-1,2-benzanthracene to fall well outside the active range with $\Delta E_{1,\bar{3}} = 1\ 2824\,\gamma$ For those molecules which remain to be tested theory predicts

(i) the 1′- and 2′-fluoro-10-methyl-1,2-benzanthracenes should be active, (ii) both the 5 and 8 isomers fall just a little outside our present 'active' range with $\Delta E_{1,\bar{3}}$'s of 1 3286 and 1 3327 respectively, since we have no molecules the activities of which are known with such low values of $\Delta E_{1,\bar{3}}$ we cannot be certain as to the probable activities of these compounds, and (iii) the 9-fluoro isomer has $\Delta E_{1,\bar{4}}$ lying within the active range There seems no reason why the fourth unfilled level should not be used as the resonating charge transfer-level so that we would predict this molecule to be active Evidence of inactivity would indicate that a common resonating level must be used by all the molecules of an isomeric series and would clearly be of considerable interest

TABLE 1 DIFFERENCES IN ENERGY BETWEEN THE HIGHEST FILLED AND SUCCESSIVE UNFILLED LEVELS IN UNITS OF γ, THE RESONANCE INTEGRAL

Molecule	$\Delta E_{1,\bar{1}}$	$\Delta E_{1,\bar{2}}$	$\Delta E_{1,\bar{3}}$	$\Delta E_{1,\bar{4}}$	Activity
5,9,10 Trimethyl-	0 8711	1 1025	1 3868	1 5509	+
6,9,10 Trimethyl-	0 8733	1 1129	1 3919	1 5593	+
9,10 Dimethyl-	0 8769	1 1154	1 3978	1 5637	+
8,10 Dimethyl-	0 8842	1 1266	1 4122	1 5752	+
5,10 Dimethyl-	0 8848	1 1244	1 4114	1 5747	+
5,9 Dimethyl-	0 8851	1 1312	1 4156	1 5292	+
1′10 Dimethyl-	0 8909	1 1484	1 4229	1 5895	+
10 Methyl-	0 8909	1 1379	1 4229	1 5885	+
5,8 Dimethyl-	0 8867	1 1376	1 4252	1 5860	–
9 Methyl-	0 9931	1 1463	1 4287	1 5966	+
5,6 Dimethyl-	0 8910	1 1469	1 4309	1 5956	+
5 Methyl-	0 8965	1 1517	1 4387	1 6021	+
3 Methyl-	0 8977	1 1592	1 4390	1 6048	+
8 Methyl-	0 8974	1 1552	1 4409	1 6038	+
4 Methyl-	0 8975	1 1562	1 4411	1 6039	+
6,7 Dimethyl-	0 8984	1 1569	1 4413	1 6062	+
2′6 Dimethyl-	0 8985	1 1605	1 4428	1 6071	–
7 Methyl-	0 9002	1 1587	1 4462	1 6097	+
6 Methyl-	0 9014	1 1644	1 4462	1 6133	+
3′6 Dimethyl-	0 9004	1 1671	1 4473	1 6119	–
3′7 Dimethyl-	0 8996	1 1618	1 4477	1 6087	–
4′ Methyl-	0 9022	1 1724	1 4480	1 6144	–
2′ Methyl-	0 9012	1 1631	1 4485	1 6114	–
1′ Methyl-	0 9046	1 1777	1 4523	1 6186	–
3′ Methyl-	0 9038	1 1703	1 4536	1 6167	–
1′ Fluoro-10-methyl-	0 8806	1 2190	1 4126	1 6184	?
2′ Fluoro-	0 8274	1 0728	1 3794	1 5061	?
3′ Fluoro-	0 8622	1 1402	1 4304	1 5736	+
4′ Fluoro-	0 8173	1 1397	1 3379	1 5542	Toxic
3 Fluoro-	0 7911	1 0519	1 2824	1 4560	–
4 Fluoro-	0 8116	1 0381	1 3462	1 4609	+
5 Fluoro-	0 8161	1 0097	1 3286	1 4836	?
6 Fluoro-	0 8205	1 0771	1 3341	1 5773	+
7 Fluoro-	0 8321	1 0482	1 3667	1 5271	+
8 Fluoro-	0 8035	1 0233	1 3327	1 6199	?
9 Fluoro-	0 7806	1 0802	1 2712	1 4141	?

Note For the methyl group, $>C_2-C_1\equiv H_3$, the following parameters have been used

$$\alpha_{C_2} = \alpha_C - 0\ 1\beta$$
$$\alpha_{C_1} = \alpha_C$$
$$\alpha_{H_3} = \alpha_C - 0\ 5\beta$$
$$\beta_{C_1\equiv H_3} = 2\ 5\beta_{C=C}$$
$$\beta_{C_1-C_2} = 0\ 7\beta_{C=C}$$

For the fluorine atom

$$\alpha_F = \alpha_C + 1\ 0\beta$$
$$\beta_{C-F} = \beta_{C=C}$$

The interpretation of the activities of the methyl benzanthracenes has always been one of the most demanding requirements of any electronic theory of the mechanism of carcinogenesis The success of the π complex model in this sense together with its explanation of the differences in activity between the 3- and 4-fluoro-10-methyl-1,2-benzanthracenes must be regarded as some further justification for its acceptance On the other hand, the original difficulties of the model remain The calculated energy-levels are based on an approximation which ignores the singlet-triplet separation of states arising through π electron excitation, moreover, this separation is not constant for all the carcinogenic alternant hydrocarbons although it is likely that it will be similar for the molecules discussed here There is also the problem of the effects of mutual polarization on the energy-levels of the protein and hydrocarbon and its bearing on the likelihood, or otherwise, of the presence of resonating levels in the π complex The attractiveness of the model undoubtedly lies in its simplicity and its ability to predict critical experiments Davis *et al* [7] have measured the enhanced semiconductivity of protein complexes and interpreted the results in a way consistent with the mechanism put forward here Spectroscopical investigations of the hydrocarbon-protein complexes formed *in vivo* would clearly be also useful in defining the nature of the interaction From the theoretical point of view, we hope to be able to examine the problem more realistically by the use of more sophisticated models for the carcinogens and protein since the present approach does not offer convincing evidence that the charge-transfer hypothesis is a unique, rather than the most economical, description of the carcinogenic interaction

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BIOCHEMISTRY

Hydrolysis of Hippuric Acid in Electrolytic Desalters

IN 1947 Consden, Gordon and Martin[1] published the design of an apparatus for the removal of salts from solutions of amino-acids and peptides by electrolysis Since that time this method has been frequently used for the preparation of samples for the analysis of urinary amino-acids by paper chromatography Whenever this has been done little account seems to have been taken of the fact that hippuric acid, which is present in relatively large quantities in some urines, is a fairly strong organic acid, and for that reason approaches the semipermeable membrane of the desalter at an early stage in the electrolysis, and is there hydrolysed to benzoic acid and glycine The purpose of this communication is to show that glycine released by the hydrolysis of hippuric acid is retained in the cathode compartment of the desalter, where it adds to the amount of free glycine already present in the urine, and that the increase in free glycine is dependent on the initial concentration of hippuric acid and salt.

In the course of work on the determination of amino-acids in urine using electrolytic desalting followed by paper chromatography[2-4], it was found that the mean 24-hr excretion of glycine from ten subjects of European stock was 3 43 m moles/24 hr , whereas the mean value obtained by Stein[5] using an ion exchange procedure was 1 76 m moles/24 hr , and that obtained by Gutman and Alexander[6] using a chemical method was 1 60 m moles/24 hr The fact that some microbiological methods were giving values in the region of 10 m moles/24 hr [7], and that these high values were attributed to a greater activity of the micro-organism concerned for hippuric acid, led to the possibility that the high results obtained in my experiments could have been due to the retention of glycine in the cathode compartment subsequent to the hydrolysis of hippuric acid at the semipermeable membrane

All the results quoted here were obtained with a small 'Perspex' electrolytic desalter using the same basic principle as that of Consden, Gordon and Martin[1], but designed for the rapid desalting of solution volumes not greater than 1 ml In each case a starting current of 0 9 amp and a voltage rising to about 200 V was applied to the electrolysing solution. The fact that the hydrolysis of hippuric acid and the factors affecting it are not peculiar to a particular design of electrolytic desalter was shown when similar results were obtained from the electrolytic desalter of Astrup, Stage and Olsen[8]

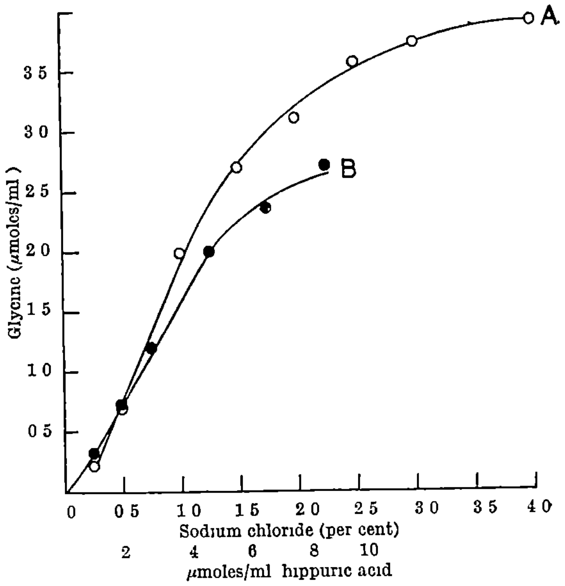

Fig 1 *A* shows the amount of glycine retained in the cathode compartment when the initial concentration of sodium chloride is varied and the initial concentration of hippuric acid is constant at 5 0 μmoles/ml *B* shows the amount of glycine retained in the cathode compartment when the initial concentration of hippuric acid is varied and the initial concentration of sodium chloride is constant at 1 0 per cent

The initial possibility that hippuric acid hydrolysis took place and resulted in the retention of free glycine in the cathode compartment of the desalter was examined by desalting a solution of hippuric acid in 1 2 per cent sodium chloride A sample of the desalted solution was chromatographed and glycine was found to be present A control was carried out by electrolysing a pure solution of hippuric acid for the same length of time, and this produced no free glycine Desalting a solution of glycylalanine in 1 2 per cent sodium chloride produced no free glycine, which would seem to indicate that neutral peptides are unaffected

A series of experiments was then carried out to determine the effect of changing concentrations of salt on the rate of hydrolysis of constant amounts of hippuric acid, and, also, the effect of changing amounts of hippuric acid in solutions in which the initial concentration of sodium chloride was kept constant The results of these experiments are shown in Fig 1 Each electrolysis was carried out by placing 1 ml of the test solution in the desalter No loss of glycine occurs during electrolysis in this desalter[9] The desalting time varied from 2 to 15 min depending on the initial concentration of sodium chloride

Curve *A* shows that the amount of free glycine retained in the cathode compartment approaches an equilibrium between 3 and 4 per cent sodium chloride for a constant amount of hippuric acid Curve *B* also shows a tendency to approach an equilibrium, and it is clear that the position of this equilibrium will be determined by the initial concentration of salt Unfortunately, it was not possible to extend curve *B* any further due to an uncontrollable amount of heat being evolved at higher concentrations of hippuric acid

All efforts to prevent the hydrolysis of the hippuric acid either by varying the starting current and voltage or the concentration of the sulphuric acid flowing through the anode compartment resulted in failure

No attempt has been made to examine experimentally the possible mechanisms involved in the retention of glycine in the cathode compartment, or of the hydrolysis of the hippuric acid at the semipermeable membrane It seems reasonable to suggest, however, that the basic factor involved is the very low *p*H which, according to Consden, Gordon and Martin[1], prevails in the region of the semipermeable membrane At the commencement of electrolysis the amount of chloride ion passing through the semipermeable membrane should be dependent on the initial concentration of sodium chloride This movement of chloride ions from the cathode towards the membrane is presumably balanced by a similar movement of hydrogen ions supplied by the sulphuric acid on the anode side of the membrane The concentration of hydrogen ions available in the region of the membrane would thus be determined by the amount of chloride ion approaching the membrane and would in turn determine the rate of hydrolysis of the hippuric acid together with the degree of retention of glycine in the cathode compartment

These results show that great care must be taken when examining paper chromatograms of urinary amino-acids after electrolytic desalting, since the amount of free glycine found on the chromatogram will be seriously affected to a varying degree depending on the initial concentration of hippuric acid and salt in the urine In general, electrolytic desalting should be avoided in any work involving the quantitative determination of glycine in urine

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Purification of Fluorescent Protein Conjugates : Comparison of Charcoal and 'Sephadex'

REMOVAL of unreacted fluorescent material from fluorescent protein conjugates is essential for unambiguous interpretation of microscopical findings in immunofluorescence[1] Many procedures have been suggested for conjugate purification, but extraction with powdered activated charcoal[2] and gel filtration with 'Sephadex'[3,4] are at present the only efficient methods suitable for general application These two methods have been compared as purification procedures for conjugates of lissamine rhodamine *B* (*RB* 200) and fluorescein isothiocyanate We have confirmed the inefficiency of dialysis[5] after exhaustive dialysis (7–10 days), conjugates still contain appreciable unreacted fluorescent material which can be removed by shaking with charcoal or passage through 'Sephadex'

The powdered activated charcoal supplied by British Drug Houses, Ltd, for general decolorizing purposes has been found to be the most efficient material for removal of unreacted fluorescent material It may contain soluble substances which affect the *p*H of the solution, but after thorough washing in phosphate-saline (0 15 *M* sodium chloride, 0 01 *M* phosphate, *p*H 7 1) and water, and drying at 100° C, it remains highly adsorbent The effect of shaking different amounts of charcoal for 1 hr with samples of the same conjugate has been examined by measuring the ratio of dye to protein concentrations This ratio decreases to a constant value for both *RB* 200 and fluorescein isothiocyanate conjugates, as the amount of charcoal is increased (Table 1) For *RB* 200 conjugates, 15 mgm charcoal per ml of original serum is sufficient to reduce the ratio to its lowest value, but for fluorescein isothiocyanate conjugates, at least 75 mgm per ml is required, double these quantities are normally used to allow for small variations in conjugates Only 5–10 per cent protein is lost from *RB* 200 conjugates, but the larger amount of charcoal for fluorescein isothiocyanate conjugates removes 30–40 per cent protein

Table 1 RATIO OF DYE TO PROTEIN IN CONJUGATES EXTRACTED WITH CHARCOAL (µGM /MGM)

Mgm charcoal per ml original serum	0	15	30	75	150	300
RB 200 conjugate	36	16	17	17	17	—
Fluorescein isothiocyanate conjugate	33	—	27	23	22	22

Purification by gel filtration is achieved by passing the centrifuged conjugate through a column of 'Sephadex *G*–25', which has been washed with phosphate-saline The protein conjugate is eluted by phosphate-saline and travels more rapidly than the unreacted fluorescent material A clear region develops between two coloured bands and the purified conjugate is collected first, there is very little loss of protein The elution is continued to remove unreacted fluorescent material and clean the column for further use Dilution of the conjugate (about three-fold) is of little consequence since the globulin fraction may be precipitated and reconstituted

The antibody content of conjugated rabbit serum (anti-bovine-serum-albumin) has been measured by precipitation before and after purification by charcoal and by Sephadex' The percentage recovery of antibody has been the same as the total yield of protein in each case, indicating that antibody is neither selectively removed from the conjugate nor denatured *RB* 200 and fluorescein isothiocyanate conjugates of rabbit serum (anti-klebsiella) and goat serum (anti-rabbit-globulin), purified by each technique and brought to equal protein concentration, were examined for specific and non-specific staining and compared with dialysed samples Both direct and sandwich staining were tested with a large series of histological and bacteriological preparations No significant differences were found between charcoal- and 'Sephadex'-purified conjugates, both gave substantially less non-specific staining than the dialysed conjugates

The convenience of a simple batch process recommends powdered activated charcoal as the most suitable routine purifying agent, it has always given uniformly satisfactory results with *RB* 200 conjugates Our original experience with fluorescein conjugates was less satisfactory[5], but since using pure fluorescein isothiocyanate for conjugation and adopting the present standard procedure of charcoal extraction, consistently good results have been obtained The 'Sephadex' method, although time-consuming and less simple, is as efficient as charcoal extraction and may be preferred for fluorescein isothiocyanate conjugates of any weak sera which cannot be handled by sandwich staining

We thank Dr G S Warner for gifts of pure fluorescein isothiocyanate, and Prof W O Kermack and Dr M G McEntegart for helpful advice The research programme has been supported by a grant from the Scottish Hospital Endowments Research Trust

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Gas Chromatography of Androst-16-en-3α-ol and Related Steroids

DURING the course of work on the *in vitro* biosynthesis of androst-16-en-3α-ol (androstenol, I) from 2-^{14}C-acetate or ^{3}H-acetate by rabbit and guinea pig testes[1], it was found that the highly radioactive saponifiable lipids present could be only partially separated from the slightly active I on alumina, using as eluant light petroleum/benzene (1 1, v/v) Although in later experiments the neutral lipids were removed by saponification before column chromatography, it was considered that the technique of gas chromatography might provide an alternative method of separating (I) in view of recent results obtained in the steroid field[2-5]

We have used as stationary phase a non-polar silicone gum, *MS* 2211 (Midland Silicones, Ltd.) This was dissolved in toluene and stirred with sufficient acid-washed 'Celite 545' (85–100 or 100–120 mesh) to give 0 5, 1 5, 10 and 15 per cent (w/w) as already described[1] Fig 1 shows the separation of (I)[6] from a mixture of fatty acids up to C_{18}, using 15 per cent silicone gum on 'Celite 545'. With this relatively high concentration of stationary phase a fairly good separation was obtained but the retentions of columns having only 0 5 or 1·5 per cent were too small to allow adequate separation Although such columns could well be of use in the analysis of bile acids (cf. VandenHeuvel, Sweeley and Horning[7]), they were not investigated further in relation to (I)

Using this 15 per cent column, an attempt has also been made to separate androstenol from a group of four steroids, similar to it both chemically and in their mobility on fully acetylated paper[1] These compounds, all generously supplied by Dr C L Hewett (Organon Laboratories, Ltd), were the 3β-epimer of I (androst-16-en-3β-ol, II), ætiochol-16-en-3α-ol (III), androst-5 16-dien-3β-ol (IV), and œstra-1 3 5 16-tetraen-3-ol (V) They were each applied to the column as 10 per cent (w/v) solutions in distilled methyl laurate, which contained enough myristic ester to serve as a reference substance Analyses were carried out at six temperatures within the range 145–225°, using appropriate argon flow-rates and suitable chart-speeds in the recording potentiometer[8] The results, together with the retention of each steroid relative to myristic ester and the calculated number of theoretical plates, are shown in Table 1.

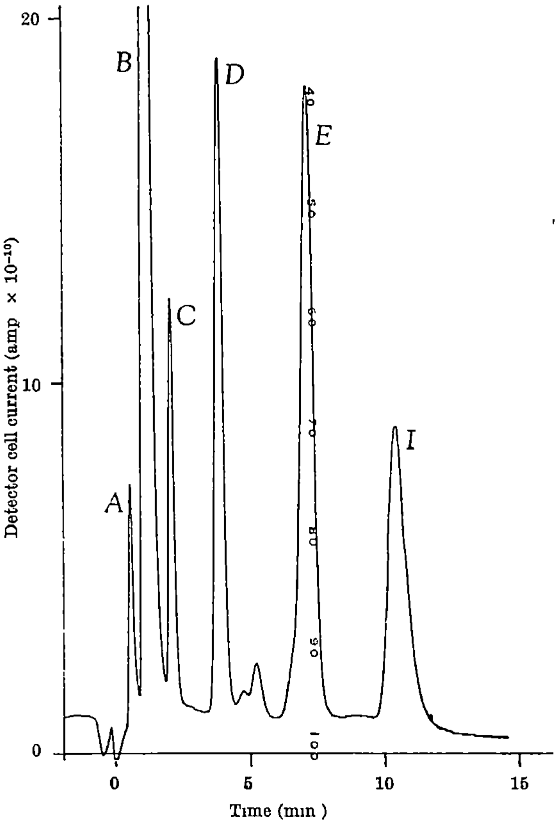

Fig 1. Vapour phase chromatogram of 10 μgm. androsten-3α-ol (I), showing separation from methyl esters of (*A*) capric, (*B*) lauric, (*C*) myristic, (*D*) palmitic and (*E*) stearic acids Stationary phase, 15 per cent silicone gum *MS*2211 on 'Celite', at 206° C , argon flow-rate 35 ml /min

Compounds III and V could be resolved from each other and from I, II and IV without difficulty, but the latter group of three steroids showed very similar properties and only partial resolution was achieved Separation did not alter significantly over this range of temperature. Evidence of the thermal instability of I, II and IV was noticed at 185°, increasing markedly as the column was run at higher temperatures Below 185°, however, the substances appeared stable even though they were retained for considerable periods

After plotting the reciprocal of the absolute temperature against $\log_{10}$ (relative retention), it was clear that effective resolution of I, II and IV from one another would be possible with silicone gum as stationary phase in the range 150–180°, provided that columns equivalent to 45,000 theoretical plates were used This should be possible with capillary

Table 1 RETENTIONS RELATIVE TO METYHL MYRISTATE OF SOME STEROIDS AT DIFFERENT TEMPERATURES ON A SILICONE GUM COLUMN

Temperature (° C)		145	153	164	185	206	225
Steroids*	I	6 18	6 31	5 94	5 37	5 04	5 02
	II	6 45	6 45	6 05	5 43	5 11	5 06
	III	—	—	—	4 86	4 47	—
	IV	6 48	6 21	5 82	5 25	4 96	4 96
	V	—	—	—	6 81	5 96	—
Gas flow (ml /min)		35	34	32	40	35	21
Theoretical plates (for methyl stearate)		4,210	1,950	2,240	1,460	1,200	930

* I, Androst-16-en-3α-ol, II, androst-16-en-3β ol, III, ætiochol-16-en-3α-ol, IV, androst-5 16-diene-3β-ol, V, œstra-1 3 5 16-tetraen-3-ol

columns about 50 feet long[9]. The preparation of such a column is in hand

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Effect of Insulin on Pathways of Glucose Oxidation in the Rat Lens

EXPERIMENTAL evidence as to whether lens is a tissue which responds to insulin has been inconclusive. Some investigators have found that glucose uptake by the isolated intact lens is increased after administration of insulin *in vivo*[1,2] However, neither a direct effect *in vitro* on glucose uptake could be demonstrated[1,3], nor did insulin influence the accumulation of labelled glucose by the intact lens[4] Recent work in this laboratory has shown that insulin causes a small but consistent increase of glucose uptake by the intact lens in Krebs–Ringer bicarbonate medium[5] Kinoshita and Wachtl[6] have found that lens is capable of metabolizing glucose by both the Embden–Meyerhof pathway and the hexose monophosphate shunt This communication deals with the effect of insulin on the two oxidative pathways in the rat lens and demonstrates an alteration of glucose oxidation in lenses of diabetic rats

Lenses of rats of body-weight 100–105 gm were excised as described in ref 5 Diabetes was produced by intraperitoneal injection of 14 5 mgm of alloxan/100 gm. body-weight; those animals having blood glucose-levels, when fed, of at least 350 mgm. per cent after three weeks were considered to be diabetic Glucose-1-^{14}C and glucose-6-^{14}C were obtained from the Radiochemical Centre, Amersham, England. Carbon-14 labelled carbon dioxide produced during incubation was collected and counted as barium carbonate on a windowless gas-flow counter, corrections to infinite thinness were made according to an experimentally determined self-absorption curve Results were calculated according to the following formula

$$\mu\text{moles of added glucose converted to } ^{14}\text{CO}_2 = \frac{\text{c.p m as barium carbonate}}{\text{specific activity of medium glucose}} \times 10^3$$

Values were multiplied by 10^3 in order to present the results as whole numbers since the absolute amount of conversion was low

The stimulatory effect of insulin on the oxidation of glucose-1-^{14}C to carbon-14 labelled carbon dioxide may be seen in Table 1 This activation of the hexose monophosphate shunt by insulin was small (about 20 per cent) but significant ($P < 0\ 01$) and was consistently observed in all experiments At the same time it may be seen that the oxidation of glucose-6-^{14}C to carbon-14 labelled carbon dioxide was depressed in the presence of insulin by about 50 per cent This finding, although unexpected, has also been noted in experiments with mammary glands[7]

Table 1 EFFECT OF INSULIN ON THE OXIDATION OF GLUCOSE-1-^{14}C AND GLUCOSE-6-^{14}C TO $^{14}CO_2$ IN RAT LENSES

	Insulin	No of exp	μmoles of glucose converted to $^{14}CO_2$ × 10^{-3}	Insulin effect (per cent)
Glucose-1-^{14}C	–	6	12 39 ± 0 43	
	+	6	15 01 ± 0 48	+ 21
Glucose-6-^{14}C	–	4	0 55 ± 0 04	
	+	4	0 29 ± 0 04	– 46

Incubation medium 1 2 ml of Krebs–Ringer bicarbonate buffer *p*H 7 4, containing 5 8–6 5 μmoles non-labelled glucose plus labelled glucose to give specific activities of 147,000–176,000 c p m /μmole glucose Insulin, when added, was 0 1 unit/ml Incubated at 37° for 2 hr in 95 per cent oxygen–5 per cent carbon dioxide Each Warburg vessel contained three lenses (average weight, 25 2 mgm) from rats fasted for 16 hr Potassium hydroxide was injected through a special rubber stopper at the end of incubation, and after addition of sulphuric acid from side-arm the carbon dioxide released was collected for 45 min

When these experiments were performed in a medium of Krebs–Ringer phosphate instead of bicarbonate, no insulin effect could be observed in either of the oxidative pathways

Investigations on lenses from diabetic rats (Table 2) indicated that the production of carbon-14 labelled carbon dioxide from glucose was depressed in the diabetic state by about 20 per cent The extent to which the oxidation of this sugar via the hexose monophosphate shunt was diminished in diabetes was of the same order of magnitude as the amount of activation observed when insulin was added *in vitro* to normal lenses No consistent alteration in carbon-14 labelled carbon dioxide production from glucose-6-^{14}C could be demonstrated in lenses from diabetic rats

Table 2 OXIDATION OF GLUCOSE-1-^{14}C AND GLUCOSE-6-^{14}C BY LENSES OF DIABETIC RATS

	Lenses used	No of exp	μmoles of glucose converted to $^{14}CO_2$ × 10^{-3}	Percentage of change in diabetes
Glucose-1-^{14}C	Normal	4	13 82 ± 0 78	
	Diabetic	4	10 84 ± 0 09	– 22
Glucose-6-^{14}C	Normal	6	0 40 ± 0 04	
	Diabetic	4	0 37 ± 0 06	none

Incubation medium and conditions the same as in Table 1, except rats were fasted for two days

The stimulatory effect of insulin on the hexose monophosphate shunt found in these experiments does not necessarily indicate that insulin is acting directly on the enzyme systems of this oxidative pathway. One of the present theories of insulin action is that it mediates in the transport of glucose into the cell It is possible that insulin might, in this case, facilitate the transfer of glucose to that site in the lens where the hexose monophosphate shunt is operating

Simultaneously with the increased activity of the direct oxidative pathway, a diminished production of carbon-14 labelled carbon dioxide from glucose-6-^{14}C takes place in the presence of insulin Similar shifts, but in the opposite direction, have been reported by Lerman[8,9], who found that the hexose monophosphate shunt was depressed in galactose-fed and in old rats while production of carbon-14 labelled carbon dioxide via the Embden–Meyerhof and Krebs cycle was increased Our experiments have shown that, in diabetes also, the hexose monophosphate shunt is depressed; the corresponding increase in carbon-14 labelled carbon dioxide production from glucose-6-^{14}C was, however, not observed, possibly because the absolute amount of radioactivity resulting from this pathway was so low that small differences could not be detected. It would appear that at least one common denominator exists in all three of these cataractogenic conditions, namely, a diminished activity of the hexose monophosphate shunt

This work was supported by research grant *B*-2298 of the Public Health Service, United States National Institutes of Health

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Isolation of an Unknown Auxin from a Solution of Indole-3-Acetic Acid labelled with Carbon-14

IT is desirable in certain investigations in metabolism to work with as stable a compound as possible, or use as an indicator of the basic structure of the compound some form of marker or tag Such is the case with plant growth regulators and auxins

In the case of indole-3-acetic acid (IAA) it is known that the carboxyl group is quite reactive and even the methylene group may be degraded Thus, there is advantage in labelling an atom in one of the rings This has been done by Pichat *et al* [1] at position 2 in the indole ring

This material was obtained for work on translocation of IAA in the *Avena* coleoptile Approximately four months after its synthesis very little activity could be found in the labelled material The substance was known to be unstable from radiolysis (internal irradiation from the isotope) by the carbon-14 on some part of the molecule

The solution contained 2 5 mgm of IAA in methanol Its specific activity was originally 6 9 mc /mm No suggestion has been made as to the degradation product(s) This may prove to be one of those recently suggested by Fawcett[2]

In order to determine the purity of the solution and to find out where the radioactivity was located, that is, if in the IAA or some breakdown product, a chromatographic separation of the methanol solution was made

This had been reported (private communication) by K V Thimann in June 1960 He had made a chromatographic analysis of the solution and found that most of the radioactivity was in a spot near the solvent front and very little in the IAA at 0 30 The method of Stowe and Thimann[3] was used with isopropanol/ammonia/water (10 1 1) to identify the auxin The chromatogram for the IAA solution gave two spots when sprayed with Salkowski reagent[4], one at about R_F 0·33 (for IAA) and another (salmon-orange colour) at about R_F 0 90

The strip was placed under a Geiger counter and read for radioactivity All of the counts were at R_F 0 90 and none at R_F 0 33 This corroborated the results of Thimann

A test was made to determine which compound was an active auxin The spots (before development with Salkowski) were cut from the paper strip, placed in cold peroxide-free ether in flasks in the refrigerator overnight The flasks (30 ml) were placed on a shaker for a short time, then the ether was evaporated down before being applied to 1 5 per cent agar medium

A Went *Avena* bioassay was run on these materials A 50 μgm /l sample was run as a control This gave a normal curvature of about 13°

The ether extracts from the IAA spot produced curvature However, when the eluted spot was sprayed with Salkowski after extraction, an easily detectable spot was developed This indicated that much of the IAA was not eluted from the spot by ether

When the same test was made on the *X* substance, curvature resulted On spraying with Salkowski reagent, no colour developed at the spot for the *X* compound

One further test was conducted to see if possibly the IAA was being carried along to the *X* spot and resulting in a tail between the two spots There was no radioactivity in any area other than at the *X* spot of R_F about 0 90

Strips were cut from the paper between the IAA spot and *X* spot and eluted with ether No curvature resulted from these areas, whereas it consistently occurred for the *X* compound

Because of the lack of more compound (original sample only 2 5 mgm) only three runs were made on the *X* compound and the blank areas between the two spots

An attempt was made to obtain an ultra-violet spectrum for the substance, but due to interfering (brown) degradation colours of indole derivatives, no satisfactory spectrum could be obtained

Although closer to the chemical structure of IAA, the new auxin described by Post[6], skatylglyoxal hydrate, does not appear to coincide in both requirements Its *Avena* curvature seems similar, especially in being more active than IAA, but the chromatographic analysis does not behave in the same pattern, although in no case was the solvent system exactly the same

An unknown, *X* compound was shown to contain all the radioactivity from an initial preparation of β-(^{14}C-2-indole) acetic acid This compound also possessed auxin properties as shown by its *Avena* coleoptile curvature ability Further investigations will be attempted when more of the labelled IAA is available and more quantitative runs can be made

Other compounds were run in both the bioassay and chromatographic analysis, but none possessed the characteristics of the *X* compound Such compounds as indole, skatole, mixtures of IAA and the former two, other indole compounds, and the two non-indolic auxins of Crosby and Vlitos[5], 1-docosanol and 1-octadecanol, were run None was found to fit the dual patterns of the *X* compound , none showed *Avena* curvature It does not seem likely that the two compounds of Crosby and Vlitos could be formed from the original compounds or the degradation products in the Pichat synthesis[1]

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Distribution of Trypsin Inhibitors in the Sera of Various Animals

TRYPSIN inhibitors have been found in pancreas, serum, colostrum, egg-white, soya bean, and lima bean[1] They have all been crystallized except from egg-white[2] Thus, examination of the distribution of trypsin inhibitors in proteins of them appears to be out of the question Recently, Nakamura *et al* [3] have demonstrated, by two-dimensional paper electrophoresis, that two trypsin inhibitors were contained in the extract of soya-bean Hence the distribution of trypsin inhibitors in other biological fluids deserves to be re-investigated to see whether there are also two inhibitors in them or not

In Fig 1 is shown one of the 'crossing diagrams' of human serum against trypsin The electropherogram shown was obtained by two-dimensional electrophoresis as follows Human serum was applied on line *AB* and the first electrophoresis was carried out in direction 1 A solution of trypsin was then applied on line *XY* and the second electrophoresis was carried out in direction 2 Under the given experimental conditions, trypsin migrates towards the cathode and serum proteins towards the anode. Thus the former migrates over the lines of the latter. As can be seen from Fig 1, the lines of serum proteins were partially digested by trypsin In this way the front of the trypsin line, which is named the crossing diagram' of the serum against trypsin, appeared across the zones of serum proteins It shows two grooves (or peaks, if viewed conversely), one in the zone of α_1-globulin and the other in that of α_2-globulin At the grooves in the line, trypsin is retarded The retardation of the migration of trypsin must have been caused by the reaction with substances in serum, which combined with it and inhibited its activity Therefore from the 'crossing diagram' of serum proteins against trypsin the distribution of trypsin inhibitors in serum can be investigated easily Fig 1 shows that human serum contains two trypsin inhibitors, one in α_1- and the other in α_2-globulin

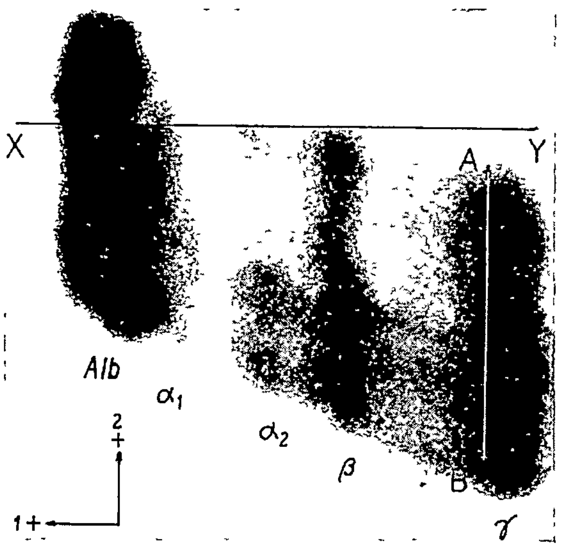

Fig 1 'Crossing diagram' of human serum against trypsin First electrophoresis, 0 04 ml /6 cm of human serum were applied on line *AB* and run at 100 V for 16 hr Second electrophoresis, after the first one, 0 1 ml /14 cm of 1 5 per cent crystallized trypsin solution was applied on line *XY*, and run at 100 V for 8 hr Barbiturate buffer, *p*H 8 6 and ionic strength 0 05 Stained with bromophenol blue

Table 1 shows some of the results obtained by the same method with sera of various animals From the results obtained so far, it can be inferred that sera of many animals contain two trypsin inhibitors (although some contain only one) and the distribution of them in the serum proteins is characteristic of the species The circumstances are the same as for the 'crossing diagrams' of serum against protein *J* of jack beans[4]

Table 1 DISTRIBUTION OF TRYPSIN INHIBITORS IN SERA OF VARIOUS ANIMALS

Animal	Albumin		α		β γ
Human		+++	+		
Dog				++	
Cat		++		++	
Horse	++		++		
Cattle			+++		
Sheep			+++	+	
Pig			+++		
Rabbit		+++		+	
Guinea pig		+++		+	

The number of (+) denotes rough estimation

The method of two-dimensional electrophoresis to obtain the 'crossing diagram' is not strictly quantitative, but from the height of the peak, if other conditions were the same, a rough comparison can be made between the sera of different animals In human sera particularly, the changes of distribution of trypsin inhibitors in diseases deserve further investigation

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Trehalose in the Cellular Slime Mould *Dictyostelium mucoroides*

IN 1925 Iwanoff[1] demonstrated the presence of the non-reducing disaccharide, trehalose, in isolated spores of the acellular slime mould, *Reticularia lycoperdon* (Myxomycetes). Recent work[2] on soluble carbohydrates in the cellular slime moulds have not mentioned the presence or absence of this sugar The present report concerns the occurrence of trehalose in the cellular slime mould *Dictyostelium mucoroides* (Acrasiales), and preliminary work on the role of this sugar during development

The fruiting body of *D mucoroides* consists of a long cellular stalk that bears a spherical mass of spores On germination each spore releases a small 'amœba' These amœbæ feed on bacteria, grow, and divide When the bacteria have been consumed, aggregation of the amœbæ occurs at various locations on the culture dish forming cigar-shaped masses of cells, pseudoplasmodia The pseudoplasmodia migrate and each constructs a stalk as it does so. Eventually migration ceases and those cells that have not become stalk cells are transformed into spores In certain strains of this species post-aggregative development results in the formation of a multi-cellular encystment stage, the macrocyst, instead of a fruiting body The present work is concerned mainly with spores and macrocysts, but vegetative amœbæ and pseudoplasmodia were also investigated

Spores were plated together with *Escherichia coli* on the nutrient agar medium of Bonner[3]. When fruiting occurred, the spores were harvested, washed, and homogenized in a glass homogenizer with 80 per cent methanol Macrocysts were collected by plating spores of the proper strain on the same medium and flooding the culture with about 3 ml of sterile distilled water Under these conditions very few fruiting bodies and large numbers of macrocysts were formed. The latter were collected, washed, and homogenized in 80 per cent methanol Following centrifugation the alcoholic extract was evaporated to dryness and redissolved in distilled water This solution was chromatographed on Whatman No 1 chromatographic paper for the separation, elution, and quantitative determination of carbohydrates[4] The solvent systems used were *n*-butanol/95 per cent ethanol/acetone/water (5 4 3 2), *n*-propanol/ethyl acetate/water (7 1 2), ethyl acetate/pyridine/water (8 · 2 1), and *n*-butanol/acetic acid/water (4 1 : 5)

When extracts of various stages of the life-cycle were analysed by paper chromatography, large amounts of an alcohol-soluble carbohydrate were detected in the spores and macrocysts but not in the other stages This substance migrated with known disaccharides and was shown to be trehalose by the following criteria it was non-reducing unless oxidized first with periodate[4] After elution from the chromatogram it reacted with the anthrone reagent[5] and on hydrolysis with 1 *N* hydrochloric acid liberated only glucose as judged by use of the glucose oxidase system ('Glucostat', obtained from the Worthington Biochemical Corp , Freehold, New Jersey, was used for the assay of glucose) When an eluted sample was co-chromatographed with authentic trehalose, no separation was observed in the four solvent systems listed above Treatment of an eluted sample with a partially purified preparation[6] of the specific hydrolytic enzyme, trehalase, readily hydrolysed the sample exclusively to glucose

Quantitative work carried out on isolated spores showed that trehalose constituted more than 7 per cent of the dry weight, and a similar concentration was found for the macrocysts. Trehalose could not be detected in the other life-cycle stages (limits of detection < 0 1 per cent of the dry weight) but small amounts of glucose were always present as judged by paper chromatography Fructose has been reported present in *D discoideum*[2], but we have not detected this sugar in our material It appears that trehalose and small amounts of glucose are the main, and perhaps the only, soluble carbohydrates present in *D mucoroides*

The restriction of trehalose to the spores and macrocysts implied that this sugar was utilized during germination Accordingly, we incubated spores for 12 hr under conditions conducive to germination, and then analysed the resulting amœbæ for trehalose content No trehalose was detected Although a more complete balance sheet is necessary before any conclusions can be drawn, it appears that trehalose might play some part in the germination process of this organism, possibly as an energy source for this activity

Trehalose has been found in large amounts in the resistant or dormant stages of several other organisms such as the ascospores of *Neurospora*[7], the eggs of *Ascaris*[8] and *Bombyx mori*[9], and the resistant embryo of *Artemia*[10] In addition, we have found large amounts of trehalose in the desiccatable eggs of *Aedes aegypti* and dried statoblasts of the bryozoan *Plumatella* (unpublished observations). Moreover, in the majority of these cases the amount of trehalose decreases sharply during the resumption of 'activity', again indicating a role for this sugar during the transition from the dormant to the active state We are at present extending our observations to resistant stages of representatives from other phyla

This work was supported by grants from the National Institutes of Health (*E* 2358) and the National Science Foundation (*G*–4549) One of us (J S C) received support from a National Institutes of Health predoctoral fellowship

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Structural Investigation of a Laminaran isolated from *Eisenia bicyclis*

In 1939 Nisizawa[1] isolated a soluble laminaran from the fronds of the brown alga, *Eisenia bicyclis* No further investigations, however, were made at that time, beyond observations on some of its chemical properties and behaviour towards enzymes Recent detailed structural investigations are now reported

In contrast to the modified Kylin method previously used by Nisizawa[1], the small pieces of frond were extracted with about ten times their volume of 1 per cent hydrochloric acid at room temperature for five days. The crude laminaran was precipitated from the extract by the addition of five volumes of

Table 1

Oligo-saccharide fraction	*F*1	*F*2	*F*3	*F*4	*F*5	*F*6	*F*7	*F*8	*F*9	*F*10
Yield (gm)	12	11 5	1 6	0 9	2 2	0 8	0 5	0 4	0 4	0 1
$[\alpha]_D^{20}$	+9 6	+20 4	—	−1 5	+2 4	−2 4	—	—	—	—
D P *	2 0	2 0	3 1	3 0	3 1	3 1	3 9	3 9	3 9	4 0
Disaccharide produced from each fraction †										
Laminaribiose	−	+	−	+	+	+	−	+	+	+
Gentiobiose	+	−	+	+	−	+	+	+	+	−

* Ratio of the reducing powers before and after complete hydrolysis

† Identified by paper chromatography

methanol. Purification was effected by repeated treatment with charcoal and precipitation with methanol from aqueous solutions

The final product, obtained as a white powder after electrodialysis, was very soluble in water and nitrogen-free (Kjeldahl) Ash (direct), 0·1 per cent Found, C, 44 44 per cent, H, 6·22 per cent, $(C_6H_{10}O_5)_n$, requires, C, 44 44 per cent, H, 6 17 per cent

Eisenia laminaran reduced hypoiodite equivalent to an average chain-length of 18 This is close to the value, 21, obtained by cryoscopy[1] and thus almost all molecules of this laminaran, in contrast to the insoluble[2,4,5] and soluble[2] laminarans from the genus *Laminaria*, appear to possess a reducing group No mannitol was detected by paper chromatography of the laminaran hydrolysate after removing glucose by alkaline degradation and by fermentation

The specific rotation of the laminaran was the same as that reported by Nisizawa[1], $[\alpha]_D^{20}$, − 45 5° (in water), although the extraction method was essentially different The acetyl derivative had $[\alpha]_D^{20}$, − 42 2° (in chloroform) Intrinsic viscosities were 1·35 (in water) for free laminaran, and 3 87 (in *n*-cresol) and 5 9 (in chloroform) for laminaran acetate.

The laminaran consumed 0 73 mole periodate and released 0 37 mole formic acid per mole anhydroglucose when oxidized by 0 03 *M* sodium metaperiodate at room temperature for 24 hr. These values were much higher than those for insoluble laminaran[3,4]

To obtain partial hydrolysis products, laminaran (150 gm) was acetolysed at 35° C for 96 hr and the mixture of deacetylated saccharides (121 gm) was fractionated by charcoal-'Celite' column chromatography Ten different oligosaccharides were isolated The yield $[\alpha]_D$, D P and the identity of disaccharides produced from the oligosaccharides on partial acid hydrolysis are shown in Table 1 Of the ten oligosaccharides obtained, two were disaccharides, four were trisaccharides and another four were tetrasaccharides. All these appeared to contain only β-1 3- and/or β-1 6-links Table 2 shows the periodate oxidation data for these oligosaccharides and their possible structures Maximum periodate consumption and formic acid release increased by 2 moles and 1 mole, respectively, for some oligosaccharides when 0 06 *M* periodate was used in place of 0 03 *M*, whereas the values for the others were the same at both periodate concentrations. The former group may be terminated by a reducing glucose residue which was subject to over-oxidation, possibly joined by a β-1 · 3-link, whereas the latter group may be terminated by a reducing residue which was resistant to over-oxidation, possibly being joined by a β-1 6-link. From the maximum values obtained on periodate oxidation and the results in Table 1, the fractions from *F*1 to *F*6 were identified as gentiobiose, laminaribiose, gentiotriose and 6-β-laminaribiosylglucose, laminaritriose and 3-β-gentiobiosylglucose, respectively

Table 2

Oligosaccharide fraction	Maximum value observed (moles) Periodate	Maximum value observed (moles) Formic acid	Possible structure	Maximum theoretical value (moles) Periodate	Maximum theoretical value (moles) Formic acid
*F*1	4 9 (4 8)	3 9 (3 8)	G1–6 (G)	5 (5)	4 (4)
*F*2	5 3 (3 2)	2 7 (1 9)	G1–3 (G)	5 (3)	3 (2)
*F*3	7 1 (6 8)	4 9 (—)	G1–6G1–6 (G)	7 (7)	5 (5)
*F*4	5 2 (4 9)	3 8 (3 7)	G1–3G1–6 (G)	5 (5)	4 (4)
*F*5	4 7 (2 6)	2 9 (1 8)	G1–3G1–3 (G)	5 (3)	3 (2)
*F*6	7 2 (4 8)	4 0 (3 3)	G1–6G1–3 (G)	7 (5)	4 (3)
*F*7	8 9 (8 7)	5 8 (6 2)	G1–6G1–6G1–6 (G)	9 (9)	6 (6)
*F*8	6 8 (6 9)	4 9 (—)	G1–6G1–3G1–6 (G) G1–3G1–6G1–6 (G) G1–3G1—6 (G) 6–1G	7 (7)	5 (5)
*F*9	7 0 (4 9)	4 0 (2 7)	G1–3G1–6G1–3 (G) G1–6G1–3G1–3 (G) G1–3G1–3 (G) 6–1G	7 (5)	4 (3)
*F*10	5 2 (2 8)	2 9 (2 7)	G1–3G1–3G1–3 (G)	5 (3)	4 (3)

Note G, β-linked glucose residue, (G), glucose residue with reducing group For the figures without parentheses, 0 06 *M* sodium metaperiodate, at room temperature, for 144 hr For the figures with parentheses, 0 03 *M* sodium metaperiodate, at room temperature, for 24 hr

Although 3 6-di-*O*-β-glucosylglucose would be expected to produce the same maximum values on periodate oxidation as laminaritriose when 0 06 *M* periodate was used, they may be differentiated on the basis of their maximum values in 0 03 *M* periodate since only laminaritriose would be expected to show a lowered value Furthermore, *F*5 did not give gentiobiose on partial acid hydrolysis and gave glucose after being oxidized with periodate and hydrolysed. This behaviour indicates the absence of 3 6-di-glucosylglucose The absence of this trisaccharide suggests in all probability a linear structure for *Eisenia*- laminaran Results with methylation also support this assumption Although three different methylglucoses were obtained, one of which was identified as 2 3 4 6-tetramethylglucose and the other two as 2 3 4- and 2 4 6-trimethylglucoses on the basis of their methoxyl contents and R_F-values, no dimethyl glucoses were found on the paper chromatogram Of the four different tetrasaccharides, *F*8 and *F*9 could not be identified, while the characteristic patterns of maximum values for *F*7 and *F*10 suggest that the latter two may be gentiotetraose and laminaritetraose, respectively It should be noted that gentiotriose and gentiotetraose have never been found in the partial acid hydrolysates of *Laminaria* laminarans[2,3]

The alkaline degradation of *Eisenia* laminaran in 4 *N* as well as 2 *N* sodium hydroxide solutions at 30° C ceased after the loss of about one-third of the weight of the laminaran, whereas periodate uptake and formic acid release by the residual polysaccharide was nearly 1 4 times that of untreated laminaran The part of the laminaran removed appears, therefore, to consist of β-1 3-linked glucose residues which would not contribute to the periodate consumption. The cessation of alkaline degradation may reflect the presence of a β-1 6-linkage in the molecule, as discussed in ref 6 Thus it may be suggested that *Eisenia* laminaran is a linear β-glucan with a D P. of about 20 containing both 1,3 and 1,6 linkages (in the approximate proportion 2 1), and since gentiotetraose was isolated from this laminaran by partial acid hydrolysis, at least three β-1 6-linked glucose residues must be present as a block among the β-1 3-linked residues, probably occupying about the central region of the molecule

We are indebted to Prof T Miwa for his interest and encouragement and to Dr B A. Stone, who kindly read this communication.

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Occurrence of Sedoheptulose in Carpophores and Basidiospores of *Inocybe* Species

ALTHOUGH basidiospores of various xylophilous, coprophilous and lignicolous Hymenomycetes germinate quite readily, and in high percentage, those from mushrooms with proved mycorrhizal associations rarely, if ever, respond to standard mycological techniques Among the latter are species of *Amanita, Lactarius, Cortinarius, Russula, Hygrophorus,* and *Boletus* After completing an extensive series of experiments on the above problem, Kneebone[1] concluded that the low respiratory-rate of the spores is probably not limited by a lack of metabolic reserves but rather by some deficiency of the organism which prevents efficient utilization of available respiratory substrates

We are investigating physiologically active constituents in the carpophores of various species of *Panaeolus* and *Inocybe* native to the Pacific northwest of the United States *Inocybe* species are thought to be mycorrhizally associated and have proved extremely difficult to sub-culture either from carpophore tissues or spores Information on the nature of the carbohydrate reserves of carpophores and spores was needed

In a paper chromatographic study of extracts of carpophores of 91 species representing 29 genera of fleshy fungi, Bonnet[2] found mannitol in 86, trehalose in 82, and glucose in 34 The results of Sussman and Lingappa[3] suggested that trehalose is the probable substrate the utilization of which is correlated with the germination of ascospores of *Neurospora tetrasperma* However, Kneebone had reported that the stimulatory effects of heat shock and of furfural on the germination of *Neurospora* ascospores could not be duplicated on spores of the mycorrhizal-associated fungi which he tested

Dried carpophores of several species of *Inocybe*, ground to a No 40 powder, were each shaken for 18–24 hr at room temperature with 20 ml of 70 per cent ethanol per gram of powder The marcs were discarded and the clear filtrates extracted with petroleum ether to reduce the fat and oil contents

Following evaporation *in vacuo* at 38° C , the residues were dissolved in 1 5 ml of 50 per cent isopropanol, transferred to vials and chilled overnight to remove interfering salts[4] Portions (25–50 μl) were spotted on sheets of Whatman No 1 filter paper and irrigated by the circular method of Rutter[5] in 150-mm Petri dishes with *n*-butanol/pyridine/water (6 4 3) or *n*-butanol/ethanol/water (10 1.2), for 2 and 7 hr respectively When processed with the ammoniacal silver nitrate spray of Partridge[6] or the periodate–permanganate spray of Lemieux and Bauer[7], three to five distinct zones appeared. One of these was identified as mannitol by comparison of its R_F value with that of reference mannitol

Volemitol (D-*manno*-D-*talo*-heptitol[8]) was isolated many years ago by Bourquelot[9] from carpophores of *Lactarius volemus* The possibility that seven-carbon saccharides might also occur in *Inocybe* species, plus the apparent specificity of colour reactions for ketoses reported by Benvenue and Williams[10], prompted us to utilize a spray containing orcinol After irrigation in the solvent systems previously mentioned, the papers were sprayed with water-saturated *n*-butanol–orcinol–trichloracetic acid[11] and heated for 10 min at 105° C Blue bands with low R_F values were noted from carpophore extracts of the following species of *Inocybe* · *picrosma* Stuntz, *kauffmanii* A H Smith, *cinnamomea* A H Smith, *lilacina* (Boud) Kauff , *mixtilis* (Britz) Sacc sensu Kuhner, *subdestricta* Kauff , *geophylla* (Fr.) Quél var *lateritia* Weinm , *agglutinata* Peck, and *lacera* (Fr) Quél *I albodisca* Peck, *I sororia* Kauff , and *I xanthomelas* Boursier and Kuhner in Kuhner did not show detectable amounts

The concentrations of the ketoheptuloses in the extracts were very low. *I kauffmanii* gave the strongest tests but our supply of dried carpophores was limited The mobilities of ketoheptulose from *I cinnamomea* and *I kauffmanii* extracts, which had been partially purified by a series of treatments including defatting, deproteinization, and ion-exchange were compared with those of crystalline standards of L-*gala*-heptulose, D-*gluco*-heptulose, D-*manno*-heptulose, and D-*allo*-heptulose Partially purified sedoheptuloses from *Cotyledon glauca* Baker and *Sedum spectabile* Bor were also included

A 48-hr descending irrigation technique, which allowed the solvent mixture (*n*-butanol–pyridine–water) to drip off the paper, was necessary for clear-cut separation Although comparative R_F values could not be obtained, the mobilities of heptulose from the two species of *Inocybe* were identical with reference sedoheptulose, separately and in admixture

The smooth-walled spores of *I cinnamomea* were subjected to low-temperature cavitation in a Raytheon oscillator (10 kc /s) About 60 min were required to disrupt approximately 90 per cent of 100 mgm of spores in 20 ml of water together with some 0 2 mm diameter glass paving beads The mobility of spore heptulose in the descending system was the same as that obtained from the sporocarps of *I cinnamomea* A second ketose was also present in the partially purified spore extract It gave a bright yellow colour with the spray containing orcinol and had a mobility similar to that of fructose Angular spores from *I mixtilis* and *I xanthomelas* were far more refractory to treatment and similar amounts did not give positive heptulose tests Much greater quantities of spores would be required to obtain enough heptulose for positive chemical identification This would indeed be difficult considering that approximately 6,000 caps of *I. cinnamomea*, averaging about 1 in in diameter, yielded only 800 mgm of spores

This constitutes the first report of the occurrence of sedoheptulose in extracts of mushroom tissues

The possibility that one of the oxidative pathways in these spores may proceed through the pentose-phosphate cycle remains to be proved

We are indebted to Dr N K. Richtmyer, National Institutes of Health, Bethesda, Md , for suggestions and supplies of several different crystalline heptuloses and heptulosans, and to Dr Howard Douglas, Department of Microbiology, University of Washington, for the use of the Raytheon oscillator The support of the U S Public Health Service grant No *RG*-7515 is gratefully acknowledged

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Inhibition of Dopamine β Oxidase by Adrenalone

WE have reported that several structural analogues of dopamine are inhibitors of dopamine β oxidase Dopamine β oxidase catalyses the conversion of dopamine to norepinephrine and the enzyme activity was determined by fluorimetric estimation of norepinephrine formed, by the method of von Euler *et al* [1] However, when adrenalone was tested as a possible inhibitor of dopamine β oxidase, the fluorimetric assay of norepinephrine was unsatisfactory This was due to the fluorescence quenching of norepinephrine by adrenalone The fluorescence quenching by other keto compounds has been reported[2], and such quenching may represent a serious obstacle to the fluorimetric determination of catecholamines in the presence of ketones such as adrenalone

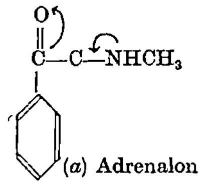
Therefore, in these investigations, where adrenalone was tested as a possible inhibitor of dopamine β oxidase, dopamine-^{14}C was used as a substrate The amount of dopamine-^{14}C which disappeared from the incubation mixture as well as the norepinephrine-^{14}C formed in the incubation mixture were calculated from the radioactivity of each of these compounds The incubation procedure as well as the separation of dopamine-^{14}C from norepinephrine-^{14}C was carried out as already described by us (ref 3) The degree of the rate of inhibition of dopamine β oxidase was determined by comparing the amount of norepinephrine-^{14}C formed and the amount of dopamine-^{14}C which disappeared in an incubation mixture which contained only the substrate and in an incubation mixture which contained adrenalone and the substrate

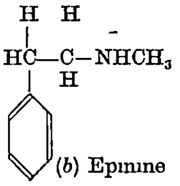
Table 1 shows the rate of inhibition of adrenalone on dopamine to norepinephrine conversion For comparison, the rate of inhibition of dopamine to norepinephrine by epinine is also presented in Table 1 It is evident that adrenalone is a more potent inhibitor of the dopamine to norepinephrine conversion than epinine

Table 1 INHIBITION OF DOPAMINE TO NOREPINEPHRINE CONVERSION BY ADRENALONE

Amount added (μmoles)			Norepinephrine formed	Dopamine disappearing
1 5	none	none	0 60 ± 0 06	0 80 ± 0 08
1 5	none	12	0 45 ± 0 05	not estimated
1 5	12	none	0 20 ± 0 02	1 2 ± 0 1

The structure of adrenalone (*a*) differs from epinine (*b*) only in that the former possesses a ketone group in the β position.


(*a*) Adrenalone


(*b*) Epinine

The electron-withdrawing effect of the carbonyl group in adrenalone will tend to make the nitrogen of the secondary amino group less basic This is consistent with our previous findings that primary amines (which are weaker bases) are also stronger inhibitors of dopamine β oxidase[3] A second possibility is that the electrophilic centre of adrenalone interacts with the nucleophilic centre on the enzyme surface

It should be stressed that while epinine itself is also a substrate of dopamine β oxidase and its inhibition of dopamine to norepinephrine conversion is due to a substrate competition, it is more likely that adrenalone is a non-competitive inhibitor

This work was supported by grants from the National Institutes of Health

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Acidic Dissociation of 7 : 9-Dialkylguanines and its Possible Relation to Mutagenic Properties of Alkylating Agents

Watson and Crick[1] proposed as a possible mechanism for mutation that changes in the tautomeric structure of a base of deoxyribonucleic acid (DNA) could induce its pairing with an anomalous base In considering the possible correlation between the action of alkylating agents on DNA and the mutagenic activity of these agents, we mentioned in a recent report[2] that it was not obvious how the initial action of alkylation of DNA, which has been shown to occur at $N_{(7)}$ of guanine moieties[3,4], could affect base-pairing in DNA according to the Watson–Crick model We therefore suggested that the subsequent loss of the alkylated guanine moiety from DNA (refs 4 and 5) might be the chemical action necessary for mutation to result

We now wish to present preliminary evidence that alkylation of guanine nucleosides or other 9-substituted guanines at $N_{(7)}$ results in an immediate change in the molecular structure of these bases at neutral *p*H in that part of the molecule involved in hydrogen-

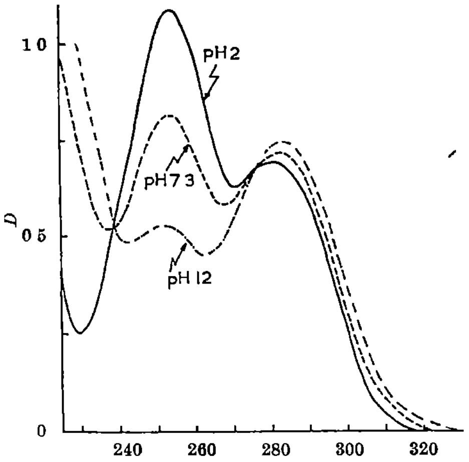
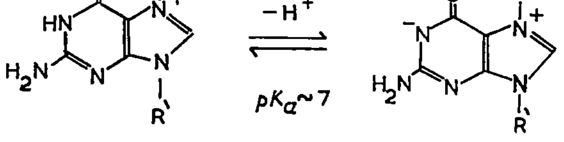


Fig 1 Absorption spectra of 7 9-di-(2′-hydroxyethyl)guanine (0 0267 mgm /ml) at *p*H 2, *p*H 7 3 and *p*H 12, 20°

bonding as required by the Watson–Crick model This follows from investigation of the acidic dissociation of 7 9-disubstituted guanines by spectrophotometric titration The absence of a basic *pKa* in the region of 3 for 7 9-dialkylguanines has previously been noted[6] However, with increasing *p*H in the region of *p*H 7 the absorption spectrum of 7 9-dialkylguanines changes, the change being complete at about *p*H 9, and these changes are consistent with the occurrence of an acidic dissociation in the region of *p*H 7, that is about two units of *p*H lower than for the corresponding 7 or 9-monosubstituted guanines Fig 1 shows spectra for 7 9-di-(2′-hydroxyethyl)guanine and Fig 2 for 7-methylguanosine

The spectrum of the molecular species of 7 9-disubstituted guanines with zero net charge, that is, at *p*H greater than 9, resembles that of 7- or 9-monosubstituted guanines in alkali This suggests that the proton has been lost from the $N_{(1)}$ ring-nitrogen atom Conventional formulation of these bases must therefore be as a zwitterion, as represented in (I), or with the negative charge on the extranuclear oxygen atom. It may be noted that the dissociated proton is normally required in formation of one of the hydrogen bonds between guanine and cytosine moieties of DNA according to the Watson–Crick model



(I)

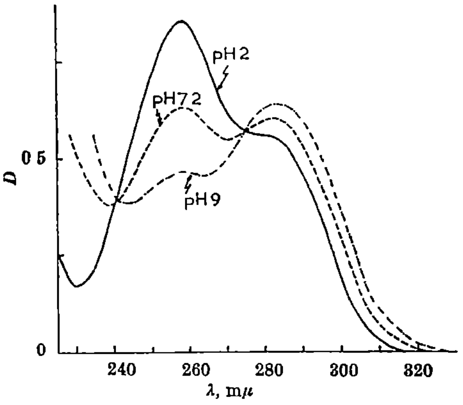


Fig 2 Absorption spectra of 7-methylguanosine (0 0234 mgm / ml.) at *p*H 2, *p*H 7 2, and *p*H 9, 20°

It appears, therefore, that at neutral *p*H the effect of alkylation of DNA at $N_{(7)}$ of guanine moieties would be to weaken the bonding between guanine and cytosine, since the tendency for the alkylated guanine to exist in the form shown in (I) would be much greater than for normal guanine Furthermore, it is possible that during duplication of DNA the specificity of base-pairing would be weakened, since the number of hydrogen bonds which could form between alkylated guanine in form (I) and cytosine would be reduced by one It may be noted that the configuration of (I) is such as to permit two hydrogen bonds to form with thymine, involving $N_{(1)}$ and the extranuclear amino group of the guanine moiety (Fig. 3) The configuration of this base-pair is such that it could fit into the DNA structure in place of a normal guanine-cytosine pair

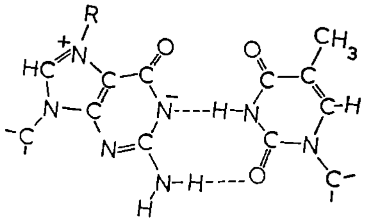


Fig 3 Possible base-pairing of alkylated guanine and thymine

We suggest, therefore, that the mutagenic action of the alkylating agents could be due to their ability to alkylate DNA at $N_{(7)}$ of guanine moieties, thus strengthening the acidity of the —NH—CO— group in alkylated guanine so that its dissociation would cause a tendency for alkylated guanine to base-pair with thymine instead of cytosine during DNA duplication. After such anomalous base-pairing, in subsequent DNA duplications an adenine-thymine pair would replace the original alkylated guanine-cytosine pair

This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U S Public Health Service

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PHYSIOLOGY

A Central Action of 5-Hydroxytryptamine and Noradrenaline

THE experiments reported here were based on the observation that an intra-carotid or intravenous injection of *d*-lysergic acid diethylamide (LSD-25) depresses the transmission of orthodromic impulses through the lateral geniculate nucleus of the cat[1]. The nature of this depression suggests that LSD-25 can compete with the excitatory transmitter for post-synaptic receptor sites. Consequently the known antagonism between LSD-25 and 5-hydroxytryptamine (5-HT) at receptors of smooth muscle[2] indicates the possibility that the as yet unknown central excitatory transmitter might be related to 5-HT. It is probable that the failure to demonstrate an action of 5-HT on these neurones[1] was due to the blood-brain barrier, which is relatively impermeable to this substance[3].

In the present investigation, 5-HT and a series of structurally similar compounds, including some catecholamines, have been applied electrophoretically to single neurones in the lateral geniculate nucleus of a cat anæsthetized with pentobarbital. The direct extracellular application of drugs circumvents the blood-brain barrier and the actions of four different compounds could be compared on single cells by the use of five-barrel electrodes[4]. The spike responses, 0·2–1·0 mV in amplitude and of negative–positive voltage sequence, were recorded by means of the central barrel of the electrode, which contained 4 *M* sodium chloride. Most substances were passed as cations from saturated aqueous solutions of *p*H 3–4. These included 5-HT creatinine sulphate, tryptamine, bufotenine, LSD-25, 2-bromo-LSD(BOL-148), psilocin[5], noradrenaline bitartrate, adrenaline bitartrate and 3-hydroxytyramine. L-Glutamic acid and psilocybin[5] were applied as anions from solutions of the sodium salt at *p*H 8.

When an electrophoretic current was used to apply cations from a saturated aqueous solution of 5-HT creatinine sulphate, there was suppression of the orthodromic responses of neurones which responded monosynaptically, and occasionally repetitively, to an optic nerve volley. Such was not the case when the electrode contained creatinine sulphate instead of the 5-HT creatinine sulphate complex, hence it is reasonable to assume that the depression observed with the complex was due to 5-HT. The latency of this effect was reciprocally related to the magnitude of the electrophoretic current and for currents of 20–100 mμamp was about 10–2 sec. Following the termination of the current flow the spike responses recovered in 5–20 sec. When L-glutamate was applied from another barrel, the excitation of the neurones, measured by the frequency of the firing evoked by the amino-acid[4], was unaltered by 5-HT. Furthermore, the antidromic spike responses of these neurones, evoked by stimulation of the optic radiation beneath the ipsilateral gyrus marginalis of the cerebral cortex, were unaffected by 5-HT. These actions of 5-HT are illustrated in Fig. 1.

All the substances mentioned here, except creatinine and L-glutamic acid, had actions identical with those of 5-HT. Although it is difficult to compare various substances quantitatively by these electrophoretic techniques[4], an approximate estimate of the potency of the compounds can be given. 5-HT was the most effective depressant of these cells, as determined by the magnitude of the electrophoretic current necessary to block the excitation of single cells within a given time. The other agents were less effective, the descending order of potency being tryptamine, bufotenine, LSD-25, psilocin and psilocybin, 3-hydroxytyramine, noradrenaline and adrenaline, BOL-148. The latter compound had a very weak action. Several experiments were carried out to test whether BOL-148 interfered with the effect produced by 5-HT and no antagonism could be detected. In addition to being less effective than 5-HT, some of the other active compounds were more prolonged in their action after the applying current was terminated. Thus the recovery from an application of LSD-25 using an electrophoretic current which produced depression in 30–40 sec often took 10–15 min.

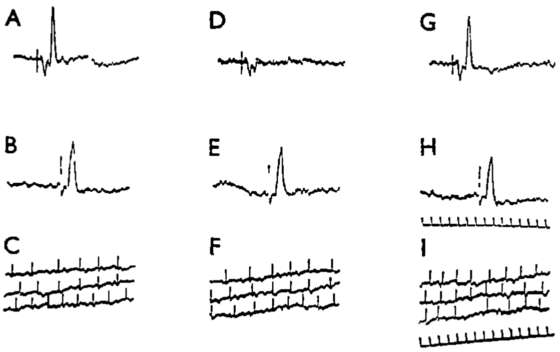

Fig. 1. Spike responses of a single neurone in the lateral geniculate nucleus responding monosynaptically to a contralateral optic nerve volley (*A*, *D*, *G*), antidromically to a stimulus to the ipsilateral optic radiation (*B*, *E*, *H*) and repetitively to L-glutamic acid (*C*, *F*, *I*) which was applied from one barrel of a five-barrel electrode using an anionic current of 100 mμamp. After the control responses *A* and *B*, a current of 20 mμamp. passed cations from an aqueous solution of 5-HT creatinine sulphate (*p*H 3·3) for 12 sec. The responses *D* and *E* were recorded 5–7 sec. after the current started and *G* and *H*, 5–7 sec. after it terminated. *C*, *F*, *I* were recorded with lower amplification (all spikes are approximately 0·5 mV. in amplitude) in another series. *C*, control; *F*, 31 sec. after a current of 20 mμamp. passed 5-HT creatinine sulphate into the environment of the cell. At this time orthodromic responses were suppressed. *I*, 2 min. after the 5-HT application ceased, the orthodromic responses having recovered fully. Time, msec. for *A*, *B*, *D*, *E*, *G*, *H* beneath *H*; 10 msec. for *C*, *F*, *I* beneath *I*

Several conclusions can be drawn from these observations.

The failure of 5-HT to affect either the antidromic firing of these neurones or the firing evoked by L-glutamic acid indicates that 5-HT does not significantly modify the membrane conductance of the cells. Thus it is very unlikely that it was combining with the sub-synaptic receptors of inhibitory synapses and activating the associated receptor membrane, or that it was depressing the electrically excitable membrane of the cells in some other fashion. It is probable that the depression of the orthodromic responses was due to a reversible interaction between 5-HT and the receptors at excitatory synapses which are specialized for the transmitter released from the optic tract terminals. However, the possibility that 5-HT was depressing the release of this transmitter cannot be excluded until the transmitter is identified and applied to the synapses.

The antagonism which is exhibited between 5-HT and the other compounds, when tested on smooth muscle, was not apparent at these central synapses. Indeed these 'antimetabolites'[3] had actions similar to that of 5-HT. Presumably all these depressants interacted with the same receptors, the potency and the duration of action of a particular compound being determined by the stability of the drug-receptor complex

The possible interaction of these substances with post-synaptic transmitter receptors suggests that the excitatory transmitter acting on the lateral geniculate neurones might be related structurally to them Experiments are proceeding with a large number of indoles and catecholamines in order to determine those structural features necessary for a compound to interact with the transmitter receptors

Grateful acknowledgment is made for the following gifts 5-HT creatinine sulphate, creatinine sulphate and bufotenine (Dr M E Speeter, The Upjohn Co , Michigan), LSD-25 and BOL-148 (Sandoz, Ltd , Basle), psilocin and psilocybin (Dr Hofmann, Sandoz, Ltd)

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Hormones and Electrolyte Excretion in Sheep

MERINO sheep in hot environments effectively reduce the rate of excretion of water but that of sodium and potassium may not change, and often increases during periods of dehydration[1]. Renal hæmodynamics may be concerned in this adjustment of kidney function but endocrine activity has been examined in the search for possible mechanisms

Merino ewes were accustomed to life in metabolic cages on a fixed feeding regime at room temperatures between 22° and 24° C Electrolyte excretion was followed throughout periods of 36 hr using a fraction collector to take specimens at ½ hr intervals There was considerable variation in excretion, mainly following morning feeding which produced a rise in sodium and potassium output by day and a low rate of excretion at night Sheep were therefore trained to take lucerne chaff during a period of 3 hr between 10 a m and 1 p m It was observed that within 20–30 min of beginning to feed, normally alkaline (pH 8 2–8 6) urine became acid (pH 5 2–6 6) for about 1 hr While feeding continued (Fig 1c) the urine returned to a pH of 8 2 Production of saliva with a high bicarbonate content could leave the blood depleted of alkali and an acid urine could result

Infusion of small amounts of hormone intravenously over an hour was undertaken Six hormones were examined Mixed arginine and lysine vasopressin ('Pitressin'), infused at the rate of 12 milliunits/min , had two types of effect At urine flows lower than 1 ml /min there was an increase of water, sodium, potassium and chloride excretion per unit of time (Fig 1a) At flows greater than 2 ml /min there was a reduction of flow of urine and increased output of electrolyte The osmolar excretion-rate increased at both high and low levels of urine flow under the influence of vasopressin This occurred both in the field (the tropics at 21° S latitude) and in the laboratory During diuresis the osmolarity of urine was 100–200 m osmol /l , and at low flows it was 1,400–1,800 m osmol Vasopressin produced urine between 500 and 1,700 m osmol /l always more concentrated than plasma

Lysine vasopressin gave identical results Although vasopressin does not produce the changes of urine flow found in a hot environment, it could contribute to the sodium excretion which can occur in such circumstances At both high and low rates of urine flow, vasopressin increased glomerular filtration (measured by creatinine clearance) so that differences in renal dynamics do not account for the phenomena It appears that the effect of the polypeptide is on electrolyte movements (mainly potassium) in the tubule.

Infusion of adrenaline at a rate of 15 µgm./min. into sheep with urine flows of 0 4–1 ml /min (Fig. 1b) led to excretion of 3–6 ml /min of water with no increase of electrolyte output In partially dehydrated animals there was little effect on water flow Noradrenaline infused at a rate of 5 µgm./min. increased water, sodium and potassium excretion. The effect of adrenaline in increasing water excretion. is the opposite of that found during dehydration Aldosterone increased the re-absorption of sodium in sheep given salt supplements (Fig. 1c) but it did not increase potassium excretion as in carnivores or man[2] There was no change in pH and little in water output under the influence of an infusion of 10–300 µgm d,l-aldosterone in the course of an hour.

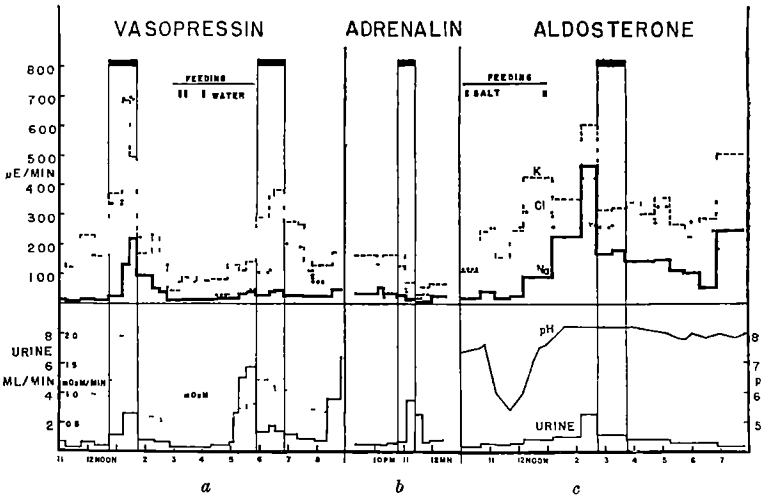

Fig 1 Infusion of hormones intravenously in sheep resulted in urine excretory changes those for potassium, chlorine and sodium being shown in the upper panel while water and osmolar excretion are shown below *a*, Lysine vasopressin (12 milliunits/min) increased low urine flow and decreased high urine flow, but osmolar and electrolyte flow increased in both *b*, Adrenalin (15 µgm /min) caused water to be excreted, but electrolytes were lost at a slower rate *c*, During feeding, urine pH fell, but rapidly returned to the normal 8 2–8 5 Ingestion of sodium chloride in 2-gm aliquots increased sodium excretion and this was reversed for 4 hr by 0 5 µgm /min d,l-aldosterone Excretion of potassium was not increased by this substance

The sheep is thus different from most laboratory animals and man[3] in having a large acid shift of its urine during feeding, in increasing the excretion of salts and water during the action of vasopressin, and in showing no increased potassium excretion under the influence of aldosterone

We are indebted to the Wool Trust Fund and the Rural Credits Development Fund of the Commonwealth Bank for support in this work, to the Ciba Laboratories for the supply of aldosterone, and to Sandoz Laboratories for lysine vasopressin

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Paradoxical Effect of Phlorizin on Glucose Uptake and Intracellular Accumulation of Amino-Acids in Rat Kidney Slices *in vitro*

THE discovery by von Mering[1] that phlorizin causes glycosuria in the dog and the observation that phlorizin prevents renal tubular re-absorption of glucose[2,3] have evoked considerable interest in this substance as a tool in the investigation of cellular transport mechanisms Much work with phlorizin has shown this drug to be a potent inhibitor of penetration of hexoses and other sugars in gut preparations[4,5], kidney slices[6], ascites tumour cells[7], and red cells[8] Significant alteration of intracellular metabolism has also been noted This literature has recently been reviewed by Lotspeich[9] and Crane[10]

We have reported[11] that rat kidney cortex slices possess an active transport system for the intracellular accumulation of amino-acids against a concentration gradient This system is dependent on oxidative metabolism as shown by the inhibition produced by anaerobiosis and dinitrophenol In view of the known inhibition by phlorizin of sugar penetration and re-absorption in the kidney, experiments were carried out to ascertain whether this drug similarly affected the penetration of amino-acids into kidney cells This communication demonstrates the unexpected finding that, in contrast to the inhibition of glucose uptake, phlorizin increases the intracellular concentration of amino-acids

Renal cortical slices from male Sprague–Dawley rats were cut with a Stadie Riggs microtome Following a 10-min pre-incubation at 25° C in isotonic Krebs–Ringer bicarbonate buffer, the slices (about 100 mgm /flask) were transferred to 25-ml Erlenmeyer flasks containing the amino-acids labelled with carbon-14 in 2 ml Krebs–Ringer bicarbonate buffer *p*H 7 4 The flasks were gassed with 95 per cent oxygen, 5 per cent carbon dioxide, sealed and incubated in a Dubnoff metabolic shaker at 37° C At the end of the incubation period the slices were dipped in isotonic saline, blotted, weighed on a torsion balance and placed in 2 ml of boiling distilled water for 5 min Aliquots of the incubation medium and the aqueous tissue supernatant were then counted in a liquid scintillation spectrometer, and the ratio of c p m per ml intracellular fluid to c p m per ml extracellular fluid calculated Extracellular space was estimated using inulin labelled with carbon-14 Glucose disappearance from the medium was assayed with glucose oxidase

The results of these experiments are shown in Table 1 Glucose uptake was reduced at phlorizin concentrations of 5×10^{-4} *M* and higher, reaching a 50 per cent reduction at 3×10^{-3} *M* The ratio of intracellular to extracellular amino-acid concentration, however was increased for all the amino-acids studied The increase was most pronounced at the highest phlorizin concentration employed, the intracellular concentration of α-amino-isobutyric acid (AIB) being increased by 77 per cent at 3×10^{-3} *M* The magnitude of the effect on basic amino-acids was somewhat less than that seen with neutral ones For AIB the increase in this ratio was observed at the same concentration of the drug which was noted to block glucose uptake Although the fact that the divergent effects on glucose and amino-acids occur in the same concentration-range suggests the two effects may be inter-related, the amino-acid effect is independent of the presence of glucose in the medium

Table 1 EFFECT OF PHLORIZIN ON GLUCOSE UPTAKE AND INTRACELLULAR CONCENTRATION OF AMINO-ACIDS BY RAT KIDNEY SLICES THE 90-MIN INCUBATION WAS CARRIED OUT IN TRIPLICATE

		Ratio of intracellular to extracellular amino-acid concentration				
Phlorizin* concentration (*M*)	Glucose† uptake (γ/100 mgm)	AIB‡	Glycine	L-Phenylalanine	L-Lysine	L-Histidine
None	258	3 49	5 76	1 85	3 52	2 28
10^{-5}	247					
5×10^{-5}		3 99				
10^{-4}	230	3 89				
5×10^{-4}	157	4 71				
10^{-3}	143	5 09	7 53	2 20		2 61
3×10^{-3}	129	6 18	9 49	3 42	4 55	3 41

* Phlorizin (Mann Co) was twice recrystallized
† Initial glucose concentration, 1 mgm /ml. (5 6 m*M*)
‡ Initial amino-acid concentrations ranged from 0 05 to 0 17 m*M*

In contrast to the kidney slices, experiments with isolated rat diaphragm have demonstrated a slight reduction in the intra-cellular concentration of AIB at 3×10^{-3} *M* phlorizin This has been reported by others[12] Thus, increased intracellular amino-acid concentration as a result of phlorizin action is not a common property of all tissues

Various components of the phlorizin molecule, the aglycone, phloretin, and phloroglucinol were also studied in order to see if the complete glycoside was needed to produce the effect on amino-acids Neither phloretin nor phloroglucinol produced a similar increase in intracellular amino-acid concentration Phloretin is known to be weakly active in inhibiting tubular reabsorption of glucose[13] The complete glycoside appears to be necessary for both the glucose and the amino-acid effect

The mechanism of action of phlorizin-induced inhibition of sugar penetration into cells is unknown although the general view is that the drug affects membrane permeability independently of the observed inhibitory effects on oxidative metabolism[14] as well as various enzymes[10] The explanation for the amino-acid effect is unknown Since these results are seen with a non-utilizable amino-acid (AIB), the phenomena cannot be related to inhibition of amino-acid metabolism *per se* The intracellular concentration of the amino-acid is an equilibrium between influx and efflux rates Phlorizin may produce the observed increased intracellular amino-acid concentration either by augmenting the transport-rate of amino-acids into, or by inhibiting the transport rate out of, the cell. Phlorizin is known to inhibit the tubular excretion of organic acids[15], perhaps indicating a selective effect on amino-acid efflux-rates. However, an induced binding of amino-acids within the cell must also be considered as an explanation. The possible basis for the observed phenomenon is under investigation.

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Heart-rates and the Sub-lingual Temperatures of Heat-acclimatized People

PEOPLE resident at sea-level in the equatorial tropics are considered to be 'naturally' acclimatized to heat[1]. There is evidence that the indigenous peoples of these regions have low forearm blood flows and systolic blood pressures in relation to their surface and deep-body temperatures[2-4]. This evidence seems to indicate that the cardiovascular systems of heat-acclimatized people are relatively insensitive to increases of body temperature. If this is true, the relationship between the heart-rates and sub-lingual temperatures of tropical people would be expected to differ from that of people who are not acclimatized to heat. The relationship between the heart-rates and sub-lingual temperatures of young English subjects, at rest in a temperate climate, has been investigated by Tanner[5]. Comparable data are not available for the indigenous peoples of the tropics.

In order to provide these data, measurements of heart-rates and sub-lingual temperatures have been made on thirty-two male Asian subjects in Singapore. The subjects were twenty-two Chinese, eight Indians and two Malays; their average age was 22.7 yr. (*S.D.* = ± 3.1) and their mean body-weight was 57.3 kgm. (*S.D.* = ± 7.2). Each subject wore his normal clothing[4] and lay on his back for 90 min. before any measurements were made. Heart-rates and sub-lingual temperatures were measured every 5 min. for 30 min. and the average of readings for each subject was taken. The mean environmental temperatures during all experiments were 28.9 ± 1.0° C., dry bulb and 25.6 ± 0.9° C., wet bulb. Heart-rates were counted from electrocardiograms and sub-lingual temperatures were measured by means of a 40 s.w.g. copper-constantan thermocouple.

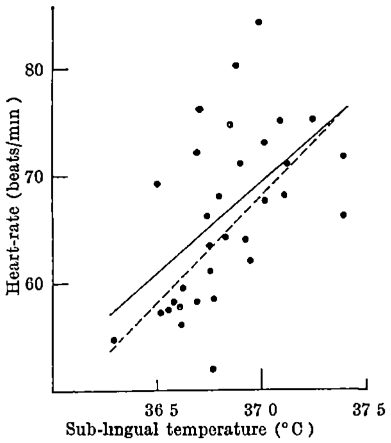

Fig. 1. Heart-rates and sub-lingual temperatures of 32 Asian subjects in Singapore. The continuous line is the fitted regression of heart-rate on sub-lingual temperature for these data. The broken line is the regression of heart-rate on sub-lingual temperature for English subjects, calculated from data presented by Tanner (ref. 6)

The heart-rates and sub-lingual temperatures of the thirty-two subjects are shown in Fig. 1. The correlation coefficient between heart-rate and sub-lingual temperature was 0.554 (0.01 > *P* > 0.001). The equation for the regression of heart-rate on sub-lingual temperature was

Heart-rate (beats/min.) = (17.24 × sub-lingual temperature in ° C.) − 569.19

Also included in Fig. 1 is the regression line for heart-rate on sub-lingual temperature calculated from data presented by Tanner[6]. Tanner's data were obtained from forty-six English subjects whose mean age was 22.1 ± 4.1 yr.; measurements of heart-rates and sub-lingual temperatures on these subjects were made while they lay on a couch at a room temperature of 17.5–20.0° C. The correlation coefficient between heart-rate and sub-lingual temperature in the forty-six subjects was 0.510 (*P* < 0.001) and the equation for the regression line of heart-rate on sub-lingual temperature was

Heart-rate (beats/min.) = (19.54 × sub-lingual temperature in ° C.) − 655.10

The heart-rates and sub-lingual temperatures of the heat-acclimatized people in Singapore tended to be higher than those of Tanner's subjects in England. The slope of the regression line of heart-rate on sub-lingual temperature was slightly greater for the English subjects. However, the correlation and regression coefficients for the two groups of data were not significantly different (*P* > 0.10).

These results have not provided any evidence that acclimatization to heat is associated with a significant general variation in the sensitivity to temperature of the tissues of the cardiovascular system. The possibility remains that changes of sensitivity to temperature are localized in discrete areas of the body such as the forearm blood vessels or centres in the brain. The latter possibility would be in agreement with a previous suggestion that acclimatization to heat involves adaptive changes in the central nervous system[7].

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BIOPHYSICS

Elimination of the Liquid Junction by using Glass Electrodes

It is assumed in measuring potential with hydrogen or alkali-sensitive glass electrodes[1] that the salt bridge which connects the reference electrode to the solution generates a negligibly small potential at the liquid junction. This is a reasonable assumption so long as the unknown solution contains no colloids, and its ionic strength is approximately that of the buffers used in standardization. Unfortunately these ideal conditions are seldom met in biological systems. The resultant error is often of a serious magnitude, as shown in experiments on *p*H in colloidal systems[2,3].

In many inorganic systems the liquid junction potential has been eliminated by the use of silver–silver chloride and other metallic reference electrodes placed directly into the unknown solution without a salt bridge[4]. The metallic reference electrodes function poorly in most biological systems because of the various interfering oxidizing and reducing substances which are present.

Glass electrodes are not affected by oxidation-reduction reactions and are well suited for use in biological media. With these advantages in mind, a system was designed and tested in which a hydrogen-sensitive glass electrode functioned as a reference electrode for sodium activity measurements with a sodium-sensitive glass electrode. The *p*H of the solution being tested was kept at 7·21. Any error in sodium determination which might have occurred if the *p*H were allowed to shift would tend to be automatically balanced out because the sodium-sensitive glass is also sensitive to hydrogen ions. The sodium concentration was varied by addition of 1 *N* sodium chloride to the buffer solution (*tris*-hydroxymethylamminomethane buffer[5]).

The sodium-sensitive glass electrode was of the bulb type (Electronic Instruments, Ltd., glass *BH* 104) while Corning 015 glass was fused to a lead glass stem to form the hydrogen-sensitive MacInnes–Dole type electrode. All potentials were measured with the Vibron model 33*B* electrometer and model *C*33*B* 'bucking' circuit.

Hydrogen ion sensitivity of the Corning 015 glass electrode was checked with buffers from the National Bureau of Standards (from *p*H 4·65 to 9·18), using a saturated potassium chloride–calomel reference electrode. The liquid junction between the saturated potassium chloride and buffer being tested was made through a porous ceramic plug. The sodium ion sensitivity of the *BH* 104 glass was tested in a *tris* buffer solution (*p*H 7·21) by varying the concentration of sodium with 1 *N* sodium chloride. The reference electrode was again the saturated potassium chloride–calomel with a porous ceramic plug. Representative data (circles) for the sodium-sensitive glass electrode and calomel reference are shown in Fig. 1. The hydrogen ion-sensitive glass electrode was then substituted for the calomel reference and the sodium ion measurements repeated in the *tris* buffer solutions (triangles in Fig. 1).

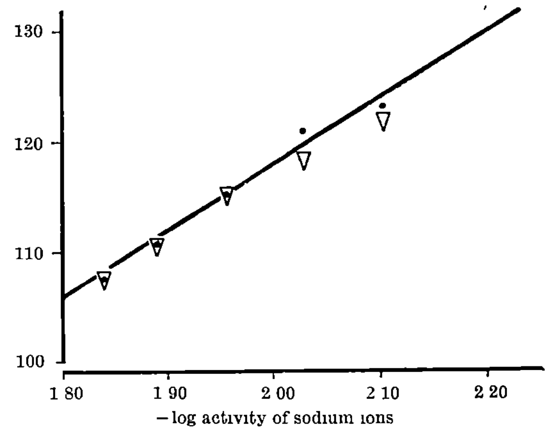

Fig. 1. Representative sodium-sensitive glass electrode potentials (circle) using a calomel reference electrode and (triangle) using a Corning 015 MacInnes–Dole glass electrode as the reference electrode. The straight line plots the slope of the theoretical potential given by the Nernst equation $E = E^0 + 2{\cdot}303RT/F$ (log a_{Na^+}) while the points are experimental measurements. The value of 2·303*RT*/*F* was taken as 59 mV at 23° C. The negative logarithm of the activity of Na^+ is plotted on the abscissa using an activity coefficient of 0·73 based on the presence of a constant 0·2 *M* buffer solution.

The equivalent response of the sodium ion electrode with either the standard saturated potassium chloride–calomel reference or the Corning 015 glass reference illustrates the ability of the glass electrodes to function in a system as both an ion-specific electrode and reference electrode. The simplicity of this method of eliminating the liquid function should prove useful to workers in many fields, especially when the specific cation to be measured is a solute in a complex solution.

This work was aided by U.S. Public Health Service research training grant No. *HTS* 5454.

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Mechanism of Nicotinic Action at the Adrenal Medulla: Calcium as a Link in Stimulus-secretion Coupling

In experiments to probe the mechanism of transmission of sympathetic activity at the adrenal medulla we have found that acetylcholine, which is believed to be the chemical transmitter, evokes catecholamine secretion through some calcium-dependent process. Our evidence, obtained in experiments in cats' adrenal glands perfused *in vivo* or *in vitro* with Locke's solution or variations of it, may be summarized as follows

Acetylcholine failed to release catecholamines when calcium was omitted from the perfusion fluid (in similar experiments in which sodium or potassium was omitted acetylcholine still had a strong stimulant effect) The amount of catecholamines released by acetylcholine varied with the calcium concentration of the perfusion fluid over a wide range, being increased about 50 per cent above the normal with 8 8 m*M* calcium and being about doubled with 17 6 m*M* calcium Calcium itself (2 m*M*) following a period of calcium-free perfusion caused a vigorous secretion of catecholamines This evidence, considered along with the known ability of acetylcholine to increase membrane permeability at other junctional sites, led us to postulate that acetylcholine evokes secretion of catecholamines by causing an influx of calcium ions into the adrenal medullary cells[1]

In support of this postulate, one of us (W W D) has recently found in experiments with Dr A M Poisner that acetylcholine (10^{-5} gm /ml) greatly increases the uptake of calcium-45 by the adrenal medulla We have in fact proposed that calcium has a role in the process we have termed 'stimulus-secretion coupling' analogous to its supposed role in 'excitation-contraction coupling' in muscles A full account of these experiments will be published elsewhere[2]

We have now obtained evidence that other substances which stimulate the adrenal medulla and which belong to the family of drugs with 'nicotinic' actions evoke secretion in a similar fashion The present experiments were carried out on cat's adrenal glands acutely denervated and perfused *in situ* through the aorta with Locke's solution In such experiments the 'resting' secretion of catecholamines appearing in the effluent collected from the adrenal veins was usually equivalent to about 50–100 mμgm adrenaline (base)/min The addition of 10^{-5} gm / ml nicotine, tetramethylammonium, lobeline or dimethyl-4-phenylpiperazinium iodide (DMPP) to the perfusion fluid caused an enormous increase in the catecholamine output Not uncommonly the secretion evoked by these drugs, particularly DMPP and nicotine (which seemed to be especially active), was more than 100 times the control 'resting' secretion

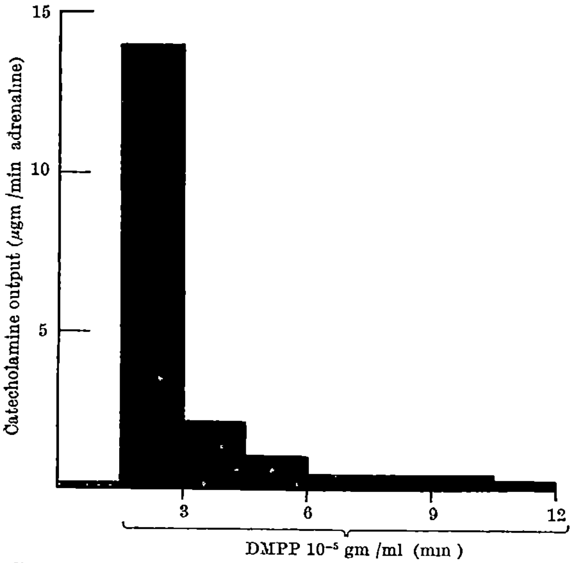

Fig 1 A typical secretory response of perfused cat's adrenals to DMPP 10^{-5} gm /ml Both adrenals were perfused with Locke's solution and the effluent assayed in terms of adrenaline (base) by bioassay with the spirally cut rabbit aorta Before addition of DMPP the gland was perfused for 20 min during which the 'resting' output was determined the output in the last 1½ min of this period is indicated

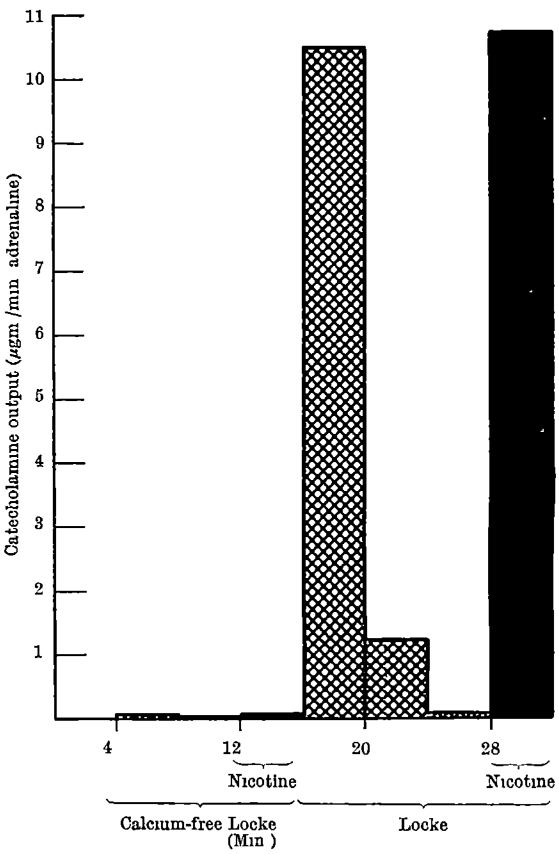

Fig 2 Failure of nicotine (10^{-5} gm /ml) to evoke catecholamine secretion in the absence of calcium The thickening of the baseline to the left indicates the 'resting' outputs (min 4–12) and the negligible response to nicotine (min 12–16) in calcium-free Locke's solution The cross-hatched bars which follow indicate the stimulant effect of re-introducing calcium (in the form of Locke's solution) The solid bar to the right indicates the control response to nicotine in Locke's solution

A typical response is shown in Fig 1, from which it can be seen that DMPP increased the rate of secretion to more than 13 μgm /min This high rate of secretion was not maintained, but fell progressively so that in about 10 min it was near to the resting value despite the continued presence of the drug The cause of this progressive diminution in the response was not determined for the present purpose tests were made with each of these substances by perfusing them in this same concentration (10^{-5} gm / ml) for periods of 4 min. In each experiment one or the other drug was first tested in Locke's solution free of calcium and then in Locke's solution. In each instance the drugs were active in Locke's solution but inactive (or almost so) in calcium-free Locke's solution A typical experiment is shown in Fig 2 After a period of perfusion with calcium-free Locke's solution (usually 16 min) perfusion was switched to calcium-free Locke's solution with the addition of nicotine (10^{-5} gm /ml) This raised secretion very little, if at all, above the resting value Perfusion was then switched back to Locke's solution This change in itself caused a transient but very vigorous secretion which, as mentioned above, is due to the re-introduction of calcium (it occurs also without prior exposure to any of the stimulant drugs) When spontaneous secretion had returned to a low level perfusion was once more switched, this time to Locke's solution plus nicotine Nicotine then exerted its familiar powerful excitant effect raising secretion to more than 10 µgm /min Similar results were obtained with TMA, lobeline and DMPP

It thus appears that these various drugs of the 'nicotine group' may all evoke catecholamine secretion by causing calcium ions to penetrate the medullary cells

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PHARMACOLOGY

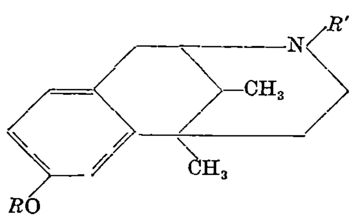
A New Potent Analgetic Antagonist

The discovery of a new potent analgetic with a reduced liability to addiction[1] in the benzomorphan series, namely, 2′-hydroxy-5,9-dimethyl-2-phenylethyl-6,7-benzomorphan[2] (I), has suggested further work in the series 2′-Hydroxy-5,9-dimethyl-2-allyl-6,7-benzomorphan (III) is a potent antagonist of phenazocine, morphine, meperidine, and other analgetic agents This compound is of special interest in that combinations of phenazocine and III in ratios of 5 1 to 100 1 retain analgetic activity in animals yet seem to be relatively devoid of physical dependence properties in the monkey in both acute and chronic experiments (biological details of this work will be described elsewhere)

The demethylation of 2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan has been described[2] Reaction of the nor-compound (II) with a slight excess of allyl bromide in ethanol containing sodium bicarbonate for 18 hr under reflux gave, after filtration, concentration and trituration with ether, the product (III), m p 141–144° in 46 5 per cent yield Anal calc $C_{17}H_{23}NO$, C, 79 33, H, 9 01, N, 5 44 Found C, 79 06, 78 99, H, 9 19, 9 29, N, 5 64, 5 78

A mixture of 5,9-dimethyl-2′-methoxy-6,7-benzomorphan (IV), prepared by treating 5,9-dimethyl-2′-hydroxy-6,7-benzomorphan[2] (II) in ethanol with diazomethane in ethanol containing sodium bicarbonate was treated with allyl bromide under reflux After appropriate working up, the residue was dissolved in acetone and treated with ether to give the desired product (V), m p 216–218°, for the hydrochloride salt Anal calc for $C_{18}H_{25}NO$ HCl C, 70 22; H, 8 51, N, 4 55 Found C, 70 00, H, 8 50, N, 4 78

The present antagonist (III) combined with phenazocine in a 1 1 ratio showed no analgesia, and precipitated abstinence phenomena in the morphine-addicted monkey (Deneau, G A, and Seevers, M H, private communication) With a ratio of 9 parts of phenazocine to 1 of the antagonist (III), the analgesic effect was as good as with phenazocine alone, and there was a precipitation of abstinence in addicted monkeys as seen with nalorphine, instead of a suppression Mixtures of 25 : 1 and 50 1 acted similarly, with a 100 1 mixture there was no precipitation of abstinence and no suppression up to a dose of 16 mgm /kgm The retention of good analgesic effect without suppression of abstinence, indeed with the presence of nalorphine-like effects with respect to precipitation of abstinence in morphine-addicted animals, seems very promising and these mixtures will be studied in man The antagonist (III) itself showed no significant analgesic effect in animals



I $R=H, R'=CH_2CH_2C_6H_5$

II $R=H, R'=H$

III $R=H, R'=CH_2{-}CH=CH_2$

IV $R=CH_3, R'=H$

V $R=CH_3, R'=CH_2CH=CH_2$

An analgesic effect of nalorphine in man, and retention of the analgesic effect of morphine in appropriate mixtures of morphine and this antagonist, were first reported by Beecher and Lasagna[3] Later it was shown that morphine–nalorphine mixtures administered repeatedly to former addicts (Fraser, H F, and Isbell, H, personal communication) produced a milder degree of physical dependence than equivalent doses of morphine alone N B. Eddy, M Piller, L A Pirk, O Schrappe and S Wenda[4] have shown that when mixtures of morphine and levallorphan (an antagonist of the morphinan group) were administered for chronic pain there was a definite trend toward delay in the development of tolerance and physical dependence under clinical conditions

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Anti-Asthmatic Effect of Phenazone and Amidopyrine

Many chronic asthmatics state that they obtain relief from proprietary asthma powders available in Germany without medical prescription Most of these powders contain either phenazone or amidopyrine, besides much smaller quantities of various other ingredients In the list of German proprietaries (the "Red List"), we have found 53 such preparations, of which 27 contained phenazone, 22 amidopyrine and 4 both

As little seems to be known about the anti-asthmatic effects of phenazone and amidopyrine, we have investigated the question in 46 patients with chronic asthma, who were trained in spirometry In each experiment the patient's vital capacity was recorded successively three or more times, and he was then given placebo or drug by mouth 1 hr later a second series of vital capacities was recorded as before The

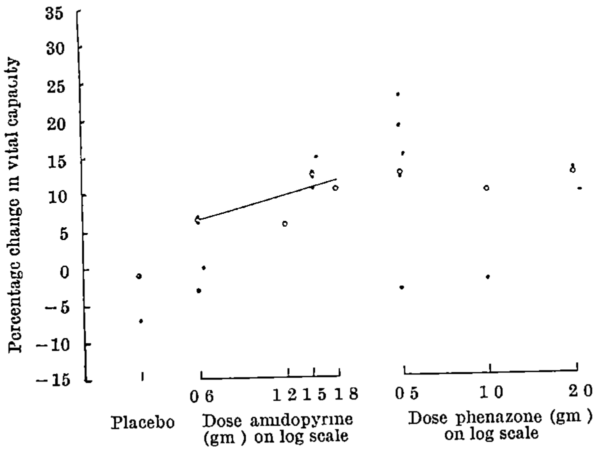

Fig. 1. Percentage change in vital capacity for placebo and doses of amidopyrine and phenazone. ●, Individual experiments; ○, mean response at each dose

means of vital capacities before and after treatment were calculated and results expressed as percentage change of vital capacity. On some occasions we also recorded peak flow and forced expiratory volume, but recording the latter tended to increase bronchial spasm.

Twenty-seven patients received phenazone in doses of 0·5, 1·0 or 2·0 gm. in 63 experiments, and 26 patients received amidopyrine in doses of 0·6, 1·2, 1·5 or 1·8 gm. in 43 experiments. Placebo was given in 17 experiments. The percentage changes in mean vital capacity from the basal values before treatment in each experiment and the overall mean values after placebo and at each dose of drug are plotted in Fig. 1. A representative example of the phenazone effect is given in Fig. 2.

Compared with the basal values before treatment, the increases in vital capacity after amidopyrine or phenazone are evident from Fig. 1, while the change after placebo is negligible. The differences between placebo and all doses of phenazone or of amidopyrine are highly significant ($P < 0{\cdot}001$ in both cases).

The calculated regression line for amidopyrine, which has significant slope ($P < 0{\cdot}05$), is also given in Fig. 1. A regression line was not calculated for phenazone, because it clearly would not have a significant slope. Where forced expiratory volume was recorded we found no definite effect, but the peak flow usually increased when the vital capacity increased.

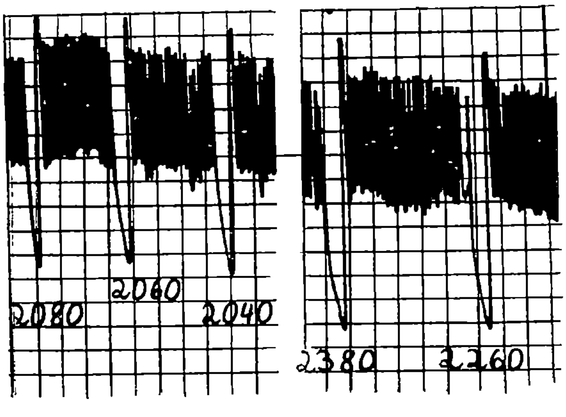

Fig. 2. Effect of phenazone on vital capacity of a chronic asthmatic. Left-hand panel, before treatment; right-hand panel, 1 hr. after phenazone, 2·0 gm. by mouth. The figures give the vital capacities in ml.

These results show that phenazone and amidopyrine lessen respiratory obstruction in most chronic asthma patients examined. Results with aspirin, so far unpublished, have been comparable. Our experience with other agents, such as histamine and isoprenaline, has shown that an increase or decrease of vital capacity by less than 4 or 5 per cent remains subjectively unnoticed, but greater changes are felt by the patient. Since most increases of vital capacity exceeded 5 per cent after all doses of both drugs, most patients felt relief from phenazone and amidopyrine, as was to be expected.

A search in the literature has so far revealed no previous reference to the anti-asthmatic action of amidopyrine. Two reports, one of 1889[1] and one of 1890[2], have been found on phenazone. In each of these the drug was claimed to have relieved asthma in one patient. Our results indicate that in many asthma cases both drugs play a more active part than was hitherto supposed.

The anti-asthmatic action of phenazone, amidopyrine and aspirin, which also inhibit histamine release in anaphylaxis[3], is particularly interesting because these drugs specifically antagonize bradykinin broncho-constriction in the guinea pig[4,5], and because inhalation of bradykinin aerosol reduces the vital capacity of chronic asthmatics[6]. We are therefore investigating the possible relationship between bradykinin and asthma; but in 30 experiments in which one of these analgesic-antipyretic drugs was given orally before the patient inhaled a bradykinin aerosol, the reduction of vital capacity by bradykinin was inhibited on only seven occasions. This result points against the antagonistic effect of these drugs to bradykinin in man, but work on this problem continues. Results will be published in detail later.

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PATHOLOGY

A New Method for Local Chemical Analysis of Human Tissue

As a consequence of the original work of Castaing[1] on the electron beam micro-analyser, widespread application has been made in the field of physical metallurgy[2]. It is the purpose of the present communication to record the applicability of this technique to biological problems. In the Castaing instrument a beam of electrons, focused to a spot 1μ in diameter, excites fluorescent or secondary X-rays in the specimen, which are analysed in a crystal spectrometer with Geiger counter detection. An optical microscope with a reflecting objective permits simultaneous viewing and analysis of the

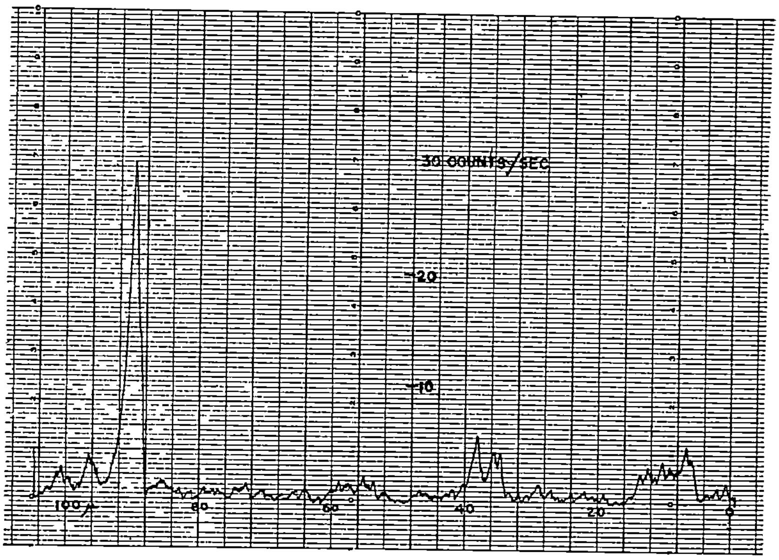


Fig 1 Iron distribution in a synovial membrane

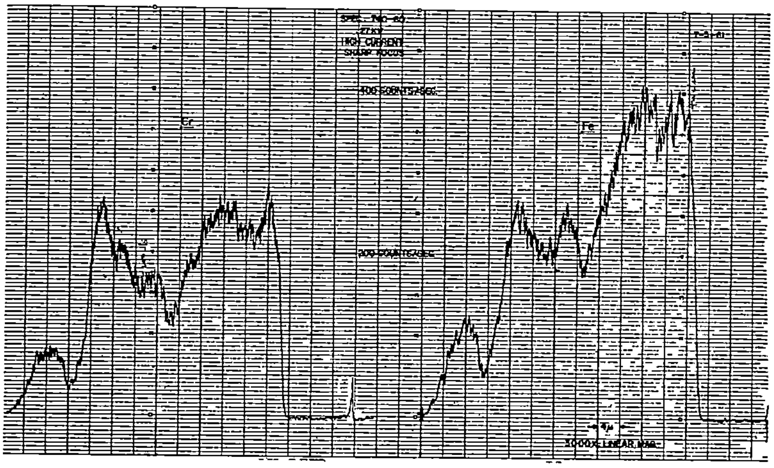


Fig 2 Iron and chromium distribution in joint capsule (hip joint prosthesis)

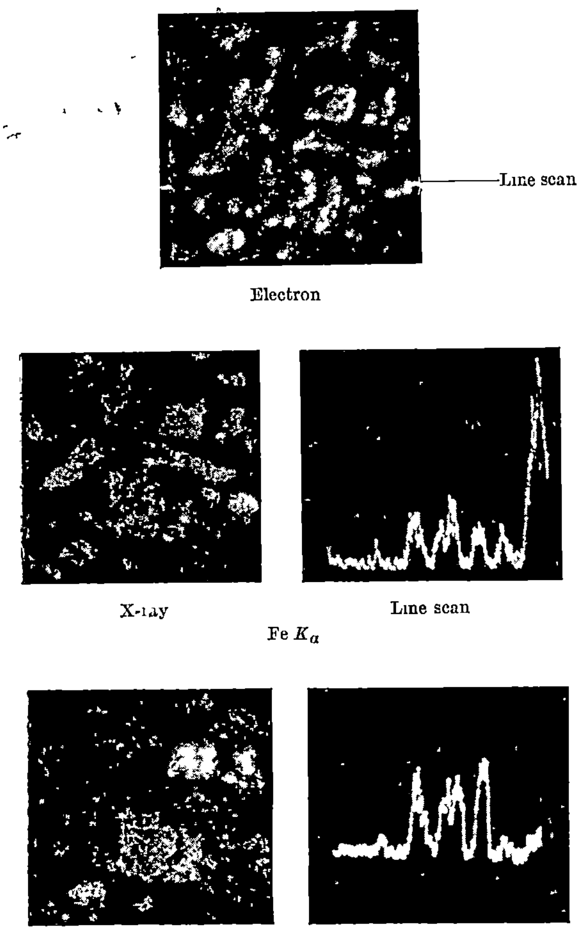
irradiated spot Mechanical stage motion allows the selection for analysis of any desired spot in the specimen The range of elements at present detectable coincides with that of modern X-ray spectrographs, that is, elements lying above sodium in the Periodic Table Two characteristics of these instruments of paramount importance to biological research are (1) a detectable limit as low as 10^{-14} gm , (2) spatial resolution of approximately 1μ Birks has recently discussed the electron probe instrument in its numerous modifications[3]

In solids which are good conductors of heat and electricity, electron bombardment in the instrument produces no damage In biological materials, however, damage to a specimen may be catastrophic under irradiation by 25-kV electrons at current densities of the order of 100 amp /cm ^{2}. Our experiments have been made on microtome sections of tissue nominally 4μ thick, mounted on polished carbon rods Sections were cut from formalin-fixed paraffin blocks, and picked up from distilled water with the carbon rods Subsequent dehydration and removal of paraffin with organic solvents was accomplished, producing a specimen of tissue firmly bonded to the underlying carbon These were analysed without further manipulation in the electron probe instrument manufactured by the Paris firm of CAMECA Although produced by techniques standard in pathology, the specimens proved to be virtually indestructible

Fig 1 shows the results of an iron analysis in human synovial membrane, pathologically diagnosed as pigmented villonodular synovitis The curve is a typical linear scan across the specimen, showing the intensity of iron K_a X-rays as a function of position Beam current was 2×10^{-8} amp at an accelerating potential of 27 kV Sharp beam focusing is indicated by the steepness of the response curves Maximum X-ray intensity observed in Fig 1 is some 30 counts/s , under identical instrument conditions a solid iron specimen emits 700 c p s Under much higher beam currents this specimen of tissue was found to yield intensities from iron-rich regions as high as 800 c p s , with a spot diameter about twice as large as that used in obtaining Fig 1 Therefore it may be estimated that the minimum detectable amount of iron in a spot of 4μ diameter is about one-thousandth of that actually present in this section of tissue

An example of exogenous material present in tissue is furnished by the curves of Fig 2 This material is a section of joint capsule taken from the vicinity of a surgical metallic implantation (hip joint prosthesis) Two linear scans show the distribution of iron and chromium along the same path The remarkable similarity of these curves implies the co-precipitation of these two elements in this particular region of the tissue Noteworthy is the low background, essentially zero, and the steepness of rise at the edge of the metal-rich areas It was observed further that sequential beam traverses over the same path gave practically identical responses, that is, for analytical purposes the method is non-destructive under the instrumental conditions used (27 kV, 10^{-7} amp , beam scanning speed, 12 μ/min)

The same specimen was investigated in a scanning probe which utilizes the 'flying spot' arrangement of Cosslett[4] In this instrument the electron beam is caused to execute two-dimensional scanning while the emitted X-rays are used to modulate the intensity of a synchronously driven cathode-ray tube Photography of the cathode tube display yields a television-like image of the distribution of a chosen element Alternatively, an image formed by back-scattered electrons may be displayed, giving information analogous to the conventional image formed by an optical microscope Fig 3 shows the same specimen as Fig 2, imaged successively by means of scattered electrons, iron K_a, and chromium K_a X-rays Identical areas at a magnification of 300 are covered by the three images Also shown are two linear



Electron

X-ray Line scan

Fe K_α

X-ray Line scan

Cr K_α

Fig 3 Same specimen as Fig 2, imaged by back-scattered electrons, iron and chromium X-rays

scans similar to those of Fig 2, plotting variations in iron and chromium concentrations across the tissue Fig 3 clearly indicates differing composition from one metal-rich area to another in this tissue No serious damage to the specimen occurred during the experiments, and the pictures of metallic localizations in Fig 3 were reproducible

It appears that the Castaing electron probe microanalyser is directly applicable to the field of human pathology With proper specimen preparation, the microtome section is amenable to extremely sensitive chemical analysis Many problems in cellular chemistry and pathology could be investigated by the use of the technique For example, preliminary investigation has shown the feasibility of measuring the distribution of calcium and phosphorus in undecalcified bone blocks

We wish to acknowledge with thanks the support and encouragement of F L LaQue, and the help of Carl Kruse, Cambridge Instrument Co , in the use of the beam scanning probe

Note added in proof An oral presentation in this subject was made on November 15, 1961, at the Eastern Analytical Symposium, New York City, entitled "Elemental Analysis (Cu, Ag Fe) at the Subcellular Level in Tissue Sections of Human Specimens by Means of the Electron Microprobe", by A J Tousimis, George Washington University, and I Adler, U S Geological Survey

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Carcinogenicity of Dibenzo(a,l)pyrene

DIBENZO(*a*,l)PYRENE (1,2,3,4-dibenzopyrene) has been shown to be present in cigarette smoke condensate and in automobile exhaust gas in small quantities[1–5] In view of this, the relative dearth of tests for the carcinogenic activity of this substance is surprising Though dibenzo(*a*,l)pyrene was regarded as a strong carcinogen by Cook and Kennaway[6] and by Pullman and Pullman[7], the only biological evidence we could find in the literature has been presented by Bachmann *et al* [8] These authors placed a 0 3 per cent dibenzo(*a*,l)pyrene solution in benzene on two mice After 6 months one mouse died The remaining mouse lived for 262 days and had developed an epithelioma To our knowledge no other group of workers has reported the carcinogenic activity of dibenzo(*a*,l)pyrene[9]

This report presents results of testing the carcinogenic activity of different concentrations of dibenzo(*a*,l)pyrene With slight modifications in the method, dibenzo(*a*,l)pyrene was synthesized by Zander's method[10] The material used for testing was purified by vacuum-sublimation, repeated column chromatography on alumina and recrystallization from benzene. The purity of this hydrocarbon (m p 224–226° C) was checked by ultra-violet absorption and fluorescence spectra and by paper chromatography[1]

Acetone solutions in concentrations of 0 25, 0 1, 0 05 and 0 01 per cent were applied thrice weekly to the backs of 6-week-old Swiss (Millerton) female mice The materials were applied with one stroke of a camel-hair brush (No 5) Re-grown hair was removed with an Otis clipper whenever necessary

Table 1 CARCINOGENIC ACTIVITY OF DIBENZO(*a*,l)PYRENE

No of	0 25 per cent			0 1 per cent			0 05 per cent			0 01 per cent		
months	S*	P†	C‡	S	P	C	S	P	C	S	P	C
1	20	0	0	20	0	0	20	0	0	20	0	0
2	20	0	0	20	0	0	20	0	0	20	0	0
3	20	0	0	20	0	0	20	0	0	20	0	0
4	20	5	0	20	5	0	20	0	0	20	0	0
5	17	65	30	20	35	10	20	15	5	20	0	0
6	10	95	50	13	75	35	18	65	10	20	5	0
7	0	100	100	6	95	70	3	95	65	18	20	10
8				3	100	85	1	100	85	16	35	20
9				0	100	95	0	100	100	12	50	35
10										8	75	55
11										2	85	85
12										0	85	85
Average conversion time in (days) from papilloma to carcinoma	21			27			29			35		
Tumour potency (ref 11)	70			63			57			39		

* Survivals, † papillomas, ‡ carcinomas

The results of the experiment are listed in Table 1 and show carcinogenic activity for all concentrations tested. Comparing Blanding potency numbers[11] with previous data for benzo(*a*)pyrene 0·05 per cent, 94 (ref. 12), 0·01 per cent, 57 (ref. 13), and for dibenz(*a,h*)anthracene 0·1 per cent, 48 and 0·01 per cent, 33 (ref. 13) shows that the carcinogenic activity of dibenzo(*a,*1)pyrene lies between that of the other two carcinogens.

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RADIOBIOLOGY

Production of Chromatid Aberrations by Diepoxybutane and an Iron Chelator

RECENT investigations have shown that chromatid breaks produced by certain radiomimetic agents in combination do not interact to produce reunions[1]. Specifically, breaks produced by 8-ethoxycaffeine do not form exchanges with those produced by maleic hydrazide, nor do the breaks produced by potassium cyanide and β-propiolactone interact with each other. Conversely, breaks induced by X-rays will interact with those produced by potassium cyanide, β-propiolactone, 8-ethoxycaffeine and maleic hydrazide. The interpretation of these results was stated in terms of differences in the bonds broken by the chemical agents, such differences precluding interaction or rejoining of broken ends of chromosomes.

As part of an investigation of radiomimetic agents and an iron chelator, a series of experiments utilizing combined treatments was performed. In addition, combined treatments with X-rays and 1,2-3,4 diepoxybutane or 2,2′ bipyridine were carried out. The material used consisted of the lateral roots of *Vicia faba* (Carter's Seville Longpod) grown at 20° C. for 7 days prior to treatment. The chemical agents were used as follows: 2,2′ bipyridine, 2×10^{-3} *M* for 15 min.; potassium cyanide, 1×10^{-3} *M* for 30 min.; 1,2–3,4 diepoxybutane, 3×10^{-3} *M* for 15 min. In the X-ray experiments, the roots were exposed to 100 r. X-rays administered in 54 sec. The source used was a 'Keleket 220-*C*' X-ray unit operated at 180 kV.p. and 18 m.amp. with 1·3 mm. aluminium and 0·8 mm. copper filtration. Irradiations were performed by Dr. John E. Edwards, of the Department of Physics, Ohio University. Roots were immersed in 1,2-3,4 diepoxybutane or 2,2′ bipyridine for 13 min. prior to irradiation, exposed while immersed, and rinsed 1–2 min. following exposure. Feulgen squashes were prepared from roots fixed 24 and 48 hr. following treatment.

Table 1. INTERACTION EFFECTS OF POTASSIUM CYANIDE AND 1,2-3,4 DIEPOXYBUTANE

Treatment	Recovery period (hr.)	Isochromatid deletions per cell*	Exchanges per cell Observed	Calculated Additive effect	Calculated Complete interaction
Potassium cyanide	48	0·24 ± 0·02	0·07 ± 0·01	—	—
Diepoxybutane	48	0·54 ± 0·01	0·20 ± 0·01	—	—
Potassium cyanide + diepoxybutane	48	0·82 ± 0·04	0·48 ± 0·03	0·27	0·51
Potassium cyanide and diepoxybutane†	48	1·57 ± 0·08	1·03 ± 0·06	0·27	0·51

* A minimum of 200 cells was analysed for each treatment.
† Simultaneously.

Table 2. PRODUCTION OF CHROMATID ABERRATIONS BY X-RAYS, 1,2-3,4 DIEPOXYBUTANE AND 2,2′ BIPYRIDINE

Treatment	Recovery period (hr.)	Isochromatid deletions per cell*	Exchanges per cell Observed	Calculated Additive effect	Calculated Complete interaction
Diepoxybutane	24	0·33 ± 0·01	0·11 ± 0·05	—	—
X-ray	24	0·26 ± 0·02	0·08 ± 0·01	—	—
Diepoxybutane + X-ray	24	0·62 ± 0·04	0·20 ± 0·02	0·19	0·39
Diepoxybutane	48	0·54 ± 0·01	0·20 ± 0·01	—	—
X-ray	48	0·13 ± 0·02	0·05 ± 0·01	—	—
Diepoxybutane + X-ray	48	0·68 ± 0·04	0·24 ± 0·02	0·25	0·46
2,2′ Bipyridine	24	0·07 ± 0·005	0·01 ± 0·002	—	—
X-ray	24	0·26 ± 0·02	0·08 ± 0·01	—	—
2,2′ Bipyridine + X-ray	24	0·20 ± 0·02	0·10 ± 0·02	0·09	0·15
2,2′ Bipyridine	48	0·05 ± 0·006	0·01 ± 0·002	—	—
X-ray	48	0·13 ± 0·02	0·05 ± 0·01	—	—
2,2′ Bipyridine + X-ray	48	0·13 ± 0·02	0·04 ± 0·01	0·06	0·11

* A minimum of 200 cells was analysed for each treatment.

The peak effect of combined treatment with potassium cyanide and 1,2-3,4 diepoxybutane occurs in early interphase as indicated in the roots fixed 48 hr. after treatment. This result is consistent with my preliminary investigations. The combined applications of potassium cyanide and 1,2-3,4 diepoxybutane have elicited two unexpected results (Table 1). When the two agents were administered one following the other, in either order, the resultant frequency of exchanges clearly indicated interaction between the breaks produced by the two compounds. The implication is that the breaks produced are of a similar or identical chemical nature. This result is contrary to that found with potassium cyanide and beta-propiolactone[1]. The other unforeseen result arises from the simultaneous treatment with potassium cyanide and 1,2-3,4 diepoxybutane. The aberration frequencies were so high as to make analysis difficult. It is probable that in this case potassium cyanide as a metabolic inhibitor prevents the restitution of broken ends of chromosomes. Thus, all the breaks produced by the two agents are available at the same time and for a longer period of time favouring reunions and deletions. Potassium cyanide is known to inhibit the terminal stages of cellular respiration, and in this way reduces the available energy necessary for restitution[2]. Results of this type were also obtained in *Allium* roots treated with X-rays and carbon monoxide in the dark[3].

The results of the X-ray experiments are presented in Table 2. The frequencies of exchanges and isochromatid deletions produced by treatment with X-rays and 1,2-3,4 diepoxybutane indicate a strictly additive effect. This result is expected for the deletions but not for exchanges. Earlier work has indicated that, for the most part, breaks produced by radiomimetic agents interact with those produced by X-rays[1,4,5]. An explanation of these results may lie in the difference in the peak time of effect of the two agents Early interphase is the most sensitive period with respect to 1,2-3,4 diepoxybutane activity, whereas late interphase appears to be more sensitive to X-ray treatment[6]. Therefore, the majority of breaks produced by the two agents would not be available at the same time to facilitate reunions This argument has been presented previously by Auerbach[4] The additive effect produced by X-rays and 1,2-3,4-diepoxybutane is evident for both 24- and 48-hr recovery despite the fact that 1,2-3,4 diepoxybutane has an appreciable effect in late interphase (Table 2, 24-hr recovery period) This is also the time during which X-rays have their maximum effect Presumably, a large number of broken ends are available at this time for interaction, but interaction does not occur Since X-rays have an essentially random effect in producing chromosome breaks, certain of these breaks must involve bonds similar to those broken by 1,2-3,4 diepoxybutane as well These breaks of similar bonds would be expected to undergo reunions with each other to increase the total yield of exchanges The effect, therefore, cannot be explained in terms of one mechanism, but probably depends on considerations of both a temporal nature and a qualitative difference between the breaks produced by the two agents.

The results of the X-ray investigations with bipyridine (Table 2) are similar to those with 1,2-3,4 diepoxybutane. There is no enhancement of X-ray effect with 2,2′ bipyridine, nor is there any indication of interaction of breaks produced by the two agents The effect is additive for isochromatid deletions and exchanges at both recovery periods The result is in basic agreement with similar work by Kihlman[7] Normally, one would expect at least an additive effect of the two agents for both aberration types In the results reported here, even an additive effect is lacking in most instances This may indicate that the effect of 2,2′ bipyridine is to reduce the total yield of aberrations in some manner The sticky effect and clumping of chromosomes produced by 2,2′ bipyridine may cause a masking of the true breakage frequencies and may account for the apparent lack of an additive effect This aspect of the results requires further investigation before a clear explanation can be presented The absence of interaction between 2,2′ bipyridine and X-ray-induced breaks is again contrary to previous results 2,2′ bipyridine appears to act over a long period during interphase, part of this period coinciding with the time during which the peak effect of X-rays occurs Thus, the presence of breaks at the same time, and the random effect of X-rays, would be expected to permit interaction The mechanisms of breakage by the two agents are possibly different or there may be a qualitative difference in the breaks produced The low frequency of aberrations produced by 2,2′ bipyridine alone further complicates the situation, and the role of 2,2′ bipyridine as a metabolic inhibitor is even more difficult to evaluate from these results

This work was supported in part by the U S Atomic Energy Commission, under contract *AT*(11–1) 826

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Radioactivity in Wool

KULWICH *et al* recently reported[1] the presence of unidentified γ-ray activity in several samples of wool The bulk of the observed activity in the wool was shown to be most probably associated with its impurities and hence due to natural and fall-out contamination

In May 1960 we examined a sample of wool for its potassium-40 content in conjunction with an experiment for the determination of lean-to-fat ratio *in vivo* by determining the potassium content of the animal Since then, we have again examined two samples of raw wool for their associated radioactivity Each sample consisted of approximately 500 gm of the material, and the work was performed in a low-background room (iron room) of the Biological and Medical Research Division of the Argonne National Laboratory A 5 in × 4 in sodium iodide (thallium) crystal detector was used in conjunction with an Argonne type 256-channel pulse-height analyser

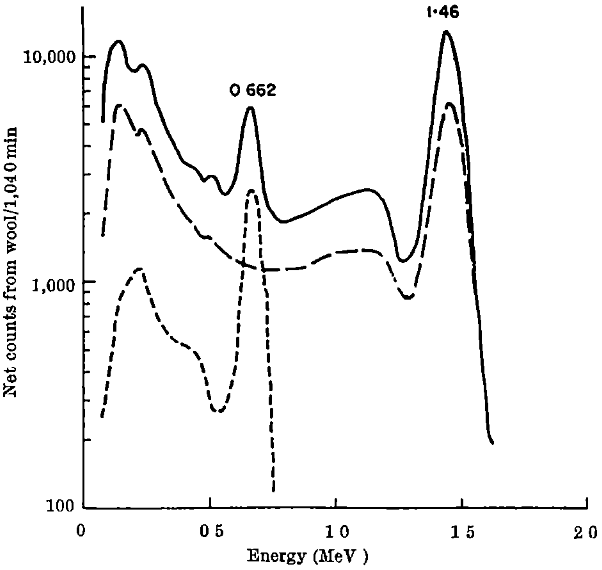

Fig 1 Net γ-ray spectra of raw wool samples (——), compared with spectra for potassium-40 (– – –), and cæsium-137 (- - - - - -) standard

Our work confirms the presence of γ-emitting radionuclides in the wool of Hampshire ewe and Rambouillet ram lambs, and allows tentative identification of the major components of such activity Fig. 1 shows the net γ-ray spectrum of one of the two wool samples as well as γ-ray spectra of potassium-40 and cæsium-137 for comparison The spectra from the two samples of wool are identical in shape but differ in absolute amount of the activity present. Except for trace quantities of other nuclides only two prominent nuclides are observed, namely, potassium-40 and cæsium-137. In practice, the sample to be encountered probably will vary widely in activity, hence our values shown in Table 1 indicate only the order of magnitude to be expected

Since no natural activity aside from that of potassium-40 was observed, one can conclude that the potassium-40 activity is present in wool grease, suint,

Table 1

	Hampshire ewe	Rambouillet ram
gm potassium/kgm wool	32	14
μμc ^{137}Cs/kgm wool	356	438
μμc ^{137}Cs/gm potassium	11	31

and dead skin in the wool[2] Cæsium-137, on the other hand, probably comes from two different sources, namely, from fall-out contamination as well as from the sources present in the wool If an appreciable portion of the activity were due to fall-out contamination, we should observe ruthenium-106 and cerium-144 in larger than trace quantities, hence the major portion of it must be due to the second cause Public Health Service Data[3] of July 1958–July 1960, add some credence to this conclusion For example, the level of cæsium-137 in milk has varied approximately from 15 to 70 μμc /gm potassium during this period and now is running in the range 15–25 The observed values for wool are also in this range

This work was performed, in part, under the auspices of the U S Atomic Energy Commission

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BIOLOGY

Toxicity associated with Certain Samples of Groundnuts

A NEW disease, called 'turkey *X* disease' has been described since the widespread outbreaks of deaths in turkey poults in 1960[1] Post-mortem examination of dead poults from field outbreaks revealed acute hepatic necrosis, associated with generalized bile duct proliferation Siller and Ostler[2] directed attention to the similarities of the lesions to those of *Senecio*-alkaloid poisoning in the fowl described by Campbell[3]

Table 1 COMPOSITION OF DIETS

	Control basal diet (per cent)	Suspect Brazilian meal (per cent)	Control Indian meal (per cent)
Casein + 0 75 per cent L-cystine	20 15	20 15	20 15
Groundnut oil	10	10	10
Starch	57 85	37 85	37 85
Cellulose	6	6	6
Jones Foster salt mixture*	4	4	4
Wheat germ oil	1 5	1 5	1 5
Vitamins (see below)	0 5	0 5	0 5
Groundnut meal	—	20	20
Vitamin A 200 I U Vitamin D_3 21 3 I U	In groundnut oil once weekly to each rat		

Vitamins (mgm /100 gm diet) B_{12}, 0 003; folic acid, 1 5, *p*-aminobenzoic acid, 10, biotin, 0 02, inositol, 22, thiamine, 0 5, riboflavin, 0 65, pyridoxine, 0 3, pantothenic acid, 3 0, ascorbic acid, 0 5, nicotinic acid, 60, choline chloride, 400

* *J Nut* , 24, 245 (1942)

In the search for the toxic agent, no contamination with any known insecticide, solvent, plant toxin or alkaloid could be found However, Blount noted that these outbreaks of disease had followed the use of Brazilian groundnut meal in the feed. Some batches of the meal were shown to be toxic for turkeys[1], and for ducklings[4] A toxic fraction has now been isolated and shown to be free from plant alkaloids[4] This toxic material has also been found in some samples of other groundnut meals and groundnuts[5]

While the above investigations were in progress, we observed that feeding Brazilian groundnut meal to newly weaned rats did not produce the acute toxicity seen in turkey poults. However, a few weeks of feeding a diet containing 20 per cent Brazilian groundnut meal reduced growth-rate and food intake, and the continued feeding of this diet caused severe liver lesions. This communication describes the changes seen after a sample of Brazilian groundnut meal known to be toxic to turkey poults had been fed to rats for 6 months.

Three groups (9 male and 7 female) of 4-week-old rats from our inbred colony were caged singly and fed on the following diets purified diet, purified diet with 20 per cent of the starch replaced by Brazilian groundnut meal, and a diet with 20 per cent (control) Indian groundnut meal The purified diet used is our standard purified diet (Table 1)

All rats received tap-water *ad lib* , were weighed weekly and their food intakes measured at twice weekly feeding After 9 weeks, 2 male and 2 female rats from each group were killed for post-mortem The remainder were killed after 30 weeks feeding, by this time the males had eaten 650 gm of groundnut meal.

Histological examinations were carried out as follows Tissues were fixed in formol-saline, embedded in paraffin and sectioned at 7μ (hæmatoxylin and eosin), the liver sections were also stained by Gallegio, reticulin (Gordon and Sweet) and periodic acid–Schiff methods. Frozen sections of the liver and kidney were stained for fat by sudan IV

Growth-rate, food consumption and food conversion ratio were similar in the two control groups receiving the purified diet and the Indian groundnut meal. The weights of rats fed the Brazilian groundnut meal were all lower than the controls, the difference was not significant for the females, but the males reached a difference of 70 gm at the end of the test The food intake and conversion of these rats were also lower than those of the controls (Table 2) No other clinical changes were noted

Post-mortem examination after 9 *weeks* In the rats killed after 9 weeks feeding, macroscopic lesions were confined to the liver of those rats fed Brazilian groundnut meal. The surface of the liver was smooth, with numerous sub-capsular yellow focal

Table 2 GROWTH AND FOOD UTILIZATION

	Mean food intakes (gm)				Mean body-weight gains (gm)				Mean gains (gm)/food consumed (gm)			
	Males		Females		Males		Females		Males		Females	
	0–6 weeks	0–9 weeks	0–6 weeks	0–9 weeks	0–6 weeks	0–9 weeks	0–6 weeks	0–9 weeks	0–6 weeks	0–9 weeks	0–6 weeks	0–9 weeks
Control, purified diet	553 0	886 6	473 2	745 4	164 4	203 3	97 0	111 3	0 297	0 229	0 206	0 150
Brazilian groundnut meal (20 per cent)*	462 5	743 5	423 5	669 2	126 5	161 1	82 1	92 2	0 274	0 217	0 194	0 137
Control, groundnut meal, Indian (20 per cent)	567 6	887 3	482 9	744 4	167 7	208 1	98 2	111 9	0 296	0 235	0 205	0 151

* Significant difference (*P* = 0 05) between results for Brazilian groundnut meal diet and control diets

Table 3 EFFECT OF FEEDING GROUNDNUT MEALS ON LIVER WEIGHT AND TUMOUR INCIDENCE

Group	Average liver weight (gm)		Liver tumours	
	Male	Female	Male	Female
Purified diet	13 6	8 2	0/7	0/5
Purified diet + Brazilian groundnut meal, 20 per cent	28 9	16 6	5/6*	4/5
Purified diet + control (Indian) groundnut, 20 per cent	17 2	10 2	1/7	0/6
Stock diet (containing 10 per cent groundnut meal)	—	—	0/243	1/257

Rats 6–12 mths old

* One death at 6 weeks

lesions 1–2 mm diameter The kidney appeared normal, but sections showed some hydroptic change in the tubules No macroscopic or microscopic lesions were found in the liver and kidneys of the rats in either of the control groups

Post-mortem examination after 30 weeks In the rats killed at the end of 30 weeks feeding, macroscopic lesions were confined to the livers and lungs of the rats fed the Brazilian groundnut meal The other organs—heart, spleen, kidney, adrenal, intestinal and urogenital tract—all appeared normal The livers of all rats in this group were grossly abnormal, twice the weight of the controls (Table 3) with a brownish yellow irregular nodular surface, red and greenish cysts and numerous (1–2 mm diameter) yellowish focal lesions The nodules consist of liver tumours of three types solid yellowish lobulated hepatomas, and blood- or bile-filled cysts Of the 11 rats in the group fed Brazilian groundnut meal, 9 had macroscopic tumours, and two of these rats also had secondary deposits in the lungs The yellow focal lesions seen in all the livers in this group are similar to those found in the rats killed at 9 weeks

In the control animals, one hepatoma was found in the group fed the control groundnut meal, but no focal lesions were seen There were no macroscopic lesions in the rats fed the purified diet

The kidneys were all macroscopically normal, but in the group fed Brazilian groundnut meal, sections show some glomerular and tubular changes In some glomeruli there is a homogeneous eosinophilic deposit on the glomerular membrane Some tubules show a few greatly enlarged hydroptic cells, these have very large vesicular nuclei and much vacuolated cytoplasm No changes were found in the kidneys of control rats

The lungs all show various stages of the rat pneumonia complex, however, in two rats fed Brazilian groundnut meal macroscopic greyish lesions 2–3 mm in diameter with a wide surrounding zone of hæmorrhage were found Sections show infiltration with anaplastic hepatoma cells, with mitosis, and a wide outer zone of alveoli filled with alveolar phagocytes and red blood cells Control rats show only the lesions of the rat pneumonia complex

We have therefore found that the feeding of a known toxic sample of Brazilian groundnut meal to rats failed to reproduce the acute liver damage which occurred in turkey poults The growth of male and female rats was depressed, but this was accompanied by a reduced food intake and lowered food conversion ratio (Table 2) Diets containing the toxic meal were unpalatable and reduced the growth-rate in turkey poults[1]

After six months feeding of 20 per cent Brazilian groundnut meal in a purified diet, 9 out of 11 rats developed multiple liver tumours, and 2 of these had lung metastases This finding indicates that this diet is carcinogenic In comparison the breeding stock show a very low incidence of liver tumours (Table 3) The presence of liver tumours in the absence of cirrhosis, cell necrosis, or cellular infiltration suggests that the toxic agent acts directly on the hepatic cells

The nature of the toxic agent is at present unknown, so far all the attempts to show significant amounts of known toxic substances have been negative[1], and in spite of the resemblance of the liver lesions in turkey poults to those produced by *Senecio* alkaloids these have not been found in the toxic extract[4] The presence of the toxin in groundnut meals from other sources has been reported[5], but our finding of a single hepatoma in rats fed the control Indian groundnut meal is not conclusive evidence for the presence in this sample of the carcinogenic agent

Extracts of the meal are now being tested for their hepatotoxic and carcinogenic effect in rats, but we think that these unexpected preliminary results are of general interest

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LARGE numbers of turkey poults[1] and ducklings[2] died on British farms in 1960 as a result of consuming groundnut (*Arachis hypogaea*) meal imported from Brazil Afterwards, outbreaks of disease associated with the feeding of Brazilian groundnut meal were reported in cattle[3], pigs[4], and sheep (Buxton, J C , personal communication) More recently it has been shown that some samples of groundnut products from a number of other producing countries are toxic to animals[5]

Toxic groundnuts cause hepatic changes in all the species mentioned In cattle, the disease has been reported as indistinguishable from ragwort (*Senecio jacobea*) poisoning[3] In rats fed on a diet containing toxic groundnut meal for 6 months, carcinoma of the liver developed (Lancaster, M C , *et al* , preceding communication)

Earlier work[6] provided a sensitive biological method of detecting toxicity in groundnut products and the active substance was concentrated 250 times We have now further purified the toxic extracts from Brazilian groundnut meal by chromatography on alumina and obtained a crystalline though still not pure substance which produces the same biological effects in ducklings Twenty μgm. of this material given orally is fatal to day-old ducklings in 24 hr , and considerably less produces the characteristic histological liver lesions

The toxic substance is almost colourless, and on paper chromatography on Whatman No 1 paper in *n*-butanol–5 per cent acetic acid gives a single spot, R_F 0 7, which emits a bright blue fluorescence in ultra-violet light The amount of fluorescent material estimated visually to be present in the spots produced in this way from numerous samples has invariably corresponded with the toxicity of the sample as determined biologically We are now using this method to select samples for biological testing, when a little more is known of the physical and chemical properties of the toxic material we have every hope that the method will provide the basis of a routine chemical test

It was suspected that the toxic substance might be a fungal metabolite since a highly toxic sample of nuts from Uganda which had been associated with the deaths of ducklings in Kenya was seen to be heavily contaminated with fungi

Pure cultures of certain of the fungal species present in this sample were produced and those of eight isolates, grown for 7 days at 27° C on Czapek's solution agar, were extracted with chloroform. The extract from one of these was shown to contain a fluorescent material, R_F 0 7, in *n*-butanol–5 per cent acetic acid These extracts were dosed to groups of day-old ducklings and those receiving the fluorescent extract died with the characteristic histological liver lesions of toxic groundnut poisoning The dose required was also of the order expected from a consideration of the amount of the fluorescent material present The toxin-producing fungus was next grown on heat-sterilized, non-toxic groundnuts After 7 days at room temperature extracts prepared from the visibly mouldy nuts were shown to contain the blue-fluorescent material and to be lethal to day-old ducklings, producing the typical liver lesions

The toxin-producing fungus has now been identified as *Aspergillus flavus* Link ex Fries

Further work on several aspects of this problem is now being pursued Thus the toxic substance is being prepared in a pure state from cultures of *A flavus* for comparison with the crystalline material isolated from the toxic groundnut meal, and then for detailed chemical and biological study Examination of other fungi isolated from groundnuts is also being continued to discover whether they produce a toxin Development of a routine chemical test for the toxin is also in hand, while other sections of the Tropical Products Institute are studying the incidence of fungal attack on groundnuts in the tropics and the steps necessary to prevent it Finally, we are examining possible economic methods of making toxic meals safe

Since strains of *Aspergillus flavus* are among the commonest fungal contaminants of cereal grains[7], it would not be surprising if some grain samples were found to be contaminated with the toxic metabolite described We intend to study this problem

We wish to thank Mr J J Elphick, of the Commonwealth Mycological Institute at Kew, for identifying the toxin-producing fungus

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Leaf Vernalization

Vernalization is the indirect stimulating effect of low temperature on subsequent flower formation in high temperatures So far seed vernalization and plant-vernalization are known In winter annuals vernalization of germinating seed is effective In biennials seed vernalization has no visible effect, but 'adult' plants—which have passed the juvenile phase—can be vernalized

In their classical experiments, Gregory and Purvis[1,2] and Purvis[3,4] demonstrated that in *Secale cereale* variety Petkus the locus of seed vernalization lies within the embryo and more specifically in the stem apex Curtis and Chang[5] found that in celery the terminal meristems of the stem can be vernalized Further investigations on the locus of plant vernalization are unknown, but it is believed that the stem apices, or the buds, are the only organs that perceive vernalizing temperatures However, recent experiments have shown that leaves can be also vernalized

These experiments were carried out with leaf cuttings of honesty, *Lunaria biennis* According to former investigations[6] this plant is a typical cold-requiring biennial Hagemann[7] demonstrated that leaf cuttings, consisting of a complete leaf with part of its petiole but without a bud, regenerate easily and reproduce the developmental phase of the plant of origin leaf cuttings from vegetative plants remain vegetative, but those of generative plants will flower Unpublished results by me have shown that translocation of a 'flowering stimulus' from vernalized scions to unvernalized stocks may occur

The experimental approach to leaf vernalization was quite simple Leaf cuttings of juvenile and adult plants, 6 and 12 weeks old respectively, were obtained These cuttings were made of leaves of approximately the same age and were exposed to 5° C during 12 or 20 weeks and afterwards grown at ordinary greenhouse temperatures None of the cuttings from juvenile plants flowered Of the adult plants none of the 25 controls flowered, but 3 out of 17 (17 per cent) and 21 out of 24 (88 per cent) after 12 weeks and 20 weeks of vernalization, respectively, flowered quite normally They had visible flower buds after. respectively, averages of 219 and 182 days from the day of cutting The cuttings had started root formation while at 5° C , but no bud had yet regenerated Consequently, vernalization of leaf plus part of petiole without a bud had taken place

Two additional points are of interest First—and this is quite remarkable—the juvenile nature of the original plants is maintained in the leaf cuttings Secondly, leaves from adult plants require more cold than whole plants, as 9 weeks of 5° C and sometimes less are already sufficient for complete vernalization of 12-week-old plants[6]

Two possibilities offer themselves as explanations of leaf vernalization (1) The whole leaf is vernalized resulting in the production of a flowering stimulus which is translocated to the regenerating new plant The results of Hagemann and of my own grafting experiments tend to support this possibility (2) Each meristematic cell is subject to vernalization and reproduces the vernalized condition by mitosis to the newly regenerating plant It is difficult to choose between these possibilities, but experiments are under way to settle this question

The investigation has been extended by growing the leaf cuttings at ordinary greenhouse temperatures for 2 weeks before the cold treatments started This was done on the supposition that the cuttings would stand the cold treatments better After all, it has turned out to be unnecessary, but rather unexpected results were obtained. percentages of flowering between 72 and 87 per cent in the four groups of 6- or 12-week-old plants and 12 or 20 weeks vernalization It should be realized that the leaves from the 6-week-old plants were 2 weeks older when the cold treatment started, and according to former results plants of 8 weeks old are usually adult Furthermore, root formation had started when the cuttings were exposed to low temperature, while a number of them had formed buds during the cold treatment However, cuttings without buds at the end of the vernalization have flowered, while for the rest the experiments without a 2-week warm period and without buds formed in the cold are conclusive enough The favourable effect of a preceding root formation remains unexplained

Finally, some side-results with regard to the influence of low temperature on the regeneration are worth while mentioning. All cuttings formed roots quite readily All cuttings from 6-week-old plants without cold treatment regenerated sprouts after an average of 89 days Only 52 per cent of the cuttings from 12-week-old plants without cold treatment regenerated sprouts after an average of 169 days After cold treatment, however, 100 per cent regenerated sprouts after approximately the same time as the comparable 6-week groups Judging by the average numbers of sprouts per plant the low temperature had a decidedly favourable effect: 1 3–2 6 in the 6-week group and 1 2–4 8 in the 12-week group

In conclusion, leaf vernalization is possible when the leaves are taken from adult plants On the other hand, the regeneration of sprouts is much better when the leaves arise from juvenile plants Low temperature has a decidedly favourable effect on this regeneration

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A Potent, Non-Toxic Plant-growth Inhibitor from an Antibiotic Fermentation

THE production by micro-organisms of substances inhibitory to the growth of higher plants is well documented. The purpose of this communication is to report a potent inhibitor of plant growth, as measured by our duckweed (*Lemna*) test[1] Solvent extraction at *p*H 9 0 of filtrates derived from fermentations of an unidentified *Streptomyces* led to the recovery of a light tan solid, designated crude *PA*-180 This preparation exhibited considerable antibiotic activity against various micro-organisms (for example, minimum inhibitory concentrations of 3 12 and 6 25 µgm /ml against *Streptococcus faecalis* and *Corynebacterium diphtheriae*, respectively, we wish to thank Dr A R English for the anti-microbial testing and Dr T M Lees and Mr M Lynch for papergram determinations). This crude *PA*-180 preparation also was shown to exhibit remarkable plant growth activity This inhibitory effect was first noticed in a routine test being conducted for an evaluation of its growth effect and its phytotoxicity The results (Fig 1) were noteworthy since they demonstrated a strong inhibitory action with no apparent toxic symptoms The plants at all test-levels were normal except for a decreased size The original experimental transfer consisted of one rosette of four fronds These four original fronds are easily detected among the tiny daughter fronds at the 5, 10 and 20 p p m test-levels The number of fronds is not appreciably decreased, as opposed to the size and weight of the plants

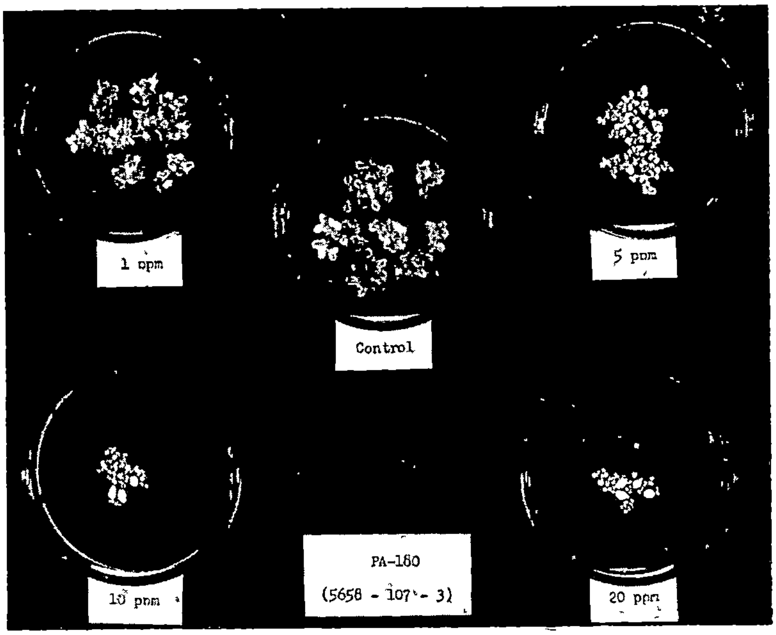

Fig 1 Effects of a preparation of *PA*-180 on the growth of *Lemna minor* under aseptic conditions on a liquid synthetic medium The dwarfing effect is apparent at 5 p p m and more The original four leaves of the rosette used for inoculation are clearly shown at these same levels

The effect of this new inhibitor on the fresh weight of *Lemna minor*, a small aquatic duckweed grown under aseptic conditions on a simple, synthetic liquid medium, is shown in Table 1 along with similar data in this test using several microbial metabolic products, including antibiotics and some well-known plant-growth and general metabolic inhibitors Present investigations are being conducted for the purpose of determining how widespread this inhibitory action is and the range of plants affected, including the lower forms Preliminary results show inhibition of (1) several plant tissues *in vitro*, (2) cucumber seed germination, and (3) growth of barley and several other seedlings

Efforts were then directed toward the preliminary characterization of crude *PA*-180 Papergram

Table 1 EFFECT OF FERMENTATION PRODUCTS (INCLUDING ANTIBIOTICS), PLANT-GROWTH INHIBITORS AND METABOLIC INHIBITORS ON THE GROWTH OF A DUCKWEED (*Lemna minor*) UNDER ASEPTIC CONDITIONS

Compound	Percentage change from control (wet weight basis)				Comments
	1 p p m	5 p p m	10 p p m.	20 p p m.	
Actidione (refs 1, 2)	dead	dead	dead	dead	
Anisomycin	−80	−90	−90	dead	leaves small, chlorotic, complete root inhibition
Azaserine (ref 3)	dead	dead	dead	dead	
Bacitracin (refs 1, 4, 5)	0	+80	+100	+130	
Catenulin (ref 1)	dead	dead	dead	dead	
Duramycin (ref 6)	+35	+35	+35	+30	root inhibition at 20 p p m
Griseofulvin (ref 1)	−10	−15	−20		
Kojic acid	+40	+70	+65	+70	
Oleandomycin phosphate (ref 5)	0	0	0	0	
Penicillin *G* (refs 1, 4, 5)	+60	+225	+230	+220	
Penicillin *S*	+100	+210	+290	+240	
Polymyxin *B* (refs 4, 7)	−15	−15	dead	dead	
Trichomycin	0	+5	+10	+35	
PA-180	−15 to −60	−85	−90	−95	tiny plants at 5–20 p p m.
Azauracil	−20	dead	dead	dead	
3-Amino-1,2,4-triazole (ref 8)	−5	−10	−50	−70	chlorosis and root inhibition at 5–20 p p m
Amo-1618 (refs 9, 10, 11)	+10	+20	+20	+25	
Amo-1619 (ref 10)	+15	+15	+10	+10	
2,4-Dinitrophenol (refs 12, 13)	−25	dead	dead	dead	
(2-Bromoethyl) trimethyl-ammonium bromide (refs. 14, 15, 16)	0	+10	+5	−55	
(2-Chloroethyl) trimethyl-ammonium chloride (refs 14, 15, 16)	+55	+20	0	0	
(2,3-*n*-Propylene) trimethyl-ammonium chloride (refs 14, 15, 16)	+40	+25	10	0	
Maleic hydrazide (ref 17)	0	−10	−25	−40	root inhibition at 10–20 p p m

investigations utilizing various chemical and physical detection techniques disclosed a heterogeneous nature for the material Bioautographs showed the presence of one dominant antibiotic substance Work with eluates of papergram sections (with several culture strains) led to detection of plant-growth inhibitory activity dissociated from that of the dominant antibiotic component, heat stability of the plant factor was also demonstrated It is apparent that the plant factor is a small but potent fraction of crude *PA*-180 Thousands of materials have been screened to date in this test, but none has demonstrated the amount, type and reproducibility of plant-inhibitory activity associated with crude *PA*-180, with the possible exception of another actinomycete fermentation preparation (at present being compared with the *PA*-180 product).

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Antennary Feeding of the Hermit Crab *Diogenes brevirostris* Stimpson

THERE exist only two previous descriptions of feeding behaviour in hermit crabs, namely, those of Thompson, who described the feeding of *Eupagurus longicarpus* Say , and of Orton on *E bernhardus* (L) Orton[1] says that both species feed by scooping up debris directly with either the third maxillipeds or with the small chela The material is brushed between the third maxillipeds, and the rejected portion falls to the ground The antennæ and the walking legs play no part in the feeding of these animals

Diogenes brevirostris Stimpson usually feeds by scraping debris towards the third maxillipeds with the second and third walking legs, and the small chela The material is picked up by the endopods of the third maxillipeds, scrubbed between the brushes of the propus, dactylus and merus, and then passed on to the endopods of the second maxillipeds. These brush the debris further and pass the food to the mouth, the inedible portions being carried away in the exhalent respiratory current Small pieces of algæ may be picked from stones by the small chela and passed on to the mouth parts and so to the mouth

The alternative method of feeding can be described as antennary cast-net feeding. The flagellum of each antenna bears two rows of plumose setæ These are about 3 mm long and set widely apart, each being separated from its neighbour by 0 2 mm The setæ of the two rows curve in towards one another so as to form an obtuse and reflex angle between them The animals normally make occasional cleaning movements of the antennæ, bringing them down alternately to the endopods of the third maxillipeds, which clean them by wiping off the debris caught on the setæ The antennæ then resume their normal resting position, the oblique angle between the setæ facing downwards and slightly laterally If, however, food particles are caught on the setæ, the antennæ begin rapid to-and-fro movements These are interspersed with cleaning movements after every three or four sweeps, and the adhering particles are removed by the maxillipeds and eaten During each sweep the antennæ are twisted so that the oblique angle between the setæ faces in the direction of the sweep. If the animals are feeding in a water current the antennæ are not swept, but merely kept with the oblique angle between the setæ facing into the oncoming current so as to catch the suspended particles. The antennæ are then cleaned in the normal manner. Sorting of the edible and inedible fractions takes place in the usual manner.

Cast-net feeding occurs in other Crustacea but appendages other than the antennæ may be used. Thus *Porcellana longicornis* (L.)[2] and *Haplocarcinus marsupialis* Stimpson[1] use the third maxillipeds as cast-nets. The use of antennæ as cast-nets is found in the nauplius larvæ of *Artemia salina* (L.)[3], while two anomuran sand crabs, *Emerita talpoidea* (Say), and *E. analoga* Stimpson, are also known to feed with their antennæ. They feed in the wash of the waves on the shore, with their antennæ extended as nets. It is reported that *E. analoga* can feed in quiet water by waving its antennæ, but that *E. talpoidea* cannot feed in quiet water at all[4]. There is, however, a distinct difference between the structure of the antennæ of *Emerita* and *Diogenes*. The flagellum of each antenna of *E. austroafricana* Schmitt has two double rows of setæ, the outer setæ of each pair bearing long setules, and the inner short setules. These crabs depend almost exclusively on cast-net feeding, and correlated with this carry on their antennæ a dense feltwork of setules which can trap both large and minute particles. This is not true of *Diogenes brevirostris*, which uses its antennæ only as accessory feeding devices.

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Spawning Mechanism in the Male Lugworm

IT has been shown[1] that the male lugworm (*Arenicola marina*, L.) can be induced to spawn by injecting into the body-cavity the lipid fraction of tissue extracts of whole worms. It was suggested that release of lipid into the body cavity might provide the immediate (internal) stimulus for spawning under natural conditions. The validity of this suggestion can only be tested when the mechanism by which the gametes are emitted is thoroughly understood.

In the lugworm the gametes develop in the body-cavity for a prolonged period and are eventually shed through the nephromixia[2]. The problem in the elucidation of the spawning mechanism lies in the fact that during their development the gametes must be rejected by the ciliated funnels of the nephromixia so that they are retained in the body-cavity, whereas when spawning commences they must suddenly be accepted by the ciliated funnels so that they are shed.

The answer to this problem suggested itself in the experiments, mentioned above, in which tissue extracts were injected into male worms. It was noted that before the worms began to spawn the spermatozoa in the cœlom, which are normally bound together in morulæ, became dissociated forming a dense suspension of free spermatozoa. It seemed possible that whereas sperm morulæ were rejected by the nephromixia, free spermatozoa were accepted by them so that shedding automatically ensued. This possibility has since been tested in a series of experiments in which samples of free spermatozoa were injected into the body-cavities of male worms which were not themselves spawning. Samples of free spermatozoa were obtained by concentrating sperm suspensions spawned naturally by centrifugation, or by treating cœlomic sperm *in vivo* or *in vitro* with lipid extract. The lipid was afterwards separated, again by centrifugation. As it could be argued that free spermatozoa obtained by both these methods might be contaminated with a natural spawning stimulant, samples of free sperm were also obtained by treating cœlomic sperm with alkali. The *p*H of the suspension was afterwards adjusted to 7·5. Untreated cœlomic sperm was administered to control worms. 1·0–1·5 ml. of one of these concentrated suspensions was administered to each test worm, the total number of free sperm injected having been estimated previously by counts using a 'Metz' hæmocytometer. With the exception of the controls, shedding ensued in every case, usually less than 1 hr. after injection of the free spermatozoa (Table 1). It would appear, therefore, that free spermatozoa are accepted automatically by the nephromixia and shed. It is possible, however, that shedding under natural conditions is not entirely passive. In these experiments spermatozoa were shed from the pores of the nephromixia much less vigorously than in normal spawning. Furthermore, estimates of the number of free sperm remaining in the body cavities of the test worms after shedding had ceased showed that in most cases only a third to a half of the total free sperm injected had been shed (Table 1).

Table 1. COUNTS BY HÆMOCYTOMETER OF FREE SPERM INJECTED INTO MALE WORMS AND OF THE NUMBER REMAINING IN THE BODY CAVITY AFTER CESSATION OF SHEDDING

Worm No.	Type or treatment of injected sperm	Total free sperm ($\times 10^9$) injected	Spawning occurred in (+)	Time of spawning (min.) after injection	Total free sperm ($\times 10^9$) in body cavity after spawning ceased
1	Naturally spawned	2·0	+	24	0·3
2	Naturally spawned	3·1	+	10	not counted infected
3	Naturally spawned	3·1	+	28	not counted infected
4	Extract (*in vitro*)	8–10·3	+	90	3·2
5	Extract (*in vitro*)	8–10·3	+	overnight	8·4
6	Extract (*in vitro*)	8–10·3	+	60	5·3
7	Extract (*in vivo*)	8–12·6	+	28	2·9
8	Extract (*in vivo*)	8–12·6	+	14	3·0
9	Extract (*in vivo*)	8–12·6	+	13	2·5
10	Alkali (*in vitro*)	11·0	+	9	4·1
11	Alkali (*in vitro*)	11·0	+	overnight	10·9
12	Alkali (*in vitro*)	11·0	+	15	4·1
13	Control untreated	1·75	—	—	—
14	Control untreated	1·75	—	—	—
15	Control untreated	2·0	—	—	—
16	Control untreated	2·0	—	—	—

While no great accuracy can be claimed for these counts due to difficulties in estimating the volume of the cœlomic fluid in each worm, it is clear that substantial numbers of free spermatozoa were usually retained in the body cavity. Even in the controls substantial numbers of free sperm were present in the suspension injected (due to handling of the suspension and to the fact that it was exposed to the air for some time) and none of this sperm was shed. This suggests that in normal spawning there is more active uptake of free sperm from the body-cavity. On the other hand, it was noticeable that when concentrated suspensions of naturally spawned sperm were injected there was more complete evacuation of free sperm from the body cavity although it was only possible to make one count (Table 1) Furthermore, when a microscopic examination was made of the suspensions of free spermatozoa produced by treatment with extract and alkali, it did appear that although the sperm morulæ had been broken down there was a tendency for the spermatozoa to continue to adhere together by their tails It may have been for this reason that many apparently free spermatozoa were not shed whereas the naturally spawned sperm was completely dissociated and thus accepted more readily by the nephromixia The correct interpretation of this evidence was suggested in similar experiments with female worms, which are reported at greater length elsewhere[3] In these it was shown that maturation of the ova immediately precedes the onset of shedding This suggested that mature eggs might be accepted by the nephromixia whereas immature eggs are rejected. Mature eggs which had been spawned naturally were collected and concentrated As in the experiments with males, these were injected into test worms which were not spawning Shedding immediately ensued, but again only about half of the material injected was emitted. It appears, therefore, that emission of the gametes in *A marina* involves primarily a change in the nature of the gametes which is associated with their maturation so that they can be accepted by the ciliated funnels of the nephromixia, and secondly, there may also be some form of active uptake of the gametes by the nephromixia

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VIROLOGY

Cytopathic Protective Factor : its Production by Normal Uninfected Serum-free Cell Cultures

At the present time two well-defined systems of viral interference by cell products have been demonstrated *in vitro* They are interferon[1] and viral inhibitory factor[2], the latter possibly being a variety of interferon Both at least have in common the fact that they are produced in cell cultures infected with virus, active virus in the case of the latter, or inactivated where interferon is concerned Other differences and similarities are discussed in a recent report by Ho and Enders[3] It is well known that virus inhibitors are present in a variety of mammalian tissues and fluids[4] and may be demonstrated either *in vivo* or *in vitro*

In the process of searching for biologically synthesized inhibitors for viruses, it was discovered that a line of serum-free mouse lung cells produced a substance which 'protected' heterologous cells from destruction by influenza virus The lung cells were originally isolated from lung tissue of new-born white Swiss mice on a medium of 10 per cent horse serum and 90 per cent medium 199 They were afterwards adapted to rapid growth on a medium consisting of 1 per cent peptone, 100 mgm per cent dextrose, and 99 per cent medium 199 (ref 5) They have been designated the *MPD*1 line of cells It had also previously been shown that the serum-free cells were equally as susceptible to vaccinia virus infection as the original serum-containing parent strain[6] Neither line of cells showed any response to dilute or undilute influenza viruses *PR*8, Asian, Lee *B*, or *B*54, when 40-hr or older cultures were used.

Two additional cell lines, one designated *UIRHM*1 (University of Illinois rabbit heart mark 1) isolated from normal rabbit heart tissue, and one designated *UIKM*1 (University of Illinois keratoacanthoma mark 1) isolated from a human keratoacanthoma lesion[7], are fully susceptible to all four of the influenza viruses previously mentioned Both these cell lines contained 10 per cent calf serum as a supplement to the base medium 199 The cytopathic effect of the virus was evidenced as a pronounced cytoplasmic vacuolation with eventual cell destruction in the *UIKM*1 cells In the case of the *UIRHM*1 cells the destruction of cells occurred without cytoplasmic vacuolation These cytopathic effects could be prevented by specific antiserum, and occurred within 48 hr of virus inoculation

Following the basic recognition that such a phenomenon occurred with *MPD*1 fluids and influenza virus, it was decided to develop and control an investigation into this 'protective' effect.

Cultures of *UIRHM*1 and *UIKM*1 cells were seeded in a volume of 5 × 10^5 cells per ml in 1-ml Leighton tubes and incubated at 37° C for 24 hr At the end of this time, the supernatant fluid was removed and the cells were covered with 1 ml of pooled 6-day supernatant fluids from *MPD*1 cells 5 min after the addition of the 6-day old fluid, the cultures were inoculated with 0 05 or 0 1 ml of a specific strain of influenza virus, either *PR*8 or *B*54, which had been grown in chick embryos Controls consisted of normal cultures, cells which were reared with maintenance media (99 per cent Hanks balanced salt solution and 1 per cent calf serum), and cells to which complete fresh peptone 199 media was added In addition to these, a third series of tubes received an overlay of pooled *MPD*1 supernatant fluid to which had been added concentrated medium 199 and calf serum so that the amount of these substances present was equal to their original concentration in the test cell media The cells were re-incubated at 37° C and viewed microscopically at 17, 24, and 40 hr The cytopathic response was graded as 0, 1+, 2+, 3+, or 4+ Under this system, 0 would indicate no cytopathic response or complete cell protection, 1+ as 25 per cent, 2+ as 50 per cent, 3+ as 75 per cent, and 4+ as 100 per cent cytopathic effect

Results showed complete protection against cytopathic effect of both influenza viruses in the two cell lines In addition, it was found that this same protection occurred when Lee *B* or Asian influenza viruses were used This protective fluid was afterwards named cytopathic protective factor

In order to standardize future work, a test system composed of *UIRHM*1 cells and *B*54 virus was chosen The standard inoculum was approximately 3 × 10^5 tissue cells per ml of fluid and 0 1 ml of *B*54 chorioallantoic fluid virus, containing $10^{7\cdot 1}$ 50 per cent chick embryo infective doses (EID_{50})

In order to determine the concentration of cytopathic factor in the supernatant fluids, the following experiments were set up Fluids from pooled 6-day harvests of *MPD*1 cells were diluted from 1 2 up to 1 512 in two-fold steps in complete growth media The fluid from 24–72 hr old *UIRHM*1 cells was decanted and the cells overlayed with specific cytopathic factor dilutions, and the standard testing procedure carried out Four tubes were used for each dilution, as well as the normal control series It was possible to dilute the cytopathic factor 1 16 and still protect 75 per cent of the cells A dilution of 1 8 afforded 100 per cent cell protection

At this point it was considered vital to know whether the cytopathic factor acted on the cell *per se* or the virus particle To resolve this problem, the fluid from 24-hr old cultures of *UIRHM*1 cells was decanted and the cell sheet was overlayed with undiluted cytopathic factor fluid and incubated for 1, 3, 6, and 20 hr at 37° C At the end of this time, the fluids were removed and replaced by complete growth media and 0 1 ml of *B*54 virus No protection occurred at any of the periods tested, showing that the cytopathic factor was not adsorbed to the cell sheet.

To test the action of cytopathic factor on influenza virus, *B*54 virus was diluted 1 10 in the undiluted factor fluid and incubated at 37° C for 1, 3, 6, and 20 hr At the end of this time, the virus was sedimented by centrifugation at 81,000*g* for 30 min The fluid was decanted and the virus re-suspended, to its original stock concentration, in complete growth media and used in the standard test system The virus was fully as toxic to *UIRHM*1 cells as untreated virus, indicating that the action of cytopathic factor is not directed against the virus *per se* The lack of action on the virus was further demonstrated by its negative reaction in hæmagglutination inhibition tests.

In order to determine the period of formation of cytopathic factor, the following experiment was designed Six 10-ml flasks of *MPD*1 cells were inoculated with 4 × 10^5 cells per ml and incubated at 37° C. At the end of 24 hr , and every 24 hr for a total of 6 days, the entire 10 ml of supernatant fluid was harvested and stored frozen When all fluids had been collected, they were tested for the presence of cytopathic factor by the dilution method mentioned The fluid was negative for protective capacity at 24 hr , became positive at 48 hr , and increased to a maximum at 5 days The period between 24 and 72 hr is undergoing further investigation

Present results indicate that the cytopathic factor differs mainly from interferon and viral inhibitory factor in that it is produced in uninfected cultures and not as a result of contact with viruses, either active or inactive It is similar to viral inhibitory factor in that it neither acts on the virus *per se* nor is adsorbed to the cell sheet Interferon, on the other hand, is readily bound by test cells The cytopathic factor appears to be a natural cell product protecting against the cytopathic effect of influenza virus in the cells from which it originates as well as cells from human and rabbit sources Further, it has not been found in the parent cultures containing serum of the *MPD*1 line Since both virus infection and adaptation to serum-free media must represent a traumatic experience to the cell, there is the possibility that other manipulations of this type will produce similar materials That the protection crosses species barriers at a cellular level is not startling, since it has been shown by Alfred and Pumper[8] that the supernatant fluid from *MPD*1 cells or purified portions of it can act as a serum replacement for human liver cells *in vitro*

Cytopathic factor had been used in attempts to protect the *UIKM*1 line of cells against coxsackie, mumps, vaccinia, poliomyelitis, and adenoviruses to which this cell line is susceptible, without success Thus it appears to be specific for influenza viruses Experiments to determine its physical and chemical properties and site of action, as well as work on its purification, are in progress

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MISCELLANY

The Grayling Fish and River-Names

The grayling fish belongs to the family Thymalidae and is closely related to the family Salmonidae It offers, I believe, a new and tenable explanation for the much-disputed origin of the river-names 'Thames' and 'Humber'

When, in British prehistory, these Rivers were so named, fishing was a vital industry, certainly more so than agriculture, and possibly more than hunting. Our rivers were, in those remote days, teeming with fish, and especially fish of the salmon family, so that nothing could be more natural than that some of these rivers should be named 'grayling-river'

'Grayling' is a name of germanic origin (cf German *grau*) But in medieval English the grayling fish retained two names of non-germanic origin *thyme* and *umbre* The 'thyme' word for a grayling is due to the fact that the fish has a smell closely resembling that of the herb of the same name The word is related to Latin *thymallus* (a fish smelling of thyme) and to Greek θύμος, the herb 'thyme' (θύω, 'to smell') The 'umbre' word for a grayling is due to its grey and shadowy appearance, and may be cognate with, if not directly descended from, Latin *umbra*, 'shade' (*umbra* was a Roman word for a grayling fish)

In his book, *Der Flussname Thamse unde seine Sippe* (1941), Prof Max Forster reaches the conclusion that the meaning of 'Thames' is 'dark river' For 'Humber', Prof Eilert Ekwall (*Oxford Dictionary of English Place Names*) suggests that the meaning is 'good-river'.

However, I submit that there is a *prima facie* case for expert investigation of the hypothesis that both the Thames and the Humber, and the groups of river-names associated with them, are so called because of the grayling fish that frequented them—the Thames on account of the smell, and the Humber on account of the colour

If this is acceptable, it has some bearing on the origins of the language spoken by the natives of Britain before the Romans arrived

John Tutin

11a Tregunter Road,
London, S W 10

FORTHCOMING EVENTS

Monday, December 18

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W C 2), at 5 30 p m —Mr F J D Taylor "Large Microwave Steerable Aerials for Communication with Artificial Earth Satellites and Space Probes".

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S W 1), at 5 30 p m —Meeting on "The Mothproofing of Wool"

Institution of Mechanical Engineers, Education and Training Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "The Teaching of Machine Tool Technology as an Academic Subject"

Tuesday, December 19

Institution of Electrical Engineers, Measurement and Control Section (at Savoy Place, London, W C 2), at 5 30 p m —Mr K G King "The Use of Controllable Semiconductor Rectifiers in Machine Control".

Wednesday, December 20

Royal Meteorological Society (at 49 Cromwell Road, London, S W 7), at 5 p m —Scientific Papers

Royal Society of Medicine, Comparative Medicine Section (at 1 Wimpole Street, London, W 1), at 5 p m —Meeting on "The Provision and Use of Pathogen-free Laboratory Animals" Speakers Dr W Lane-Petter, Dr D G Davey and Dr. A O Betts

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W C 2), at 5 30 p m —Dr R L F Boyd "Satellite Instrumentation"

Society of Chemical Industry, Corrosion Group (at 14 Belgrave Square, London, S W 1), at 6 p m —Discussion of recently published papers

Thursday, December 21

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p m —Prof B V Derjaguin (U S S R) and Mrs N D Shukakidse (U S S R) "Dependence of the Floatability of Antimonite on the Value of Zeta-Potential", Dr J D Peacock and Mr R Williamson "Radon Determination as a Prospecting Technique"

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W 1), at 5 p m —Prof G W Mackey "Some Connections between Ergodic Theory and Group Theory"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Lecturer in Mathematics—The Principal, College of Education, St Andrew Street, Aberdeen (December 26)

Organic Chemist or Biochemist (with a good honours degree or equivalent qualification), for research in structural carbohydrate chemistry and immunochemistry—The Secretary, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S W 1 (December 30)

Chair of Applied Physics—The Registrar, The University, Hull (December 31)

Chair of Sociology—The Registrar, The University, Hull (December 31)

Lecturer in Geography, a Lecturer in Physics, and a Lecturer in Psychology at the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, December 31)

Lecturer or Senior Lecturer in Genetics at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, December 31)

Lecturer (with an honours degree in biochemistry or chemistry with subsequent research and/or teaching experience in some branch of biochemistry) in Biochemistry at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, December 31)

Lecturer (with some experience in computing, preferably in connexion with electronic machines) in Computing—The Registrar University College of Wales, Aberystwyth (December 31)

Reader or Senior Lecturer in Mathematics at the University College of Townsville, University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, December 31)

Assistant in Bacteriology (Science)—The Secretary of University Court, The University, Glasgow (January 3)

Lecturer in the Electronic Computation Laboratory of the Applied Mathematics Department—The Registrar, The University, Sheffield (January 6)

Lecturer or Senior Lecturer in Statistics in the Department of Mathematics—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (Januarv 6)

Lecturer or Assistant (preferably with a special interest in solid state physics) in Physics—The Secretary, Queen's College (University of St Andrews), Dundee (January 8)

Lecturer (preferably candidate who can teach in the fields of Romano-British and/or Medieval English archæology) in Archæology—The Registrar, The University, Nottingham (January 13)

Chair of Anatomy in the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 15)

Lecturers in Physics—The Registrar and Secretary, King's College (University of Durham), Newcastle-upon-Tyne 1 (January 15)

Research Fellow (preferably with experience in elementary particle physics) in the Department of Theoretical Physics, Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W.1 (January 15)

Senior Demonstrator (with a science degree with a major in geology, and special qualifications and research experience in geomorphology) in Geomorphology in the Department of Geology —The Registrar, University of Melbourne, Parkville, N 2, Victoria, Australia (January 15)

Senior Lecturer (with special qualifications in analysis) in Mathematics, and a Lecturer and an Assistant Lecturer (with research experience in mathematics) in Mathematics in the Department of Pure Mathematics—The Registrar, University College of North Wales, Bangor, North Wales (January 18)

Professor (with a higher degree, preferably a doctorate, and research in the field of philosophy) of Philosophy at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 20)

Lecturer in Mathematical Statistics at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 22)

Reader in Physics at St Bartholomew's Hospital Medical College —The Academic Registrar, University of London, Senate House, London, W C 1 (January 22)

Chair of the History of Science and Technology at Imperial College—The Academic Registrar,. University of London, Senate House, London, W C 1 (January 25)

Turner and Newall Research Fellow (with two or three years postgraduate experience, or industrial experience, and preferably some knowledge of semiconductor devices) in Applied Science, for research concerned with novel types of electrical machines of special significance to control engineering—The Registrar, University College of North Wales, Bangor, North Wales (January 25)

Lecturers (2) in Pure Mathematics at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 26)

Chair of Engineering in the University of Malaya, Kuala Lumpur —The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Kuala Lumpur and London, January 31)

Demonstrators (graduates with some research experience) in the Department of Organic Chemistry—The Registrar, The University, Liverpool, quoting Ref CV/575/T (January 31)

Lecturer in Physics—The Registrar, The University, Hull (January 31)

Official Fellow and Tutor in Physics, or a Lecturer in Physics—The Principal, St Hugh's College, Oxford (February 1)

Reader in Mathematics at the University of Western Australia —The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, February 17)

Imperial Chemical Industries Research Fellows in Chemistry, Physics, Metallurgy, Engineering, Geology, Agricultural Science or related subjects—The Registrar, University of Wales, Cathays Park, Cardiff (March 5)

Lecturer (with a good honours degree in physics with chemistry as a subsidiary subject, and a Diploma in Education or Teachers' Certificate, or a good general degree with physics and chemistry, and at least five years teaching experience) in Physics with Chemistry in Nigeria—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W C 2, quoting Ref G 13/3

Psychologist with a Research Unit engaged on laboratory and field studies of complexity of industrial tasks—H Murrell, Department of Psychology, The University, 22 Berkeley Square, Bristol 8

Research Fellow (Senior or Junior) (with a Ph D , and preferably postdoctoral research experience), for work on biochemical activities of intact intestine and of isolated subcellular fractions of intestinal mucosal cells—Dr J W Porteous, Department of Biological Chemistry, Marischal College, The University, Aberdeen

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

World University Service Student Mental Health Report of a Conference held at Campbell Hall, London, 8th–9th April, 1961 Pp 44 (London World University Service, 59 Gloucester Place, 1961) 2*s* 6*d* [259

Institute of Personnel Management Personnel Management Salaries Report of a Survey undertaken in February/March 1961. Pp 32 (London Institute of Personnel Management, 1961) 21*s* [259

Thomas Hedley and Co , Ltd , Financial Statement for the year ended 30th June, 1961 Pp 16 (Gosforth, Newcastle upon Tyne Thomas Hedley and Co , Ltd , 1961) [259

The University of Sussex Prospectus 1961–1962 Pp 26+2 plates (Brighton The University of Sussex, 1961) [259

Research Institute, Animal Virus Diseases, Pirbright Report for the years 1956–1960 Pp 52 (Pirbright, Surrey Research Institute, Animal Virus Diseases, 1961) 2*s* 6*d*. [259

Agricultural Research Council A Bibliography of Farm Buildings Research 1945–1958 Part 6 Buildings for the Processing and Storage of Fodder Pp. 52 (London Agricultural Research Council, 1961) 4*s* [259

Corrosion Science, Vol 1, No 1 (August, 1961) (An International Journal of the Science of Corrosion and Protection of Metals) Pp 76 Annual subscription (A) For libraries, government establishments, and research institutions 140*s* , 20 dollars (B) For individuals who write directly to the publisher certifying that the journal is for their personal use 70*s* , 10 dollars (London and New York Pergamon Press, 1961) [259

Ministry of Agriculture, Fisheries and Food , Department of Agriculture and Fisheries for Scotland , Department of Health for Scotland Milk Powder Pp v+18 (London H M Stationery Office, 1961) 1*s* 3*d* [259

The Royal Institute of Chemistry Lecture Series, 1961 No 1 Atmospheric Photochemistry By Sir Harrie Massey and A E Potter Pp 27 5*s* No 2 A Chemist's Introduction to Statistics, Theory of Error and Design of Experiment By Dr D A Pantony Pp ii+38 5*s* No 3 Recent Development in Polarography By Dr G W C Milner Pp ii+41 5*s* (London The Royal Institute of Chemistry, 1961) [259

Building Research Station Digest (Second Series), No 14 Concrete Mix Proportioning and Control—2 Pp 4 (London H M Stationery Office, 1961) 4*d* [259

Department of Agriculture and Fisheries for Scotland Freshwater and Salmon Fisheries Research No 26 The Fecundity of Atlantic Salmon (*Salmo salar* Linn) By J A Pope, D H Mills and W M Shearer Pp 12 (London H M Stationery Office, 1961) 4*s* net [259

Ministry of Pensions and National Insurance Digest of Statistics Analysing Certificates of Incapacity, 1957/58 Pp 25+2 charts+42 tables (London Ministry of Pensions and National Insurance, 1961) [259

Other Countries

Organization for European Economic Co-operation Research Into Road Safety Report on the First International Meeting on Research Into Road Safety, organized by O E E C , at the Road Research Laboratory, Langley (United Kingdom), July, 1960 Pp 54 (Paris Organization for European Economic Co operation , London H M Stationery Office, 1961) [259

United States Department of the Interior Fish and Wildlife Service Circular 107 Patuxent Wildlife Research Center—Descriptive Brochure Pp 24 (Washington, D C Government Printing Office, 1961) 20 cents [259

The Conservation of Wild Life and Natural Habitats in Central and East Africa Report on a mission accomplished for Unesco, July–September 1960 By Julian Huxley Pp 113+20 plates (Paris Unesco , London H M Stationery Office, 1961) 4 50 N F , 6*s* , 1 25 dollars [259

Coryndon Memorial Museum Occasional Papers No 7 New and Little-known African Lepidoptera By R H Carcasson Pp 23+5 plates 7*s* 6*d* No 8 Notes on the Snails of North-East Tanganyika By B Verdcourt Pp 23 7*s* 6*d* (Nairobi Coryndon Memorial Museum , Cambridge Deighton Bell and Co , 1961) [259

Chicago Natural History Museum Fieldiana Zoology Vol 33, No 7 The Philippine Biting Midges of the Genus *Culicoides* (Diptera Ceratopogonidae) (Philippine Zoological Expedition, 1946–1947) By Mercedes D Delfinado Pp 627–675+plates 8–14 2 dollars Vol 39, No 48 A New Genus and Species of Frog (Leptodactylidae) from Colombia By Doris M Cochran and Coleman J Goin Pp 543–546 10 cents Vol 39, No 49 On the Nomenclature of Certain Whales By Philip Hershkovitz Pp 547–566 50 cents Vol 39, No 50 Variation in the Quail-Dove *Geotrygon frenata* By Emmet R, Blake and Gunnar Hoy Pp 567–572 15 cents (Chicago Chicago Natural History Museum, 1961) [259

Geological Survey of British Guiana Geological Maps Quarter Degree Sheet 0559 SW Potaro S W Quarter Degree Sheet 1658 SW Georgetown S W Quarter Degree Sheet 0659 SW Puruni S W Quarter Degree Sheet 0759 SW Waini S W 1 dollar (B W I) each (Georgetown Geological Survey, 1961) [259

Indian Forest Bulletin (New Series) No 230 Wood Seasoning—Experiments on the High Temperature Drying of some Indian Timbers, Part 1 By S N Sharma, B I Bali, and M A Rehman Pp ii+14 (Delhi Manager of Publications, 1961) Rs 18nP , 2*s* 9*d* [259

Canada Department of Mines and Technical Surveys Geological Survey of Canada Memoir No 316 Triassic Stratigraphy and Faunas, Queen Elizabeth Islands, Arctic Archipelago By E T Tozer Pp viii+116+30 plates (Ottawa Queen's Printer, 1961) 2 50 dollars [259

United States Department of Agriculture Agricultural Research Service—Plant Pest Control Division Picture Sheet No 4 Japanese Beetle Pp 2 (Washington, D C Government Printing Office, 1961) [259

Indian Forest Records (New Series)—Silviculture Vol 10, No 6 Indian Forest and Forest Products Terminology Part 1—Forests (Silviculture, Protection, Mensuration and Management, Ecology, Soil Science and allied subjects) Pp iv+125–215 (Delhi Manager of Publications, 1961) Rs 6 00 nP , 9*s* 6*d* [259

World Health Organization Technical Report Series No 219 Arthropod-Borne Viruses—Report of a Study Group Pp 63 3 Swiss francs , 5*s* , 1 dollar No 223 Programme Development in the Mental Health Field—Tenth Report of the Expert Committee on Mental Health Pp 56 2 Swiss francs , 3*s* 6*d* , 0 60 dollars World Directory of Dental Schools Pp 228 15 Swiss francs , 25*s* , 5 dollars (Geneva World Health Organization , London H M Stationery Office, 1961) [259

Geological Survey of British Guiana Mineral Resources Pamphlet No 10 Preliminary Report on the Ferruginous Bauxites of the Pakaraima Mountains By J H Bateson Pp 27 (Georgetown Geological Survey, 1961) 1 dollar (B W I) [259

World Meteorological Organization Technical Note No 35 Techniques for High-Level Analysis and Forecasting of Wind- and Temperature Fields Pp xv+187 (WMO-No 106 TP 45) 8 Swiss francs Technical Note No 36 Ozone Observations and their Meteorological Applications By H Taba Pp xii+48 (WMO-No 108 TP 46) 5 Swiss francs (Geneva World Meteorological Organization, 1961) [259

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Forschungsberichte des Landes Nordrhein-Westfalen No 1001 Die Informationsubertragung bei der Mikroskopie mit Rontgenstrahlen Von Günther Langner Pp 126 (Koln und Opladen Westdeutscher Verlag, 1961) 37 D M [259

United States Department of the Interior Geological Survey Professional Paper 386–A Temperature Rise Within Radioactive Liquid Wastes Injected Into Deep Formations By Herbert E Skibitzke Pp iii+8 15 cents Professional Paper 387–A Botanical Evidence of the Modern History of Nisqually Glacier, Washington By Robert S Sigafoos and E L Hendricks Pp iii+20 Professional Paper 411–A A Solution of the Differential Equation of Longitudinal Dispersion in Porous Media By Akio Ogata and R B Banks Pp iii+7 15 cents (Washington, D C Government Printing Office, 1961) [259

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United States Department of Commerce Weather Bureau Research Paper No 43 The Measurement of Secular Temperature Change in the Eastern United States By J Murray Mitchell, Jr Pp ix+80 (Washington, D C Government Printing Office, 1961) 35 cents [259

Institut Royal Météorologique de Belgique Observations Ionospheriques–Station de Dourbes Janvier, 1961 Pp 26 Fevrier, 1961 Pp 26 Mars, 1961 Pp 26 Avril, 1961 Pp 26 (Uccle-Bruxelles Institut Royal Météorologique de Belgique, 1961) [259

Organization for European Economic Co-operation—Office for Scientific and Technical Personnel New Thinking in School Chemistry Report on the OEEC Seminar on the Status and Development of the Teaching of School Chemistry, Greystones (Ireland), March 1960 Pp iii–215 (Paris Organization for European Economic Co-operation—Office for Scientific and Technical Personnel, 1961) [259

Organization for European Economic Co-operation—European Nuclear Energy Agency Third Report on the Activities of the Agency Pp 253 (Paris Organization for European Economic Co-operation—European Nuclear Energy Agency, 1961) [259

Publications of the United States Naval Observatory, Second Series Vol 17, Part 7 Photometry of Stars in Galactic Cluster Fields By A A Hoag, H L Johnson, B Iriarte, R I Mitchell, K L Hallam, and S Sharpless Pp iii+347–542 (Washington, D C Government Printing Office, 1961) [259

United States Department of Agriculture Agricultural Research Service , in co-operation with the United States Department of the Interior—Geological Survey Agriculture Handbook No 200 Selenium in Agriculture By M S Anderson, H W Lakin, K C Beeson, Floyd F Smith, and Edward Thacker Pp v+65 (Washington, D C Government Printing Office, 1961) 50 cents [410

United States Department of the Interior Geological Survey Water-Supply Paper 1330–E Water Requirements of the Copper Industry By Orville D Mussey Pp iv+181–219+plate 2 Water-Supply Paper 1706 Surface Water Supply of the United States, 1960 Part 3–B Cumberland and Tennessee River Basins Prepared under the direction of E L Hendricks Pp vii+234 Professional Paper 316–E Regional Gravity Survey along the Central and Southern Wasatch Front, Utah By Kenneth L Cook and Joseph W Berg, Jr Pp iii+75–89+plate 13 (Washington, D C Government Printing Office, 1961) [410

Meddelanden från Statens Skogsforskningsinstitut, Band 49, 1960–1961 (Reports of the Forest Research Institute of Sweden, Vol 49) Pp iv+580 Flygblad Nr 74 Skogstradens Fruktsätning År 1959 Av E Fall. Pp 4+10 figures Flygblad Nr 75 Skogsträdens Fruktsättning År 1960 Av E Fall. Pp 4+10 figures (Stockholm Statens Skogsforskningsinstitut, 1961) Skogsforskningsinstitut, [410

Arctic Institute of North America Technical Paper No 6 The Medusae of the Chukchi and Beaufort Seas of the Arctic Ocean including the description of a New Species of *Eucodonium* (Hydrozoa Anthomedusae) By Cadet Hand and Lai Bing Kan Pp 23 (Montreal and Washington, D C Arctic Institute of North America, 1961) 1 dollar [410

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TECHNICAL AND SCIENTIFIC MAN-POWER IN BRITAIN

THE debate on technical and scientific man-power and research which was opened in the House of Lords on November 15 by Viscount Caldecote and Lord Shackleton drew a major speech from Lord Hailsham, the Minister for Science, in reply, but was also noteworthy for several constructive suggestions Viscount Caldecote's purpose was to direct attention to the significance of making full use of the technical man-power of Britain and to the need to devote more resources in research and development in industry to enable exports from Britain to compete effectively in the changing conditions of world trade, and he was confident that if we made full use of the scientific factor we could lead in many more fields He quoted with approval the view of Mr S P Chambers that we could keep our balance of payments straight only by being among the world's leaders in developing new products and new processes We had to export ideas and techniques as well as products

Viscount Caldecote emphasized the importance of making full and proper use of trained man-power in order to maintain the quality of products, but he was highly critical of the magnitude of expenditure on home advertising compared with that on research and development He was doubtful whether there is adequate co-operation between the universities and industry, and thought that there may be some duplication of research and development in the nationalized industries, particularly the service industries of transport, electricity and gas, where the emphasis should be on operational research He suggested that further investigation is required into the possibilities of collaboration between industry and the universities and colleges of technology, but his suggestion for the appointment of a scientific committee akin to the Public Accounts Committee to investigate whether the nation's scientific resources are being adequately, properly and economically used seems to disregard the Advisory Council on Scientific Policy, in spite of a compliment he paid to the Council's work

Viscount Caldecote also thought that the Research Associations should be expanded, though he agreed that some of the smaller associations might perhaps, with advantage, be amalgamated He advocated larger grants, including capital grants, and an inquiry into the reasons why industrial support is so limited He also pointed out the opportunities which the use of computers in management techniques may offer in gaining the full advantage of professionally trained staff, and he pressed the need to ensure that scientific and technically trained staff should have every opportunity to reach the highest positions

By and large, Viscount Caldecote was concerned to avoid the waste of trained man-power Lord Shackleton was concerned primarily to direct attention to the recent report of the Committee on Scientific Manpower on the long-term demand for man-power, of which he was both apprehensive and somewhat critical While he recognized the reservations which the Committee expressed in the report, he seemed to think that the mere suggestion of a possible surplus of scientists and technologists was unfortunate and that the Committee had not pointed out in anything like sufficiently forthright terms the value of employing scientists and technologists outside positions that are strictly professional Lord Marks referred particularly to the problem of the dissemination of information, which he regards as the greatest single difficulty, particularly among the small and medium-sized firms Lord Fleck, however, thought that little is likely to be gained by further inquiry into the use of technical man-power, but that it is much more important to improve the training of technicians and apprentices, particularly to make better use of the higher grade of recruits who now enter on such training. He agreed, however, that we could make far better use also of the most highly trained technologists and scientists at the top, but for this he looked not to the new computer techniques and the like but to the use of the human sciences and especially psychology

Lord Walston dealt more particularly with the character of the training which scientists and technologists should receive, and, while fully conscious that the Robbins Committee is examining the whole field, suggested that both the extent of university courses and the organization of the universities may require adjustment to meet the needs of to-day. Specifically, he suggested a rearrangement of academic terms which would in effect facilitate something like 'sandwich courses' (though he did not himself use the term) and in any event promote co-operation with industry for those studying social science as well as for students of engineering and science He also advocated closer co-operation between the colleges of technology and the universities, and was anxious that the colleges should have the status that conferment of a degree rather than a diploma would give

Lord Halsbury, who strongly supported the transfer of scientists and technologists across the economic structure, referred to their importance as administrators and on occasion as salesmen, he was highly critical of the efficiency with which they are used, particularly in the Government service, and of the way in which administrative delays hinder technical progress Lord Taylor was doubtful whether we are attracting sufficient of the ablest young minds into scientific and technical careers, attributing this largely to inadequate financial rewards as compared with those open to men who take up an administrative career He stressed, however, the need for some degree of planning if the resources of Britain were not to be too diffused, but thought that free consultation and co-operation, particularly by using the statistics which planning produced, might be an effective answer

Lord Hailsham, who replied, has within the past two months delivered three major addresses discussing the problems of science and Government in a free society, research in the universities, and again, in his Fawley lecture, to which Lord Taylor referred, science and Government All these issues in some aspects or other were raised in the debate, and Lord Hailsham's reply naturally reflected a good deal of his conception of his office as indicated in those addresses Nevertheless, his address in the House of Lords on November 15 was no mere repetition, and some of his comments were timely in a debate which once or twice seemed to reflect sensibility more than sense. Lord Hailsham commented that the demands on skill and knowledge for scientific and technical man-power in Britain are not exclusive demands, and are not substitutes for the knowledge of the arts and humanities or of the human sciences. He did not think that in any large respect an advance in science can any longer be obtained by getting out of the schools a much higher proportion of scientists as against humanists.

Lord Hailsham firmly defended the report of the Committee on Scientific Manpower, pointing out that the latest report was one of a series which went back as far as 1946 and that another survey would be made in January 1962. He maintained, moreover, that the prospects for any able young man or woman entering on a scientific or technological career are, and will remain, wider and better than ever before, and it would be a disaster if misinterpretation of the report made any such young people hesitate He stated that the Government is seeking accurate information about the numbers of technicians in training, and agreed that it is essential that highly trained scientists and technologists should be adequately supported in this way. The University Grants Committee is also considering this aspect of the effectiveness of university research

On the wider use of scientists and technologists outside strictly vocational fields, Lord Hailsham was in full agreement with other speakers He referred to the critical shortage of teachers of mathematics, and in a passing reference to university research, in which he put the current research grants from the Department of Scientific and Industrial Research at a total of £2,970,000, expressed the view that the greatest need is to encourage research in industry Industry, however, must in the end meet its own needs, and, turning to the Research Associations, although he agreed that the system could appropriately be re-examined and that there are several ways in which we could stimulate co-operative research, he was convinced that an approach which relied principally or primarily on State support is ineffective In this connexion, supporting what Viscount Caldecote and Lord Marks had said about libraries and the dissemination of information, he suggested that even firms which are too small to conduct research themselves could well afford the modest subscription to their Research Associations which would make available to them so much information on other people's experience in research

The difficulty here is, of course, that many such firms are not even aware of what they are missing, and that frequently they lack people capable of appreciating the significance for their own activities of current advances in science and technology. Here there might be a place for the scientific adviser, such as Lord Marks suggested, but though Lord Hailsham thought that the Department of Scientific and Industrial Research should encourage industrial research chiefly through the Research Associations and the work of its own research stations, he thought that there are two other promising possibilities One is in the work of the Department's Economics Committee, established four years ago, in promoting investigations providing information essential in determining future policy. The Committee's preliminary work has led to more detailed and technical investigations of particular industries, two of which, on machine tools and shipbuilding, have been completed, while others are in progress

Lord Hailsham believes that the machine tool project was outstandingly successful It had effected a decisive change of atmosphere and stimulated a much greater interest by the industry in education The design scholarship course sponsored by the industry at the Manchester College of Science and Technology is proving a great success, and schools of research are being developed in four institutions, to which the Department has already awarded research grants amounting to £170,000, and the industry has now established its own research association In addition, the National Engineering Laboratory is making an impressive contribution in this field, and several projects for civil development contracts are under investigation

The civil development contract is the other line of development which Lord Hailsham regards as particularly promising, and, quite independently of the work of the Economics Committee, the Department of Scientific and Industrial Research has been concerned with the general problem of large-scale development work and has been considering the use of civil development contracts to complement the work of the National Research Development Corporation Lord Hailsham said that the Department has now been authorized to proceed with two major projects, and, if these projects succeed, we may have achieved a major break-through in the relations between Government and industry in the encouragement of science Early in his speech, Lord Hailsham had referred to the long period of time required to bring any plan to fruition—and especially in regard to education. The production of leaders in science may represent the better part of thirty years effort, and it may be for no more than a further ten that they are at the height of their powers, and perhaps fifty years before the application of their discoveries really begins Nevertheless, he reiterated, in concluding, the importance of this connexion between education and man-power and productivity, and the ultimate dependence of progress on the devotion and creative inspiration of men of dedicated genius

Although the main interest of this speech of Lord Hailsham lies in his reference to the work of the Department of Scientific and Industrial Research, and in the indication it gives of his attitude to the Research Associations, it reflects very clearly his consistent adherence to the principles which he laid down in his earlier address on science and Government. He adheres firmly to the idea of the maximum freedom for the scientist and technologist, whether in industry or in the university The main task of the Government and its own institutions is to supplement and to stimulate, to correct the balance where necessary and to identify neglected areas in which some national effort is desirable It was not appropriate on that occasion for Lord Hailsham to enlarge on the detailed implications of that policy or philosophy, though some of the questions asked might have tempted him to do so He admitted, indeed, that the British system of training craftsmen and technicians needs a thorough overhaul, but otherwise made no reference to this aspect of the effective use of trained man-power On the dissemination of information and the place of libraries and information services he made no comment, although it should be a prime concern of the Minister for Science, or any such philosophy as Lord Hailsham expounds, to see that both the National Lending Library for Science and Technology and the National Reference Library for Science and Invention are adequately conceived and planned as part of a national system, and that the resources placed at their disposal are adequate to the needs, not just of to-day, but of the decades immediately ahead A firm adherence to the principles that fundamental research is best pursued at, or in close association with, the universities and that industrial research in the main is the business of industry, implies as a corollary that the Government has the responsibility to see that such ancillary services as library services, the supply and training of technicians and craftsmen, the provision of equipment, etc , for co-operative effort are generously adequate, and commensurate with national needs and technical potentialities rather than the limited horizons which may characterize a backward industry It is in such respects that the Minister for Science has yet to convince some of those who are most ready to applaud his policy and his philosophy that his actions are worthy of his ideas and his vision

CAPTAIN COOK'S VOYAGES

The Journals of Captain James Cook on his Voyages of Discovery

Edited by J C Beaglehole Vol 2· The Voyage of the *Resolution* and *Adventure*, 1772–1775. Pp clxx+1,021 (82 figures). (Cambridge At the University Press, 1961 Published for the Hakluyt Society) 120*s* net

ONE of the greatest explorers and discoverers of the eighteenth century, Captain James Cook, was responsible for important contributions to scientific knowledge After Cook had completed his first voyage round the world in the *Endeavour* (1768–71), during which the transit of Venus was observed at Tahiti, with the support of the Admiralty he was soon able to pursue his further strategic plan of discovery in the southern hemisphere By his own first voyage and by the work of others it had been proved that no southern continent of any magnitude could exist north of 40° south The next step, therefore, was to see whether such a continent could lie south of the fortieth parallel This Cook proposed to do by circumnavigation of the globe in high southern latitudes, taking advantage of the prevailing westerly winds His aim was amply realized. Leaving London with two ships in July, 1772, he arrived back in July, 1775, after an absence of three years and eighteen days Half-way through the voyage he had been parted from the *Adventure*, under Captain Furneaux, which returned to England alone But he carried on with his plan in the *Resolution* and returned in good order, having lost but four of his crew, only one of these by sickness As part of this remarkable feat of ingenuity and endurance, using New Zealand as a primary base with Tahiti as a secondary one, he made three major incursions into the antarctic and two into the tropical waters of the Pacific, each cruise yielding significant discoveries

Crossing the antarctic circle for the first time in history, he succeeded on one occasion in getting farther to the south (71° 10′) than any other nautical explorer on record up to the present day has been able to achieve He encountered a great deal of ice of various types which led him to many interesting observations Though he did not establish the existence of the antarctic continent, and indeed considered it probable that no land would be found to the south of the ice, he did incline to the view that the ice extended in a solid body to the Pole

Apart from the observations on ice and weather in these high southern latitudes right around Antarctica, Cook and his companions were responsible for various other important scientific achievements The hypothesis of a great southern continent in the temperate zones, so persistently advocated by Alexander Dalrymple and others, was finally disposed of In the tropical waters Cook, in pursuance of his plan, was able to confirm, systematize and extend in many particulars the discoveries of Pacific islands hitherto made His observations on the Tonga group and on the New Hebrides were particularly full and significant, and his own geographical discoveries included Palmerston Island and Niue.

From the scientific point of view, one of the most important contributions of this voyage was undoubtedly the success of the chronometer for the establishment of longitude Cook carried with him several of these instruments, not all of which were reliable, but the one which triumphantly proved itself was that made by Larcum Kendall as a duplicate of the fourth instrument made by John Harrison. Known as "Mr Kendall's Watch", this instrument received high praise from Cook and greatly facilitated the research of the astronomers aboard. The botanical and zoological records made by John Reinhold Forster and his son George, together with the anthropological and other observations of Cook and his companions, have now passed into the annals of the early scientific history of the Pacific.

This volume, like its predecessor (see *Nature*, 179, 390, 1957), is a model of editing. In the introduction to his first volume Dr Beaglehole rather prematurely stated that the study of Cook's journals is more complicated for the first voyage than for the second. In the introduction to this volume he remarks rather ruefully that the more the textual problem for this volume is scrutinized, the more complicated it becomes, since there is no existing original version—properly speaking—of Cook's journal on the *Resolution*. The editor deserves, therefore, all the more congratulations for the meticulous way in which he has accomplished his task. The text primarily used for this volume is that of a holograph manuscript in the British Museum, written by Cook. This has been supplemented by material from another parallel manuscript, also by Cook, which he used as a draft for printing his own *Voyage towards the South Pole*, which appeared in 1777. Readers who have known the latter will recognize the great value of the present edition for the expansion of the text, and for the scholarly introduction and abundance of footnotes which assist a great deal in the interpretation. In addition, extracts from officers' records, including that of Furneaux; the journal of William Wales, the astronomer, papers from the Board of Longitude respecting instruments and their use, some letters by Cook; and an admirably annotated calendar of documents add much to the story of the voyage. Of particular human interest in the penumbra of scientific activity is the account of the abortive attempt by [Sir] Joseph Banks (afterwards president of the Royal Society) to assume a leading part in the voyage. In his introduction, Dr Beaglehole seems to be rather hard on Banks. But his reproduction of Banks's own letter to the Earl of Sandwich, as First Lord of the Admiralty, makes it quite clear that Banks's concern for the size and accommodation of his own retinue weighed with him at that time more than a proper thought for the scientific objectives of the voyage. The volume is completed by an important series of maps and plans and many very effective illustrations, including a large number by Hodges, the official artist.

R. Firth

FLORA OF NEW ZEALAND

Flora of New Zealand

By H. H. Allan. Vol. 1: Indigenous Tracheophyta—Psilopsida, Lycopsida, Filicopsida, Gymnospermae, Dicotyledones. Pp. liv+1085. (Wellington: Government Printer, 1961.) 105s.

The publication of a new Flora of New Zealand is an event of supreme importance, and the work now available is a remarkable tribute to the industry and critical botanical sense of the author. Unfortunately, Dr Allan did not live to complete his formidable task of compilation and elucidation, and this book, which includes all indigenous vascular plants except the monocotyledons, was most worthily finished and seen through the press by Miss Lucy B. Moore. It is thirty-six years since the last comprehensive descriptive account of New Zealand plants was published by F. T. Cheeseman, and in that period collectors and investigators have been most active in the study of the flora. Dr Allan played a leading part in these researches and in field-work, and his wealth of knowledge of plants, botanical literature and the classic collections preserved in Britain equipped him admirably for the forbidding exercise of preparing a new Flora.

Students of the vegetation of New Zealand are well aware of the problems of unusual taxonomic complexity with which the flora of the country bristles. In many groups delimitation of acceptable taxa is notoriously difficult because of the high degree of polymorphy and the prevalence of natural hybridity. There is a wide range of habitats, and the comparatively small area of New Zealand has developed an extraordinary concentration of critical genera. Possibly, as has been suggested in the case of the north-temperate flora, some disturbance of the genetical balance during glacial periods has led to the intense and critical diversification which poses the multitude of taxonomic problems. Most informative, adequately referenced, supplementary notes throughout the work add greatly to its value and assist the reader to gain a better understanding of the many specific and generic complexities.

There is still ample scope for research, and Dr Allan's work reveals most pertinently the many challenging features of the New Zealand flora. Within the compass of little more than a thousand pages he has produced a concise, convenient and eminently satisfactory Flora which will be of immense help in identifying New Zealand plants. Many beautiful New Zealand species have enriched gardens in other parts of the world, and horticulturists will certainly welcome this informative and valuable work.

There is an excellent chronological bibliography and, throughout, copious references are given to relevant literature. This is a work thoroughly to be recommended to all interested in the New Zealand flora—indeed, it is an indispensable tool.

George Taylor

SAMPLING AND ANALYSIS OF COMBUSTION PRODUCTS

Gas Sampling and Chemical Analysis in Combustion Processes

By Prof. G. Tiné. (AGARDograph No. 47.) Pp. xv+94. (London and New York: Pergamon Press, 1961. Published for and on behalf of Advisory Group for Aeronautical Research and Development, North Atlantic Treaty Organization.) 42s. net.

This latest AGARDograph is devoted to a review of the experimental technique which forms its title, with particular reference to combustion phenomena of aeronautical interest. A proper appreciation of this subject involves a full understanding of many aspects of chemistry, aerodynamics and heat transfer, and it is rare to find an author with an equally detailed acquaintance of all three branches of science. It is clear from the introduction (Section *A*, 3 pages) that Prof. Tiné is well aware of the great importance of chemical phenomena in combustion processes but from the remainder of the book it is apparent that this is not where his own interests lie and that he is much more at home when discussing sampling (Section *B*, 53 pages) than he is when describing gas analysis (Section *C*, 31 pages). Indeed in the conclusion (Section *D*, 1 page), Prof Tiné points out that "if sampling procedures were absolutely reliable, chemical analysis would raise no problem at all". This, in the reviewer's opinion, epitomizes one of the principal weaknesses in what is otherwise a useful and well-constructed book The author gives the impression that chemical analysis is essentially straightforward and easy and thus—to quote but one example of this point of view—we read on p. 69 that "qualitative and quantitative analysis of alcohols and ethers is exhaustively described in most analytical textbooks", a statement that would surprise many expert analysts The section on sampling, however, gives a masterly account of the various difficulties encountered in obtaining representative gas samples; it deals fully with such topics as the disturbance of gas flow by a probe and the problems involved in bringing about rapid quenching

Prof. Tiné has made a noble effort to write a book which attempts to bridge the gaps which always tend to exist between different formal branches of learning It contains a wealth of information, it is well produced and copiously illustrated with diagrams and tables and even if it is in some ways unbalanced in its treatment, it nevertheless provides the most useful and comprehensive treatment of the subject yet produced The price is, however, even on present-day standards, high for a book of this length.

C. F Cullis

THE ENZYMES

The Enzymes

Edited by Paul D Boyer, Henry Lardy and Karl Myrback Vol 2 Purine and Pyrimidine Nucleotides and Phosphagens Prosthetic Groups and Cofactors (Part A) Second edition, completely revised Pp xiii + 479 16 dollars

Vol. 3 Prosthetic Groups and Cofactors (Part B) Pp. xiv + 497 16 dollars

Vol. 4 Hydrolytic Cleavage (Part A) Second edition, completely revised Pp xx + 631 18 dollars. (New York Academic Press, Inc , London Academic Press, Inc (London), Ltd , 1960)

THE second edition of *The Enzymes* is now expected to run to seven volumes, compared with the four volumes of the original edition prepared by Sumner and Myrback. Volume 1, on general principles, has already been reviewed in this journal (*Nature*, 184, 1759, 1959). Volumes 2 and 3 cover nucleotides, phosphagens, prosthetic groups and cofactors, and the chapters are numbered consecutively through these two volumes, since it was originally intended that this material should be covered in a single volume Volume 4 is the first part of a survey of enzymes involved in hydrolytic cleavage; in this case also it has been necessary to extend to two volumes the treatment of a topic originally expected to occupy only one These changes are sufficient evidence of the wealth of material available to the editors, and of the appropriateness of a new edition at this time

At the end of Volume 3 is a chapter on "Enzymic Equilibria and Thermodynamics" which belongs to Volume 1, but was unfortunately delayed by Prof. Johnson's illness A chapter of this kind, discussing basic ideas of equilibria, free-energy changes, pK's, binding constants, and so on, is extremely valuable to the biochemist with little training in physical chemistry who wishes to understand the more quantitative work in the field of enzymology. On the whole this chapter succeeds in its purpose, although in places the treatment is perhaps too elementary, especially compared with the level of many of the chapters in Volume 1 For example, the importance of the *activity* of reactants in thermodynamic calculations might usefully have been stressed

The subject-matter of Volumes 2 and 3 is primarily chemical rather than enzymological There is a chapter each on nucleotides containing adenine, uridine, cytidine, and guanosine and inosine, and one on *N*-phosphorylated guanidines The remaining 15 chapters come under the general heading "Prosthetic Groups and Cofactors", and include chapters on pyridoxal phosphate, folic acid, glutathione, flavins, coenzyme A, pyridine coenzymes, and lipoic acid, as well as a chapter on metal-enzyme interactions and four chapters on vitamins The emphasis varies considerably from chapter to chapter, although it must be admitted that this may be due to the information available as well as to the idiosyncrasies of the authors (It should be noted here that, as in the earlier volumes of *The Enzymes*, each chapter is written by a different expert) The article on adenine nucleotides and pyrophosphate compounds by R. M Bock is a useful general review of the main groups of enzymes acting on adenosine triphosphate, and a discussion of some of the mechanisms involved; those on uridine nucleotides by L F Leloir and C E Cardini, on cytidine nucleotides by E P Kennedy, and on guanosine and inosine nucleotides by M F Utter are largely metabolic, stressing the enzyme systems through which biosynthesis is brought about by these nucleotides On the other hand, the articles on the pyridine coenzymes by N O Kaplan and on thiamine coenzymes by D E Metzler contain a much larger proportion of the pure chemistry of these compounds

Two chapters seem to me to be of particular importance since they survey fields in which there is a good deal of recent work, and which are clearly ripe for an authoritative treatment The first of these is on folic acid, which is excellently covered in 68 pages by J C Rabinowitz. The rather complicated chemistry is first set out, and then the main biochemical functions of folate derivatives are considered in turn purine biosynthesis, serine biosynthesis, methyl group biosynthesis, and the metabolism of various amino-acids Finally, the metabolism of folic acid itself is discussed. The other chapter referred to, by L Jaenicke and F. Lynen, is on coenzyme *A*, and contains nearly 100 pages This is particularly authoritative, since Lynen has himself been responsible for so much of the work which has made clear the metabolic importance of this compound The chapter finishes with a useful table of 63 enzymes requiring coenzyme A or its derivatives Another useful table is that given by A E Braunstein of enzymes requiring pyridoxal phosphate

In view of the obvious pressure on space in this new edition, it seems doubtful whether it was necessary to include in Volume 3 separate chapters on vitamin K, vitamin E, ascorbic acid, and the visual cycle. The role of vitamins K and E in oxidative phosphorylation is still a matter of some uncertainty and speculation, and cannot yet be discussed in terms of known enzymes. The chapter on ascorbic acid does in fact contain a good deal of enzymology, but treats the metabolism of the compound rather than its coenzyme function, which is not known

Volume 4 of the present series is the first of three in which the main groups of enzymes and a number of individual enzymes will be surveyed It deals with enzymes hydrolysing peptide, glycosidic and ester bonds. The chapters are arranged in groups, in each of which the first chapter is a survey of the field covered by those following In the volume under discussion the 'survey' chapters are. "Peptide Bond Cleavage", by Emil Smith, "Other C–N Bond Hydrolyses", by J E Varner, "Cleavage of *O*- and *S*-glycosidic Bonds", by E. H Fischer and E. A. Stein, and "Carboxyl Ester Cleavage", by D K. Myers This arrangement helps greatly to unify what might otherwise be merely a series of disconnected monographs. There is some evidence, too, of editorial efforts to avoid overlap between the chapters

The general standard of this volume is very high, although inevitably the time taken to assemble manuscripts and produce such a volume has meant that in some cases recent developments are not included and the articles are somewhat out of date Most of them, however, will probably remain authoritative sources for some time to come It is not possible here to mention many of the 33 chapters, however, some stand out as giving a stimulating account of an enzyme, in which a large body of factual information is critically assembled, and used as the basis for a discussion of the mechanism of the enzymatic catalysis or the metabolic importance of the enzyme Such chapters are useful reading for all enzymologists, whether or not their own work is connected with the enzymes under discussion They include those on chymotrypsin and trypsin by P. Desnuelle; papain by E L Smith and J R. Kimmel, α-amylases by D. French; β-galactosidases by K Wallenfals and O. P. Malhotra; and acetylcholinesterase by I R Wilson

The Enzymes is well printed on pleasant paper, and the volumes are strongly bound and are not unpleasantly large to handle The whole work is too large to be regarded as a text-book, but as a reference book it should be on the shelves of every biochemist whose activities demand a reasonable knowledge of enzymology Edwin C Webb

IDENTIFICATION, PROPERTIES AND USES OF INDIAN TIMBERS

Indian Woods

Their Identification, Properties and Uses By Dr K. A. Chowdhury and S. S. Ghosh. With the assistance of K Ramesh Rao and S K. Purkayastha Vol. 1· Dilleniaceae to Elaeocarpaceae Pp liii+304+30 plates (Delhi Manager of Publications, 1958) Rs. 25 50 n p., 39*s*

The publication of a new reference book on Indian timbers is a notable event, for Gamble's *Manual of Indian Timbers* (1922) is now out of date and the more recent *Commercial Timbers of India* (1932) by Pearson and Brown omits many of the timbers that are now being exploited commercially. The volume under review is the first of a series of six each dealing with the wood of 250–300 tree species that occur in the Indian sub-continent and are represented in the wood collection at the Forest Research Institute at Dehra Dun. The chief purpose of the book is to enable the timbers to be identified by characters that are to be seen with the naked eye or hand lens, and to assess the working qualities of the woods from their structural and physical properties Brief notes on the habit and distribution of the species are given, together with some particulars of their strength, seasoning properties, durability, working qualities, supplies and uses The descriptions of the woods are illustrated by photographs (× 10) of transverse sections. These illustrations, which are of excellent quality and well reproduced, add considerably to the value of the book The scientific names and the trade and common names are given in separate indexes and there are appendixes dealing in tabular form with the nomenclature of species, classification of the woods by uses and by anatomical structure respectively

After a valuable introduction of 53 pages, the main part of the book consists of descriptions of the woods under the botanical families to which they belong For the sequence of families the authors have used the modified form of the Bentham and Hooker system adopted by Metcalfe and Chalk in *Anatomy of the Dicotyledons* The volume now under review covers 20 families ranging from the Dilleniaceae to the Elaeocarpaceae and includes the important timber-yielding Dipterocarpaceae, more than 70 pages being devoted to this one family. At the end of each family there is an extensive bibliography

The authors are at pains to dispel the popular conception that India is the potential source of large supplies of many diverse timbers They emphasize that the number of timbers available in large quantities is not more than sixty or so, the most important being teak (*Tectona*), sal (*Shorea robusta*) and deodar (*Cedrus deodara*). Before 1939 the number of timbers handled commercially was 300–350, but about 500 were in use during the Second World War Now the local exploitation of relatively unknown woods is becoming more common owing to the increased demand for timber resulting from industrial expansion and to the difficulties of transport

In discussing the general question of how far wood structure can serve as an aid in taxonomy, the authors, in my opinion, take a somewhat pessimistic view. It is quite true, as the authors point out that in many families the individual species, and sometimes the genera, are not easy to separate on the basis of wood structure This, however, does not exclude the possibility of using wood characters for diagnostic purposes, and they are often valuable in determining the correct family relationships of genera the taxonomic position of which is uncertain from exomorphic characters alone The great danger in the study of systematic anatomy is to rely too exclusively on wood characters alone without taking the histology of the rest of the plant into consideration

The authors are to be congratulated on having produced the first volume of a most valuable book which cannot fail to be welcomed by botanists, foresters and wood technologists, and the publication of further parts will be eagerly awaited. It is a matter for regret that the quality of the binding does not do justice to a reference book of such undoubted importance C R Metcalfe

Introduction à l'Electrodynamique Quantique

Par Prof Daniel Kastler (Travaux et Recherches Mathematiques) Pp xxxiii + 333 (Paris Dunod, 1961) 68 N F

THIS remarkable monograph has been written with the highly commendable aim of providing a common understanding between physicists and mathematicians, so far as the elements of quantum field theory are concerned Whether this purpose has been achieved will probably be answered on the basis of the individual taste of any prospective reader The present reviewer feels that the book will prove stimulating for the highly trained theoretical physicist, provided he has already acquired a fairly comprehensive familiarity with the customary formulation of quantum field theory.

The first two chapters and Appendixes *A*, *B*, *C* provide the general mathematical tools and notions vector-spaces, Hilbert space theory, the Lorentz group and its representations, tensor- and spinor-calculus A short chapter on the method of second quantization precedes the classical and quantized theory of the scalar Klein–Gordon field, Maxwell field and Dirac field (Chapters 4–9) Chapter 10 is devoted to a general discussion of the coupled Maxwell and Dirac fields, and the next chapter surveys briefly the formalism of interacting fields in general, including the *S*-matrix theory (The relationship between the *S*-matrix and scattering cross-sections is established in Appendix *D*) Chapters 12–14 show, by examples of Compton scattering, Möller scattering and pair annihilation the applications of the theory The Dyson–Feynman technique and diagrams are discussed only in the next chapter The book concludes with a review of external field problems

The mathematical exposition is 'high-brow' and follows the pattern of best French tradition, but most physicists will probably find it difficult to rediscover within this work the familiar physical concepts they are looking for P ROMAN

Moon Atlas

By V A Firsoff Pp 32 + 7 plates + 2 fold-out maps (London Hutchinson and Co (Publishers), Ltd , 1961) 63*s* net

THE descriptive classification of lunar surface features to be found at the beginning of this atlas is a commendably lucid account of the intricacies of lunar topography Unfortunately, the illustrations appearing later in this atlas do little justice to the lunar photographs now available The four quadrant maps of the lunar surface, although artistically drawn, have the annoying property of including very few names, identifications have to be made via co-ordinates of individual craters Page 22 consists of a very handsome set of photographs of progressive lunar phases but of restricted value The relief map of the Moon, extremely ingeniously drawn, is of value to young astronomers with small telescopes. it is doubtful, however, whether these persons will be able to afford the cost of the atlas The spherical projection photographs add little value to the atlas although there are few such photographs available elsewhere, Firsoff's efforts have not succeeded in producing pictures of sufficiently good quality to contribute much to selenological thought There are good reproductions of the Full Moon, the Moon's averted hemisphere, and the well-known Palomar Observatory Clavius photograph It is misleading that these latter should be included in the section on spherical projection photographs The *Moon Atlas* closes with Firsoff's personal map of the lunar grid system The presentation (in three colours) of this chart leaves something to be desired and I personally prefer to use the small-scale reproduction appearing on the back of the dust cover BRIAN WARNER

The International Dictionary of Physics and Electronics

Second edition Pp. iv + 1355 (Princeton, N J ; D Van Nostrand Company, Ltd., 1961) 27 85 dollars, 205*s*

THE standardization of terminology in physics and electronics is not an attractive task Some national and international bodies have tackled parts of it with fair success, but because they seek agreement between organizations with widely differing experience and outlook, their progress is often slow This comprehensive dictionary, the work of a handful of scientists with an overwhelmingly academic background, is therefore very welcome, even though one can find some unevenness and lack of balance—most likely attributable to the speed of compilation—and some failure to draw sufficiently directly on the works of the established standardization bodies

Three features distinguish the second edition of the dictionary from the first, published in 1956 First, a new introduction shows how classical physics retreated before experimental findings of the early twentieth century and how new theories filled the gaps created, parts of the old introduction, on units and dimensions, are retained under appropriate headings in the body of the book Secondly, the inclusion of many new items lengthens the book by about 200 pages and, thirdly, multilingual indexes (in French, German, Spanish and Russian) are added.

The title 'Dictionary' understates the value of the book for some entries would fully satisfy, and many partially satisfy, the title 'Encyclopædia', the book is therefore educational as well as one of reference The next edition will have many new fields to cover, such as the properties of space, lasers and the applications of low-temperature physics, if it must accordingly increase in size, might not a shorter or concise edition, prepared by cutting the length rather than the number of entries, find a market as well ? J R TILLMAN

The International Zoo Year Book

Vol. 1, 1959 Pp vii + 160 19 plates. Edited by Dr Desmond Morris and Caroline Jarvis (London. Zoological Society of London, 1960) 40*s*

THE International Zoo Year Book fulfils a very long-needed want, but I feel it would be a great advantage if some check or verification could be made on the information given with regard to zoos—particularly in the Reference Section To my knowledge there are many inaccuracies, especially with regard to details concerning the smaller zoos This is my only criticism—otherwise I think the book is a very worth-while effort which will prove of great benefit to all zoological gardens

It is a great advantage to have a list of the senior staff of the zoos of the world, together with their addresses, and also the various departments of zoology The London Zoological Society should be congratulated on the way this book has been produced G S MOTTERSHEAD

THE BETTER USE OF THE WORLD'S FAUNA FOR FOOD

THAT a large proportion of the world's population, especially in the technologically under-developed countries, suffers from either chronic or seasonal under-nutrition is well known. In many of these countries there is a continuous shortage of dietary calories, the ultimate effect of which is to slow down most human activities, with the apparent exception of the rate of reproduction. Not less serious than calorie shortage in its effect on the younger groups of the population and on pregnant and lactating women is the acute deficiency of dietary protein, which may occur with or without a calorie shortage, and is most obvious in its effects on the post-weaning child up to the age of about five years. Such protein deficiency is particularly marked in animal protein, usually richer in the amino-acids essential for growth and usually accompanied by a more valuable assortment of mineral salts, trace elements and vitamins than is vegetable protein.

The Freedom from Hunger Campaign of the Food and Agriculture Organization has as its objectives the improvement in quality as well as in quantity of food consumed in the existing hungry countries. It was therefore a most timely effort of the Institute of Biology to arrange a symposium, held during October 27–28 in the Royal Geographical Society's rooms, on "The Better Use of the World's Fauna for Food".

In the opening session, under Sir Julian Huxley's chairmanship, Dr. Francis Aylward (Borough Polytechnic, London), who has fairly recently returned from an extensive nutritional mission for the Food and Agriculture Organization in one of the under-developed countries, discussed the methods of assessment: (1) of protein requirements of individuals and communities, (2) of protein supplies actually available in a country, (3) of protein deficits on a regional, national and world-wide basis. The available statistical evidence of protein deficits, though not always as accurate as one could wish, was sufficiently convincing, apart from the additional evidence afforded by direct observations, by morbidity records and by mortality statistics. Though it was highly desirable to remove some of the uncertainty regarding the quality of the statistics and for further research on physiological requirements to be carried out, the need was even more urgent to use the scientific and technical knowledge now available in an intensive effort to improve indigenous protein supplies by the adoption of a more vigorous agricultural policy (in the widest sense, to include animal husbandry, fisheries and controlled utilization of wild fauna) and also by a more thorough application of modern food science to conservation and distribution of food.

"The Relative Merits of Plant and Animal Proteins" was the title of the paper given by Dr. A. E. Bender, whose important work on the assessment of the nutritional value of proteins and mixtures of proteins is well known to British nutritionists. Dr. Bender's contribution provided a useful epitome of the methods by which an assessment may be made of the comparative dietary values of different individual proteins, or mixtures of proteins. He related the figures obtained to needs at different ages and in different physiological conditions. The proteins of cassava, plantain, breadfruit and sweet potatoes are inadequate for health of child or adult, those of rice, yams, millet and wheat can maintain the adult, but not the adolescent or the child. Some seed proteins, especially those of legume seeds (and particularly if two or more 'oil-seed' proteins, freed from oil, are mixed), will provide an adequate protein supply for all ages, if they constitute a sufficient proportion of the diet. The advantages and disadvantages of plant and animal proteins were compared, plant proteins being usually the cheaper, with a bigger yield per acre than animal proteins, recent attempts have been made to produce a cheap high-protein food mainly or even exclusively from vegetable sources (for example, 'Incaparina' in Central America). A mixture of animal with a poorer vegetable protein often gave a protein utilization result for the mixture almost as high as if the whole had been animal protein.

Religious prejudices in certain countries—not always under-developed countries—and local taboos more frequently handicapped the consumption of animal than of vegetable protein-rich foods, but the former were, for most people, tastier and more acceptable.

Though the answer to the protein-shortage problem in some countries might be intelligent vegetarianism, yet the advantages for the agricultural productivity of land of mixed farming, and the importance especially to the vulnerable groups of the population of having in the diet a reasonable percentage of protein food from animal sources, could not be overlooked.

Mr. T. J. Lansbury, in discussing potentialities of animal and particularly ruminant husbandry in West Africa, described the existing handicaps of absence of enclosure and of any intelligible system of land tenure, of 'night kraaling' with its consequent decrease in live-weight gain, of disease and particularly of the presence of tsetse fly in many of the wooded areas, of unfertilized, mineral-poor pasture and semi-starvation of stock during long periods of drought, of catastrophic overstocking where cattle and even sheep and goats were regarded more as status symbols than as sources of food, being associated with bride price, ceremonial gifts and ritual sacrifice at traditional ceremonies.

The local cattle were nevertheless capable of good growth and production if adequate and controlled water supplies and the provision of dry-season supplementary foods could be organized. Two indigenous breeds of cattle, the n'dama and the West African dwarf shorthorn had considerable resistance to trypanosomiasis and were being introduced with advantage into certain tsetse-infested areas where well-distributed rainfall gave good grass production. The substitution of nomadic husbandry by mixed farming, enabling pastures to be suitably fertilized and fodder to be conserved for the dry season, entailed formidable sociological problems as well as wide dissemination of improved techniques.

Mr. Lansbury considered that pig and poultry husbandry probably offered fewer difficulties than cattle husbandry and that it should be possible to use modern knowledge to expand the pig and poultry industry rapidly and efficiently in West Africa, with great benefit to human nutrition.

The text of a paper by Prof. A. G. Bannikov, of the Academy of Veterinary Sciences, Moscow, on the exploitation of the saiga antelope in the U S S R had been circulated, but unfortunately had to be discussed in the author's unexpected absence.

This antelope, which now occurs in increasing numbers in both European and Asiatic U S S.R territories, was in the 1920's, as the result of uncontrolled, destructive hunting, almost extinct Protective laws, introduced in 1919, intended merely to preserve the remnants of the species as a rare curiosity, had little effect at first, but in the 1930's and 1940's a rapid increase in numbers became evident and to-day the total saiga population in Europe is about 500,000, and in Asiatic U S S R about 1,500,000, mainly in the steppe plains and semi-deserts It is a fertile ruminant, congregates in large and mobile herds and is now being systematically and rationally hunted, usually at night by organized groups of marksmen equipped with motor-vehicles with powerful headlights which attract the antelopes Prof Bannikov stated that the recovery of the saiga from near extinction to profitability is a vindication of the principles of intelligent nature conservation

The use of an entirely different genus of animal for food was recorded by Dr R Orraca-Tetteh, who described the habits, collection and utilization in the diet of the giant African snail, *Achatina*

Achatina, the largest living land mollusc, with, it was stated, a foot which when fully expanded might reach 30 cm in length, is found in different parts of Africa, south of the Sahara During the rainy season the snail population in the forests is often large. The animals are particularly active at night, browsing on tender leaves and fallen fruit, and growing rapidly They are collected usually before sunrise and sent quickly to the market They are killed by dropping snail and shell into hot water, the molluscs are then removed by hand They may be eaten at once in soup or stew, or preserved for three months or more by smoking The fresh samples contain about 16 per cent of protein, with a small amount of fat, some thiamine, riboflavin and niacin, and more than 70 per cent of moisture, the protein has a high content of lysine.

In parts of West Africa the snail is a very popular article of diet, and can be purchased cheaply during the rainy season

Dr L Harrison Matthews discussed the problems and potentialities of wild-game ranching, which appears to be increasing, in Africa The existing species of wild vegetivorous fauna in large areas in Africa have been established there long enough to become adjusted to their environment, making use of all the food resources, some by grazing, others browsing, others by eating the upper leaves of trees The bio-mass of this wild fauna which is commercially exploitable is large, especially if natural predators are kept in check

Most cattle ranchers coming into these areas in the past have destroyed or driven away the wild ungulates, regarding them as pests, though the cattle brought in have made less-efficient use of the vegetable food available

The deliberate ranching of mixed wild fauna if they can be profitably cropped for human food is a commercial possibility that is now being exploited with some success In Southern Rhodesia it is said to be more profitable than cattle ranching, while in the Republic of South Africa between 2,000 and 3,000 farmers are said to be stocking game, including such animals as zebra, wildebeest, impala, eland, kudu The resistance of these animals to disease is greater than that of imported cattle Transport and marketing problems in the sale of meat and hides are being solved It was thought that once game ranching becomes commercially established, the extinction of the wild fauna concerned will be unlikely.

Efficient food processing preserves the nutritive value of the raw materials and yields a product acceptable to the consumer P L Pellet and D. S Millar, of the London School of Hygiene and Tropical Medicine, considered the processing and preservation of meat in under-developed areas from both these points of view. In tropical environments the slaughter of stock and the production of dressed carcasses either for immediate consumption or for preservation are fraught with temperature, and other, hazzrds Further, the particular, and to Western eyes rather peculiar, food preferences of the local consumers have to be taken into account, for example, "To the Ghanian, the eating of, or rather the chewing of, meat is a pleasure to be taken leisurely, and consequently he regards tender flesh as low in value" Methods of preservation can seriously lower the nutritive value of any kind of meat or fish, but traditional preserved meats such as biltong, charqui, pemmican and kavurma are highly acceptable to the local consumers, and often showed little protein damage. If traditional methods of preservation were studied scientifically, many could be extended with advantage to meet the immediate protein needs of populations of under-developed countries More sophisticated, modern methods such as refrigeration, canning, dehydration, vacuum contact drying, vacuum oil dehydration, production of meat powders, with the modern equipment necessary could be brought in as capital resources increased and indigenous food preferences and habits permitted

The waters of the Earth (seas, lakes and rivers) occupy 75 per cent of its surface, but account for only 10 per cent of the world's food supply The second day of the symposium was devoted to the problem of increasing food supplies from these sources. Mr E B Parrish (Department of Agriculture and Fisheries for Scotland, Aberdeen) introduced the complex question of how to obtain maximum yield from traditional methods of fishing The increasing intensity of fishing in certain areas such as the North Sea and north-west Atlantic has led to a decrease in the size and age of the fish caught and to a diminution in profitability of fishing To prevent 'overfishing' of these areas, international conventions have been arrived at "for the investigation, protection and conservation of the fisheries" in order to make possible the maintenance of a "maximum sustained catch" Regulations for the fixing of catch quotas, for closed seasons, for closed fishing areas, for controlling the size at which fish could be caught by the fishing gear, for fixing of minimum landing-size limits, have been tried with mixed success

In the conservation and management of fisheries large demands are made on scientific methods of investigating the population dynamics of the exploited fish stocks, these demands continue after regulations have been put into practice, to assess the responses of the exploited stock to them and give more precise adjustments of present fishing intensity to the maintenance of adequate future fish supplies.

Mr F D Ommanney gave a fascinating account of sea fisheries in tropical areas The poor gear and low status of the average tropical fisherman are slowly improving and in British dependent territories the work of fishery officers has led to major advances, largely a result of fitting fishing boats with engines—outboard for the smaller and inboard for the larger craft, and partly by improved nets. At the same time, attempts have been and are being made, as a result of increased landings of fish, to reduce the price to the consumer.

Some Governments in British dependent territories or those formerly dependent, have provided special fishing vessels for exploratory and pioneering work. Stations for fisheries investigations have been established in a few tropical countries, but owing to the vagaries of Government support have not been entirely successful. For the progress of tropical fisheries and the optimal use, in protein-hungry, maritime countries, of sea fauna for food, active and adequately supported research stations are urgently needed.

Improvements and trends of development in marine fishing methods and gear were the subject of a technical, well-illustrated contribution from Dr. J. Scharfe, of the Food and Agriculture Organization (Rome). Fish location and detection have been aided by aerial scouting and still more by fish-finding echo sounders and rangers. These need much skill and practical experience to use to full advantage. This technique is now firmly established for all medium and large fishing vessels in the developed countries. Synthetic fibres stronger and more elastic than natural fibres are being used very widely for nets and lines, and have inspired new ideas in the design and operation of fishing gear and methods.

Larger stern-trawlers of up to 3,000 h.p. with freezers, cold storage and in some cases manufacturing plant for oil and fish meal can now go quickly to distant grounds and remain away from home ports for extended periods. Fishing fleets exist with boats specialized for searching, catching, factory work, transport, hospital and recreation facilities. Mid-water trawling with echo sounding is a modern line of development which is likely to have far-reaching results.

Electrical killing or stunning of fish in confined waters has been experimented with with some success and may have a future even for sea fish.

Mr. K. F. Vaas, of Yerseke (Netherlands), pointed out that fish culture in freshwater and brackish ponds was both an ancient and a very widespread craft. *Tilapia* was cultivated in ponds in Egypt before 2,000 B.C., and a text-book on fish culture was written in China in 475 B.C. Large yields of fish per acre of water surface are obtainable under favourable conditions—the Grand Lac in Cambodia with an area at high water of 10,000 km.^{2} is said to produce 100 kgm. fish per hectare.

Tropical ponds give better yields than those of colder climates, but much depends on 'management' in the shape of fertilizers or the introduction of fish foods of various kinds. Fish culture may be combined with animal husbandry, the dung fertilizing the growth in the ponds of fish food such as diatoms, plants and crustaceans. Production of fish from inland waters has been calculated to be some 18 per cent of the total world catch.

The culture of marine molluscs and crustaceans was reported on by P. R. Walne, of the Ministry of Agriculture, Food and Fisheries, Fisheries Experiment Station, Conway. In the United Kingdom, shellfish form only 2·5 per cent, in Japan 19 per cent, in the Netherlands 28 per cent, and, rather surprisingly, in the United States 30 per cent of the total fish landings. Oysters and mussels are slow growing, the mussel requiring four years, and the oyster five, to reach marketable size. The meat of the adult oyster or mussel contains some 80 per cent of water and 9–13 per cent of protein, that is, roughly 50 per cent or more of protein on the dry weight. Oysters are probably the most carefully cultivated crop obtainable from the sea. In recent years mechanical methods, including special dredges, are being used for removal of the bivalves to new grounds from time to time, and for final harvesting. Such dredges attached to power-driven boats can work economically on quite low population densities. By this means both the mussel and the oyster stock in Holland is moved several times to assist growth and reduce starfish depredation.

Mr. Walne considered that an important future step in oyster husbandry should be what has been done with practically all agricultural plants and animals, namely, to increase the rate of growth and quality of the stock by breeding and selection.

Some recent advances in the processing of fish were described by Dr. D. L. Nichol, of the Department of Scientific and Industrial Research, Humber Laboratory, Hull, in relation to the more efficient use of this source of food.

'White' fish gutted, washed and stored in ice at sea become stale after 12–15 days, and if distant water voyages are to last longer than the average of 18–20 days (4 days each way to the fishing grounds) apparatus is needed for deep freezing at sea at least part of the catch. In trials carried out in 1956, this method of preservation of whole fish has been proved successful and more trawlers are being fitted out with the necessary deep-freeze equipment.

Deep-frozen fish is now being distributed in various forms to consumers. It is necessary to keep the temperature down all along the distribution chain, and this is not always realized, as the difference between a block of frozen fish at − 20° F. (a temperature at which no deterioration occurs in eight months) and + 20° F. (a temperature at which deterioration occurs within a month) is not obvious to the operator.

Herring processing presents different problems from that of white fish, as herring fishing is seasonal and the disposal of gluts may arise. Gluts of herrings are usually transformed into oil and fish meal, the latter being utilized in the manufacture of animal feeding stuffs and increasingly in tinned food for domestic pets.

White fish meal prepared from surplus or condemned fish and from fish offal is manufactured in the United Kingdom and abroad in substantial quantities for use in animal feeding stuffs. Fish flour, a deodorized and almost flavourless powder usually made from whole fish, is proving acceptable as an addition to human diet in certain protein-deficient regions.

A paper on the possible farming of the sea from Sir Alister Hardy concluded the symposium. He discussed the possibility of improving the production of plankton and hence of the fish that ultimately depend on plankton for food either by fertilization of limited or confined areas of sea with phosphates and nitrogen or by causing up-welling of the deeper layers of sea-water which are richer in nutritive salts than the upper layers. He also mentioned methods for diminishing the enormous loss of fish that occurs in early life and the possible transporting of young fish from crowded to less-crowded areas.

Recent research has shown that invertebrate competitors of fish on the sea bed, such as starfish, are much more important in limiting the potential supply of food available to fish than was formerly realized, and that at least in limited areas such pests might be greatly reduced in numbers by specialized trawling

To summarize, the following are among the main considerations that emerged from the symposium

(1) The rapid rise in world population makes it doubly important that there should be increased production and much better utilization for human food of land fauna, both domestic and wild, and of sea fauna.

(2) The potentialities of such increased production are large if present agricultural, animal husbandry and fisheries knowledge is effectively applied, and if the utilization of wild animals is conducted intelligently and under adequate control, these potentialities are immense if sufficient research effort is directed towards the further acquisition of knowledge in these fields, particularly in that of marine biology

(3) The rate of progress in improving the protein status of malnourished populations will depend largely on indigenous decisions to correlate their farming and their fisheries policy with indigenous nutritional needs, and actively and continuously to stimulate the intelligent knowledgeable local production of fish, flesh and fowl

The proceedings of the symposium will be published by the Institute of Biology (41 Queen's Gate, London, S W 7) H D Kay

FIFTH INTERNATIONAL CONGRESS OF BIOCHEMISTRY

The fifth International Congress of Biochemistry, organized by the U S S R Biochemical Society and sponsored by the International Union of Biochemistry and the Academy of Science of the U S S R, was held in Moscow during August 10–16, under the presidency of Academician A I Oparin The scientific sessions were held in the imposing building of the University of Moscow which stands on the Lenin Hills and overlooks the historic city The number of registered participants, about 4,500 together with an additional 1,000 or so guests of the Organizing Committee, was larger than any previous congress, and it seems that each congress, from the first, which was held in Cambridge in 1949, to the present one in Moscow, attracts more and more workers who are interested in one or many aspects of the broad subject of biochemistry

The congress held its opening ceremony in the impressive Palace of Sports of the Central Lenin Stadium and the session closed with a masterly lecture on "The Structure and Function of Sub-cellular Particles", by Prof D E Green (United States) Unfortunately, poor projection facilities reduced the enjoyment of many participants who were not fortunate enough to be seated near the front of the huge lecture hall The plenary session was followed by a most enjoyable concert given by the Moscow Symphony Orchestra

The scientific programme which followed included a series of symposia, and between two and three thousand original contributions given under the auspices of twenty-eight sections The main congress symposia were held in the large assembly hall of the University or in auditoria of ample seating capacity and were well attended throughout The symposia covered the following subjects (1) Biological Structure and Function at the Molecular Level (president, M F Perutz, Great Britain), (2) Functional Biochemistry of Cell Structures (O. Lindberg, Sweden), (3) Evolutionary Biochemistry (S Ochoa, United States), (4) Molecular Basis of Enzyme Action and Inhibition (P Desnuelle, France), (5) Intracellular Respiration Phosphorylating and Non-Phosphorylating Reactions (E C Slater, Holland), (6) Mechanism of Photosynthesis (H Tamiya, Japan); (7) Biosynthesis of Lipids (G Popják, Great Britain), (8) Biochemical Principles of Food Industry (E Pyanowski, Poland) The main speakers in these sessions were allowed up to forty minutes to develop their subject and the subsequent speakers fifteen minutes, a half to three-quarters of an hour being set aside at the end of the session for discussion The time proved to be adequate, and the discussions were usually lively The arrangements for simultaneous translations in these meetings were excellent.

Among the many important communications given in the symposia Dr. Britton Chance's demonstration of a new effect in the field of intracellular respiration predicted on theoretical grounds some years ago by Sir Hans Krebs, namely, the reversal of the direction of electron transfer in isolated mitochondria occurring on the addition of adenosine triphosphate and leading to a reduction of pyridine nucleotide was particularly noteworthy, as were Prof A L. Lehninger's report on a preparation of hitherto unrecognized components of the chain of oxidative phosphorylation and their relation to the swelling and contraction of mitochondria, and Prof Lynen's account of the advances he and his colleagues had recently made in the understanding of the role of (+)-biotin as a coenzyme of carbon dioxide fixation and transcarboxylation Prof E P Kennedy (United States) gave an outstanding lecture on lipid biosynthesis There were, of course, many other new and interesting aspects of fundamental problems presented at the congress, but the author, in common with other participants, was able to hear only a small part of them. Pre-prints of the main congress lectures and of the contributions to the symposia were available to participants before the meeting

Probably the largest part of the scientific programme was associated with the activities of the twenty-eight sections which held their meetings and discussions in nearly as many rooms scattered throughout the very large University building and in the Departments of Physics and of Chemistry situated a few minutes walk from the main building. Two of the Sections, those concerned with Immunochemistry and the Biochemistry of Malignant Tumours, held their meetings in the Sternberg Institute of Astronomy, which was unfortunately about a 15-min walk away from the site of the main congress activities

Each section had its own interpreter, and language difficulties were thereby reduced to a minimum Abstracts of the short communications were available in the form of a separate book, and where the text was submitted in Russian, an English translation was also provided.

A Russian version of the whole of the congress proceedings is to be published by the U.S.S R Academy of Science and a corresponding publication in English (10 vols.) will be made by the Pergamon Press, Ltd These volumes are in course of preparation.

There was a useful display of scientific apparatus originating from fourteen countries, and an attractive exhibition of modern books dealing with all aspects of biochemistry and published in many different countries

A significant achievement, admittedly not directly a part of the congress, was the appearance of the report of the Commission on Enzymes, of the International Union of Biochemistry This document, which is the first official attempt to put enzyme nomenclature on an international basis, is the work of a group of acknowledged experts under the chairmanship of Dr Malcolm Dixon of the University of Cambridge. It makes an outstanding and much-needed contribution to modern enzymology.

Most of those attending the congress were accommodated in the student quarters of the University or in two of the largest hotels in Moscow, Hotel Ukraine and Hotel Mockba The problems which arose through accommodating so many visitors in so short a time were finally sorted out, although only after some participants had suffered considerable inconvenience It was generally agreed, however, that once one had found the accommodation for which one had originally asked, the standard of comfort was all that could be desired Transport between the University and hotels was well maintained throughout the congress by an excellent bus service The hosts were most generous with their hospitality and Academician Oparin gave a series of most delightful receptions to the participants in the symposia There was a series of interesting tours arranged by Intourist.

The participants from the Commonwealth much appreciated the kindness of Sir Frank Roberts, the British Ambassador in the U S S R , who held a reception so that British participants might entertain their Russian colleagues who had given so much time and made so much effort to make the congress the scientific success it undoubtedly was

Plans for the next international congress are by now under serious consideration, and it must be in the minds of the organizers that it will be attended by an even greater number of scientists than were present in Moscow this year It is clearly impossible for any one person to attend more than a few of the sessions, and it is also evident that ten minutes for the presentation and discussion of each ordinary communication is inadequate It might, therefore, be worth considering whether a more satisfactory procedure for use at future congresses would be to restrict the number of speakers, without limiting the number of papers communicated by title and circulated in the form of abstracts A limitation of this kind would also reduce the number of simultaneous sessions, which, it is generally agreed, are far too many at large international congresses in which there are only four or five days to present and discuss all the papers submitted by the participants

Perhaps to-day the long notice required to publish and pre-circulate abstracts of the scientific contributions makes it unlikely that anything really new will be announced at an international congress Probably the most valuable function of such a meeting is to bring workers together to take part in symposia and to engage in informal discussion, where they can estimate their progress or thrash out their differences or misunderstandings in small rooms or quiet corners which are set apart for such gatherings, but which are within easy reach of the main meeting rooms and where light refreshments would be available.

The fifth International Congress in Biochemistry was the largest congress yet held in the U S S R and it was obviously well supported by the Russian Government It is generally recognized that the more biological aspects of science in the U S S R have up to the present received far less attention than have the physical and engineering sciences, but the impression gained was that there is a growing awareness of this relative neglect, and that there was an awakening to the fact that biochemistry and other biological subjects must in future receive the support they so justly deserve and need

The closing plenary session was especially noteworthy for the outstanding and informative lecture on "Proteins, their Function and Structure" given by Prof F Sorm (Czechoslovakia) This was followed by a number of short addresses of appreciation by delegates from several countries, after which the president closed the proceedings and formally announced that the next congress would be held in New York in July 1964

W T J MORGAN

ATTENTION AND IN-ATTENTION IN NEUROPHYSIOLOGY[*]

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RECENT advances in neurophysiological techniques have enabled objective evidence to be readily and reliably obtained during long recordings, and cortical and subcortical electrical events to be examined.

Despite this there has been little increase in the understanding of the working of the human brain as it relates to mental experience Much experimental work in the past has centred on the animal brain Transposition of the theories derived from such studies to the special problem of the human brain has resulted in the formulation of certain philosophical devices to overcome complexities by narrowing the observations within certain controllable limits

But philosophical issues must inevitably be subjected to the test of practical experiments The Dualists, in supporting a metaphysical theory of reality, regarded the mind as having an independent existence, and since this was considered to be beyond the reach of natural science discounted it in formal experiments. The psycho-physicists, exemplified by Fechner did much the same thing when they narrowed the limits of their experiments by attempting to exclude the effects of subjective factors, and then concentrated on the properties of the stimulus which they expressed in terms of mathematical formulæ. Pavlov went even further and chose to disregard the subjective factor altogether, assuming physiology to be a sufficient explanation of the mind. Psychophysical parallelism, on the other hand, while acknowledging essentially human manifestations to be accompaniments of physical activity in the brain, considered them to vary concomitantly without one affecting the other and therefore to be outside the scope of experimental method

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[*] Substance of a paper read at the Ninth Latin American Congress of Neurosurgery during a symposium on the "Physiological Basis of Mental Activity", October 1961.

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These manœuvres have resulted in any effect of mental life being disregarded, and have led to the emphasis being placed on the physical attributes of stimuli But the problems imposed are the very essence of conscious human being and to simplify them is to destroy the very quality that it is hoped to study Such writers as Helmholtz[1], Wundt[2] and James[3] were not restricted by metaphysical considerations and evinced an open-minded acceptance of the available facts in the context of the fundamental issues raised, discussing them comprehensively and elegantly By the same token Sherrington[4] and Adrian[5] reiterated the unanswered problems impressed on them by their experiments and observations on human subjects. Thus Adrian comments that "though one brain may look like another, what it does in response to a stimulus may be quite different because it depends on all the brain has done since it was first in action" This central problem remains and has become of paramount importance in neurophysiology, where inconsistent results of experiments and some routine findings on human subjects have not been fully explained by a physiological stimulus-response association[6-11].

In this article the attempt has been made to examine aspects of the fundamental psychological processes of attention and apperception, considering also the terminology that may usefully be applied and then to relate these to physiological changes Some common-place phenomena such as may be seen in routine and experimental neurophysiological recordings are considered and used for illustrative purpose to support the suggestion that results, which on superficial examination appear to be random, may be better understood if, when causal analysis is attempted, the mental no less than the physical characteristics of the human subject are accepted and used as data

An elementary instance of this approach is provided by the psychogalvanic skin response, the variation of which in occurrence was apparent during interviews recorded on this unit. (Electrophysiological experiments performed by us utilized two small rooms, one for the patient and one for the apparatus, linked by a one-way screen. The observer in the patient's room was kept informed of events in the instrument room via an intercommunicating system linked to a deaf-aid earpiece The electroencephalogram and appropriate peripheral ancillary recordings were made during interviews The sessions were recorded on magnetic tape and these were correlated with the electroencephalogram, which could also be marked by the observer using a marker-button) It was observed that incidental door bangs from the corridor usually elicited a response in subjects and patients when they were waiting for the observer to enter the room During the interviews, however, though psychogalvanic skin responses were occurring in conversation, they were rarely seen in relation to door bangs (the latency of the response to a known stimulus having been established) When a response did occasionally occur to a door noise, it was found on cursory examination that the interview material usually indicated that at such times the entry of another person (such as an unauthorized person or a nurse with an injection tray) would, for example, have been a frightening or embarrassing possibility Here it appears that the assessment of the physical properties of a stimulus is alone insufficient to account for the behaviour of these responses

The question of adaptation was considered and in the experimental situation when sound and light flash stimuli were repeated for some time without instructions, responses were produced that tended to 'habituate', that is, to decrease and disappear However, when subjects then performed a series of tasks each one commencing when the stimulus sounded, the response was shown to behave differently and to alter with each repetition of the stimulus. These tasks involved mental imagery and concentration, and there were experiments with simple hand or finger movement and variations with touch. From such experiments it appeared that when the physical conditions of an experiment, including the stimulus, remained the same, variations in the response could be causally related to mental activity We are reminded by Adrian[12] that "the method of experiment is to relate the stimulus to the resulting response but this ignores the fact of attention to the stimulus", the crucial consideration in this sense and in our experiments hinges on the meaning of the word 'attention'

When recognizing attention or mental activity one should perhaps first consider the distinction between the external world of objective reality and the reflexion of this in the internal world of psychical reality The description of a stimulus by an observer must necessarily have a limited connexion with the response, for the stimulus occurs in the external world of the subject while the response is related to his internal world The response to the stimulus will therefore be governed by the 'significance' that the stimulus has for the individual subject at any particular moment

In another context this is very obvious when one considers a child reacting with a temper tantrum to something we may think is innocuous In terms of the external world the child's reaction is inappropriate, but the situation as depicted in the inner world of the child may explain the behaviour "In a tantrum the violence of the physical struggle being due to internal changes such that [he] becomes blind and deaf to external reality[13]". This is further illustrated by an experiment of Sharpey-Schafer *et al* [14], who investigated the mechanism of acute hypotension from fear or nausea in a series of patients submitted to anæsthesia for dental operations He demonstrated that in four out of five subjects in whom acute hypotension occurred during the administration of gas, arterial pressure fell before the mask was placed on the face The dental surgeon and anæsthetist did not recognize that severe hypotension was present and were apt to call this state 'a nice quiet gas' The result was therefore attributed to the wrong 'cause' and had tradition not ordained that the patients be lain flat the outcome would have been irreversible. The author comments "It is suggested that emotional stimulation of the heart beat and a fall in cardiac filling pressure caused virtual emptying of a ventricular chamber during systole which fires the afferent mechanism of the faint reflex".

In electrophysiological experiments Hernández Peón[15] recorded the occipital responses to single light flashes using subcortical electrodes and found that the response amplitude became larger with the increased intensity of the light. Keeping the brightness constant he then suggested alterations in intensity and demonstrated that the occipital responses to light flashes showed a greater change in line with suggestions than with objective variations in light intensity. It may be said that subjective factors influenced some facet of 'attention' paid by the patient to the light flashes and this determined the variations in the occipital responses. Hence these reactions can be better comprehended when they are thought of as the result of object relationships in the internal world. This reasoning must also apply in the tantrum and dental situations.

A comparable instance was observed by us[16] during an intensive study of a case of sensory reflex epilepsy. A clear-cut electrical wave-form occurred in the left parietal cortex when the patient rubbed the right side of his face, which was also associated with the production of a fit, and the experiments showed that this could also be effectively produced by psychological means alone. During experimental sessions on this patient it was demonstrated that the electrical response, besides occurring when he rubbed his face, appeared when he made the rubbing movements without touching his face (the patient was hypnotized). It was confirmed statistically that this psychological stimulus was just as effective for the evocation of the wave-form as actual rubbing. The null hypothesis was tested that this response could have happened by chance alone and was shown to be highly improbable. The electrical response also appeared when he anticipated rubbing his face, and it occurred when he was asked to imagine rubbing his face, or when he imagined having a fit; this was demonstrated in the laboratory and while recording directly from the brain exposed at operation. At other times the occurrence of the electrical activity during conversation appeared to be related to certain topics.

Consequent to these findings another sample of recordings was examined to determine the frequency of similar episodes occurring during the resting state when stimuli were not deliberately being given. It was established that during a period of 15 hr. 35 min. recording time, the electrical response appeared only on ten occasions unrelated to any known stimulus, but on forty occasions was temporally related to happenings that had been noted on the record (for example, intermittent noises, including the telephone and people entering the room, etc.). It was then shown that during a much shorter period of 3 hr. 28 min. recording time, under experimental conditions, the response appeared on thirty-five occasions in relation to known psychological stimuli, statistically exceeding anything that could occur by chance alone. These instances show that a causal sequence may be established using data that at first sight appear to be random or spontaneous.

In another experiment performed on the same patient in the hypnotic state an attempt was made to produce an anæsthesia of the right cheek. Loss of sensation was suggested, and when the right face was touched by the observer there was no change in the electroencephalographic record. On another occasion, however, the suggestion was worded differently and the patient was told that the right side of his face was "frozen and insensitive". Contrary to what was expected the electrical response was immediately evident at high amplitude when this area was touched, but did not similarly occur when he was touched elsewhere on the face (Fig. 1). After this session the patient volunteered the information that he had been surprised and impressed to find that his right face had felt intensely cold. It was thus evident that his interpretation of the suggestion had differed from that which had been intended and that he had in effect focused 'attention' on this area of the face instead of away from it. Here the only clue to the unexpected results lies in the information the patient provides regarding his interpretation of the suggestion. It appeared that the factors governing the result included the direction of attention, the exclusion of other matters, and possibly the amount of effort involved in the task.

The evidence presented establishes that a relationship can be demonstrated between mental activity and physiological changes. But to understand something of this problem it is first necessary to examine aspects of the process of attention and then to use descriptive terms that have practical application. William James[3] and Adrian[17] describe attention as consciousness being occupied by one thing at a time, to Sherrington[4] attention was but a further application of the rule that "unlike reflexes have successive but not simultaneous use of the common path. Expressed teleologically, the common path, although economically subservient for many and various purposes, is adapted to serve but one purpose at a time. Hence it is a co-ordinating mechanism and prevents confusion by restricting the use of the organ, its 'minister', to but one action at a time. . . . The resultant singleness of action from moment to moment is a keystone in the construction of the individual whose unity it is the specific office of the nervous system to perfect. The interference of unlike reflexes and the alliance of like reflexes in their action on their common paths seem to lie at the very root of the great psychical process of 'attention'."

The application of attention to one thing at a time necessitates the simultaneous withdrawal of attention from everything else that is not attended to. This is described as 'in-attention', and produces a descriptive hiatus. In an attempt to resolve this difficulty Freud's concept of mental energy[18] is put forward for consideration. "The Pre-conscious has at its disposal for distribution a mobile cathectic energy, a part of which is familiar to us in the form of 'attention'." This idea of a constant amount of mental energy was first formally proposed by Cabanis[19] "Sensibility seems to behave like a fluid whose total quantity is fixed and which whenever it pours into one of its channels in greater abundance becomes less in others" and it was referred to again by Janet. Using this concept some resolution of the semantic problem is possible. An object engaging our attention could be said to be endowed with a special significance and meaning, and result in a redistribution of cathectic energy. While this energy is focused on one object, there is simultaneously a reduction of energy available for other objects. When an object is weakly cathected, another object may readily sup-

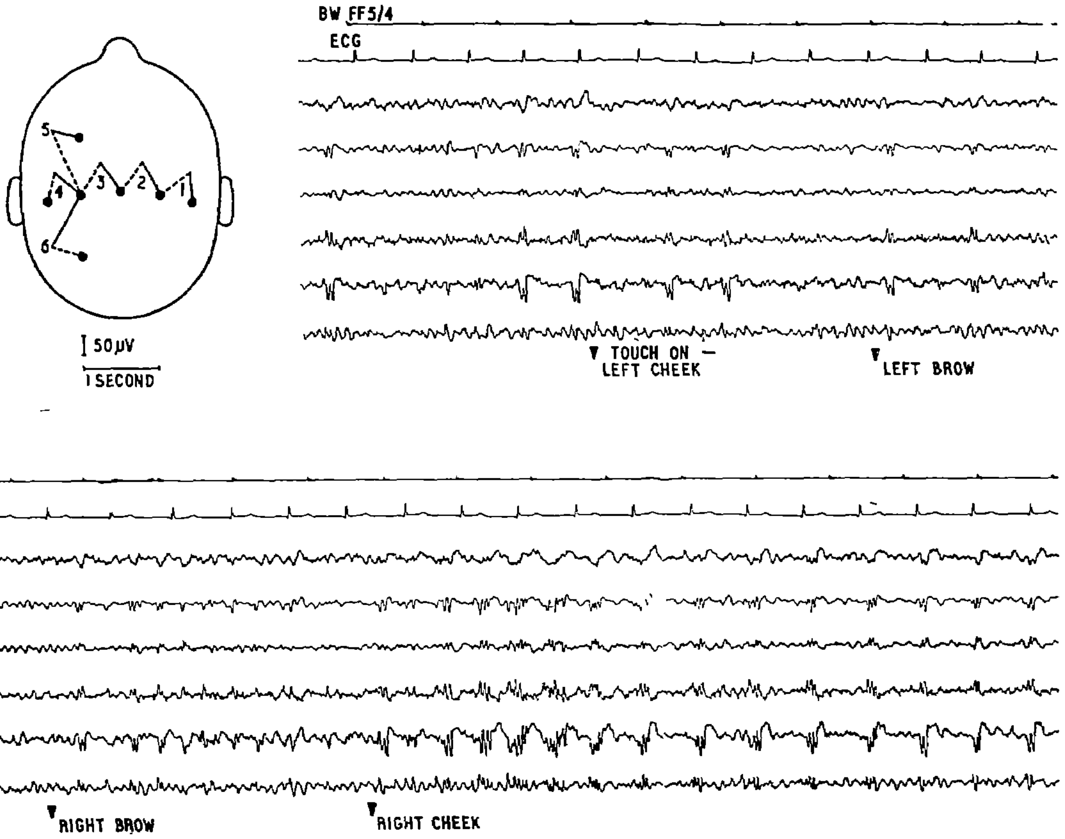

Fig 1 Time constant, 0 1 sec., high frequency, 25 per cent , amplifier sensitivity, 7 mm for 50 μV. input

plant it in common parlance it may then be said that we are 'easily distracted'.

This formulation may be applied to lambda-wave investigations[20], where it was an unequivocal finding that in the majority of control subjects tested, light, eye movement and some facet of psychological 'set' were all necessary for the evocation of lambda-waves in the occipital cortex when looking at a picture It appeared that interest, effort or the focusing of attention in a particular direction were necessary, that is, the picture had to be 'cathected' The problem, however, remains as to whether recorded changes may be due to 'attention' or 'in-attention' or due to the process of redistribution of cathectic energy Adrian[17] in 1934 and afterwards, by his exhaustive examination of the alpha rhythm on himself and his co-workers, makes the consideration of such mental processes inevitable He speaks of closing the eyes with the automatic withdrawal of attention from visual phenomena . . . "we try to see in the dark when the subject does not expect to see anything the rhythm persists" "A problem that contains the whole attention even with the eyes shut will block the rhythm, it will return as soon as the 'attention' wanders". "The rhythm is stopped if the whole attention is involved Perception of light does not affect it perception of pattern or the *attempt* to perceive it does"

Characteristically the alpha rhythm of the human, while varying enormously from subject to subject, when fully developed, remains of constant form for the individual. The phenomenon has much in common with that seen in the water beetle[17], and is presumably connected with basic biological activities necessary for survival, though in human beings the added cerebral organization obscures and complicates the connexion In man, vision is the dominant sense and the only one that can be voluntarily controlled, and it is perhaps not without reason that the alpha rhythm is most easily influenced by the act of opening and closing the eyes It may also be modified by psychological factors, the examination of which inevitably concerns us with the central process of attention Opening the eyes must involve at a primitive level the 'taking in' of visual information from the external world which confirms or denies the possibilities arising as a result of sensory input from other modalities. Similarly, closing the eyes may shut out visual stimuli arriving from the external world but also has the effect of increasing dependence on other sensory information which is interpreted in the light of past experience With such an act these interpretations become prepotent because they now lack the possibility of visual checking with reality. These considerations therefore make it necessary to try to examine the physiological changes in terms of a relationship with the mental counterparts of physical acts This possible relationship was found to occur in the case of sensory reflex epilepsy already described where the mental counterpart of the physical act was shown to be as effective in evoking the electrical response as actual rubbing of the face

That psychological factors can influence the alpha rhythm behaviour may be well illustrated by the occasions of its appearance and non-appearance and its fluctuations, during recorded interviews or silences, when the eyes were kept open or closed The significance or meaning that the situation had for the subject, and also the amount of continued 'force', 'effort', or 'cathectic energy' that was invoked appeared to be but two of the factors that could inhibit the alpha rhythm. This was particularly prominent with visualization.

It was, however, notable that the rhythm could be both evoked or inhibited when speech started or stopped. This was surprising, since one might expect the rhythm to be inhibited on the commencement of conversation and to reappear on its cessation. Furthermore, when it was shown that one subject, who had a sensitive high-amplitude alpha rhythm which was classic in behaviour, was attending closely to a discussion which at one stage involved the description of a biological film, his alpha rhythm continued unabated. Not only was the subject kept deeply involved but when electronic sound stimuli were introduced during the discussion they failed to effect any alteration in the alpha rhythm or to elicit a psychogalvanic skin response within 3 sec. of the stimulus. From these observations and some experiments involving mental tasks which produced inconsistent results it appears then that attention cannot be considered or discussed in psychologically unsophisticated terms.

That mental processes could be related to physiological functions was recognized by Darwin[21] when, for example, he wrote . . . "Blushing is not only voluntary, but the wish to restrain it by leading to self-attention actually increases the tendency" and . . . "the reddening of the face from a blush is due to the relaxation of the muscular coats of the small arteries, by which the capillaries become filled with blood, and this depends on the proper vasomotor centre being affected". Reflecting on the mental state that produces blushing he wrote: "It is not the simple act of reflecting on your own appearance, but the thinking what others think of us which excites the blush". A brief consideration of deceptively simple phenomena therefore yields complexities: paying attention to a person will not of itself produce a blush without the intervention of affective factors. In Darwin's example it may be said that an affective response has been induced by a mental image which is endowed with certain attributes: here the evocative stimulus is not the object in the environment but the feelings we have about it or its supposed intent.

With the above presumptions we may with profit re-examine other situations which remain causally enigmatic. Of the epilepsies, the 'reflex' type is perhaps most pertinent since there is an apparently specific stimulus response association. Apart from the case of sensory reflex epilepsy already quoted it was found in other cases that the causal connexion was not a simple one and that the emotional substrate had to be considered. The clearest demonstration of this was provided by three patients suffering from musicogenic epilepsy where the music alone was shown not to be directly and consistently responsible for the fits. In our experiments the metronome sounds and gramophone-record music alleged by the patients to precipitate fits were presented. Apart from rare occasions when fits occurred, the patients reported that the stimulus had not produced the feeling that occurred when a fit was imminent. Conversely, fits occurred when there was a marked emotional response to the music, and this was often accompanied by an electroencephalographic change. This observation has already been noted by Critchley[22], Shaw and Hill[23] and others. Therefore it can only be the psychological import of the stimulus that produces the evocative effect.

The function of the affective labelling of experience governing behaviour and the direction of attention may be illustrated by a patient with an obsessional neurosis which in this connexion was a case of singular interest (details of which are to be published elsewhere). In response to a specific word or allusions to it, the patient's blood pressure rose from 185/124 to 230/155. He became extremely distressed, restless and anxious, and tried by every means to avoid the conversation which was for him disturbing. This patient was seen again after he had been subjected to a leucotomy and the rise in diastolic and systolic pressure was then even more rapid and was still sufficiently alarming to prevent mention of the word. By contrast there was, however, a striking clinical change, for there was a noted absence of observed or reported experience of anxiety, though the pressor response was even more marked. There were no attempts by the patient to remove himself from the stimulus as he had done before leucotomy because his protective mechanism utilizing anxiety against a dangerous rise in blood pressure had been interfered with. Focusing of attention cannot simply be governed by the conscious discernment of certain properties of the environment, and the word presumably had a particular unconscious meaning for the patient and was attended to in a particular way because of the subjective experience of anxiety. This is but an exaggeration of the normal process whereby objects are attended to and distinguished one from another by their affective value, differentiation being ultimately impelled by pain/pleasure consideration. This complex and indirect connexion with an objective stimulus could also be said to apply to the cases of reflex epilepsy.

Our discussion has raised certain theoretical questions inherent in any work on conscious human beings. Starting with some considerations that arise out of limited neurophysiological studies it has been necessary to re-appraise the fundamental question of attention and the approach to this problem. The waking state, and therefore consciousness, is conceived of as a theoretic state which is inferred when discrimination is exercised in attending to internal as well as external stimuli; the switching of attention from one thing to another further implies an executive function performed by the ego which organizes information that is not in consciousness (pre-conscious) into a probability hierarchy awaiting entry into consciousness. Einstein[24] concerned himself with the stimulus response problem when he asked, "What, precisely, is 'thinking'? When, at the reception of sense-impressions, memory pictures emerge . . . (and) It is by no means necessary that 'concept' must be connected with sensorily cognizable or reproducible sign (word), but when this is the case thinking becomes by means of that fact communicable . . . For me it is not dubious that our thinking goes on for the most part without use of signs (words) and beyond that to a considerable degree unconsciously" (note, his brackets).

Adrian[25] said that "research has shown how information gets into the brain but not how the brain reacts to it". It is apparent from physiological studies that sensory input is always the same, from whatever organ, so that it is the arrival point that determines whether we see or hear, and contact with reality is necessarily retrospective. But to determine what in consciousness we see or hear, we have to be concerned with the psychological aspects of attention or inattention based on representations that are an amalgam of sensory input, past experience and symbolization Psychical reality consists therefore of very individual representations The focus of attention is the product of these factors and in the process the peripheral receptors may be affected[26] The relating of psychical to physiological events is therefore necessary in neurophysiological experiments. The usual causal equation of objective stimulus and objective response lacks the interposition of the psychical stimulus with the mental and emotional reactions This leads us to consider the terms that have to be used to describe such observations Generalizations based on these accounts require the use of psychoanalytic conceptions, to avoid the too-ready assumption that there is a simple psychological event directly accounting for a physiological change

Thus we are led to suggest that there are complementary points of view that are both necessary for the understanding of perceptual processes In such experiments we have two observers· the experimenter with his objective recordings, and the subject who is an introspective observer who attempts to report on the content of consciousness, that is, that on which attention is focused Since the latter's account is retrospective, he necessarily uses complementary terms to describe a state of mind, at the same time being subject to a continuous experience Philosophical issues concern us less than the epistemological difficulties of dealing with two such streams of information and the attendant problems of communication

This work was supported by a grant from the Ford Foundation, at the Institute of Psychiatry, The Maudsley Hospital, London

Thanks are due to Prof Denis Hill, Mr. Murray A Falconer and Dr J P Dewsbery, who made available patients used in this work

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AMINO-ACID SEQUENCE OF HORSE HEART CYTOCHROME *C*

Peptides released by Digestion with Chymotrypsin

THE only part of the amino-acid sequence of cytochrome *c* which has been established so far is the region to which the hæm is attached[1] through thioether bonds between the two cysteine residues in the peptide chain and the side-chains of the protoporphyrin[2] Since the conformation of the entire protein has a profound influence on the enzymatic and physico-chemical properties of the prosthetic group[3], an understanding of the interplay of structure and function in cytochrome *c*, as well as of its reactions with other components of the terminal oxidation chain, must be based on an accurate knowledge of the entire protein As a first step towards the elucidation of the structure of cytochrome *c*, a study of its complete amino-acid sequence was undertaken The present communication discusses the peptides recovered from chymotryptic digests of horse heart cytochrome *c*.

Methods and Amino-acid Sequences of Peptides

Horse heart cytochrome *c* (ref 4) was purified by twice chromatographing on 'Amberlite *IRC*-50' (ref 5) Chromatographic fraction I, the enzymatically fully active protein, containing 0 45 per cent iron, was denatured in 60 per cent ethanol The lyophilized denatured protein (100 µmoles) was digested at room temperature for 29 hr with a twice-recrystallized chymotrypsin preparation which exhibited no detectable tryptic activity The mixture was maintained at *p*H 7 8 with an automatic titration apparatus, and 2 per cent by weight of chymotrypsin was added three times during the course of the digestion The complete digest was chromatographed at 40° on a column of 'Dowex 50 × 2' 3 7 cm × 150 cm with volatile pyridine–acetic acid buffers by applying a linear gradient of *p*H and ionic strength from 0 2 *M* at *p*H 3·1 to 2 0 *M* at *p*H 5 0 The peptides in each fraction were purified further by various combinations of electrophoresis and chromatography on paper The amino-acid composition and the purity of each peptide isolated were determined by analysing suitable aliquots after acidic or enzymatic[6] hydrolysis on the automatic amino-acid analyser of Spackman, Stein and Moore[7] All purification steps were guided, and their efficacy monitored, by use of the two-dimensional electrophoresis-chromatography technique of Ingram[8] The yields of peptides were calculated from the analytical data on the final

Table 1. PEPTIDES OBTAINED FROM HORSE HEART CYTOCHROME *c* BY DIGESTION WITH CHYMOTRYPSIN

No. –*C**	Sequence	Yield (per cent)
XIX*a*	Acetyl (Asp,Gly) Val Glu Lys.Gly Lys Lys Ileu Phe (N-terminal peptide)	94
XXXIII*a*	Val Glu-NH$_2$ Lys Cys Ala Glu-NH$_2$ Cys.His Thr Val Glu Lys Gly Gly Lys His haem	60–70
XXV*a*	Lys Thr Gly Pro Asp-NH$_2$ (Leu,His) Gly Leu Phe	63
XXVI*a*	Gly Arg Lys Thr Gly.Glu-NH$_2$ Ala Pro Gly Phe	62
XIII*a*	Thr Tyr	38
XX*a*	Thr Asp Ala Asp-NH$_2$ Lys Asp-NH$_2$ Lys.Gly Ileu Thr Try	44
XVIII*b*	Lys Glu Glu Thr Leu Met Glu Tyr	47
XXIV*a*	Leu Glu Asp-NH$_2$ Pro Lys Lys.Tyr	49
XVIII*a*	Ileu Pro Gly.Thr Lys Met	47
XIV*a*	Ileu Phe	53
XXVIII*a*	Ala Gly Ileu Lys Lys Lys Thr Glu Arg Glu.Asp Leu (Ileu,Ala) Tyr	55
IV*a*	Leu	19
XXIII*a*	Lys Lys Ala Thr Asp-NH$_2$ Glu (C-terminal peptide)	44

* Peptides are designated by the prefix *C* to indicate their origin from a chymotryptic digest. The fraction number indicates the sequence *of elution from the ion exchange resin*

products and thus represent only minimal values, in some cases probably as much as 25–30 per cent less than the actual yields, because of variable losses during purification and because the original column chromatographic fractions were pooled to obtain as pure fractions as possible.

The amino-acid sequence of each peptide was established by combinations of chemical and enzymatic methods appropriate to each particular case. The chemical procedures used were the dinitrophenylation technique of Sanger[9] for the identification of amino-terminal residues and the Edman method[10] for the sequential identification of amino-acids in peptides. At each step of the Edman procedure, in addition to the chromatographic identification of the phenylthiohydantoin of the *N*-terminal amino-acid, an aliquot of the residual peptide was hydrolysed and its amino-acid composition quantitatively established. Digestion with leucine aminopeptidase[11] or with carboxypeptidase *A* often permitted identification of *N*-terminal and *C*-terminal residues or short sequences, respectively. When digestion of peptides with endopeptidases, such as trypsin and papain, was performed, the resulting derived peptides were purified and the composition and sequence of each one were established by the methods already cited.

The sequences of the chymotryptic peptides isolated from horse heart cytochrome *c* are listed in Table 1. Except for the hæm peptide, the peptides given in Table 1 are only the longest peptides isolated; in most cases shorter peptides were obtained in much higher yields than the 'overlapping' long peptides. The yields cited represent the sum of the yields of the shorter and longer peptides included in a given sequence.

In the column chromatography of the original chymotryptic digest, the main hæm-containing peptide could not be eluted from the 'Dowex-50' resin. It was prepared from another digest by column chromatography on 'Amberlite *IRC*-50'. Since the enzymatic hydrolysis in this experiment was not carried to completion, the hæm peptide obtained was larger than the hæm peptide of which the amino-acid sequence is given in Table 1 and which represents the minimal chymotryptic 'core' of cytochrome *c*.

The peptides recovered from the chymotryptic digest represent the entire amino-acid complement of horse heart cytochrome *c*, the composition of which was determined by analysis of acid hydrolysates (Margoliash, E., Hill, R. L., Kimmel, J. R., and Schmidt, W. R., paper in preparation): Try, 19 Lys, 3 His, 2 Arg, 3 Asp, 5 Asp-NH$_2$, 10 Thr, 9 Glu, 3 Glu-NH$_2$, 4 Pro, 12 Gly, 6 Ala, 2 CySH, 3 Val, 2 Met, 6 Ileu, 6 Leu, 4 Phe, 4 Tyr. The amide distribution was determined on enzymatic hydrolysates.

N-terminal and C-terminal Sequences

The first peptide listed in Table 1 behaved abnormally. It contained three lysyl residues and one each of glutamic acid and aspartic acid and would, therefore, be expected to have an overall positive charge at near neutral *p*H values; however, on electrophoresis at *p*H 6.5, it behaved as a neutral compound, indicating that either one positive charge had been eliminated or an extra negative charge added. The lysyl residues all had normal unsubstituted ε-amino groups, since digestion with trypsin resulted in practically quantitative yields of all four peptides expected. Moreover, the lysine residues were recovered as ε-dinitrophenyllysine after treatment of the peptide with fluorodinitrobenzene. On the other hand all attempts to identify an *N*-terminal residue failed, indicating that there was no free α-amino group. Thus, the peptide could not be digested with leucine aminopeptidase at ratios of enzyme to substrate fully effective for all other peptides, no *N*-terminal residue could be recovered after prolonged dinitrophenylation, two successive steps of the Edman procedure did not release any phenylthiohydantoin derivative and did not result in any change in composition of the residual peptide. Gas chromatography of a suitable acid hydrolysate established the presence of acetic acid, and it was concluded, therefore, that the amino-terminal α-amino group was acetylated. This finding explains the abnormal charge of the peptide. Incubation of the peptide in methanolic 1 *N* hydrochloric acid gave a mixture in which only aspartic acid and glycine could be identified as amino-terminal residues by the Edman procedure, thereby placing these residues at the amino-terminal end.

Since this peptide had an acetylated α-amino group, it must represent the amino-terminal sequence of cytochrome *c*. This assignment of position was fully substantiated by the work of Kreil and Tuppy and when the entire amino-acid sequence of the protein was determined (see following communications). Similarly, acetylated amino-terminal α-amino groups have been shown to occur in other proteins, for example, melanophore-stimulating hormone[12], in tobacco mosaic virus protein[13], in egg albumin[14], and in turnip yellow virus protein[15].

The amino-terminal residue of horse heart cytochrome *c* has been reported by one of us (E. M.) to be histidine[16] and by another group of investigators to be arginine[17]. Both these claims are erroneous, since the three histidyl residues and the two arginyl residues in the molecule have now been located in the interior of distinct chymotryptic peptides that must be located in the portion of the protein carboxyl-terminal of the hæm

The last peptide listed in Table 1 has a carboxyl-terminal glutamic acid Clearly this residue could not represent a possible point of attack for either chymotrypsin or trypsin Moreover, no longer sequence of which this peptide was a part could be found The last peptide listed in Table 1 was assigned, therefore, to the carboxyl-terminal position in the protein This conclusion is in accord with the results of Titani, Ishikura and Minakami[18] who identified glutamic acid as the carboxyl-terminal residue of horse heart cytochrome *c* by the hydrazinolysis method Similar findings have also been obtained by Kreil and Tuppy (see following communication)

In summary, the amino-acid sequences of the peptides obtained by digestion of horse heart cytochrome *c* with chymotrypsin have been determined and these peptides account for the amino-acid composition of the protein There is no free α-amino group in the protein The amino-terminal residue occurs as the acetyl derivative The amino-terminal chymotryptic peptide has the structure Acetyl (Gly, Asp) Val Glu Lys Gly Lys Lys Ileu Phe The carboxyl-terminal residue is glutamic acid with the terminal chymotryptic peptide having the sequence Lys.Lys Ala Thr Asp-NH_2 Glu

While this work was in progress, we learned from Prof H Tuppy that an investigation of the tryptic peptides of cytochrome *c* was under investigation in his laboratory; it is a pleasure to acknowledge the co-operative exchange of information which has led to simultaneous and joint publication of results We are indebted to our associates, Dr A Light and Dr J R Kimmel, for their help and advice This investigation has been aided by research grants from the National Institutes of Health, U S Public Health Service

A complete description of our work will be submitted for publication in the *Journal of Biological Chemistry*

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Peptides, Terminal and Internal, released by Digestion with Trypsin

According to recent analyses, horse heart cytochrome *c* contains 2 arginine (refs 1–4) and 18 (refs 1, 2, 4) or 19 (ref 3 and preceding communication) lysine residues per molecule Tryptic digestion was therefore expected to yield 21 or 22 fragments However, since the commercial trypsin used (Worthington, twice cryst) exhibited a slight chymotryptic activity, a greater number of peptides was obtained In order to minimize chymotryptic activity, the enzyme was pre-treated with dilute hydrochloric acid[5] and the duration of the enzymatic digestion of cytochrome *c* was reduced to 2 hr. The peptide mixture obtained was resolved on sheets of Whatman paper 3 *MM* with the help of a two-dimensional 'finger-printing' technique very similar to that introduced by Ingram[6] The peptides were located with the help of ninhydrin or by observing the fluorescence which appeared on heating the sheets at 105° for 10 min (ref 7) The resulting peptide pattern is shown in Fig 1

For the qualitative determinat on of their amino-acid composition, the peptides from one sheet were eluted, hydrolysed with 5 7 *N* hydrochloric acid (containing 1 per cent thioglycollic acid in the case of peptides containing methionine), and the hydrolysates were analysed by paper chromatography (Table 1) When larger amounts of peptide were required for end-group determination by the dinitrophenyl method and for sequential analysis, the material eluted from several 'fingerprints' was combined Alternatively, a larger-scale resolution of the peptide mixture obtained on tryptic digestion was carried out in two consecutive steps 8-mgm. portions of the digest were first subjected to one-dimensional paper electrophoresis so as to give a series of fractions of different mobility, corresponding fractions eluted from several electrophoretic runs were then combined and purified by chromatography on Whatman 3 *MM* paper

The methods used for elucidating the amino-acid sequence in most of the peptides occurring in the tryptic digest of horse cytochrome *c* (Table 1) were, largely, the classical ones introduced by Sanger, such as end-group determinations and partial hydrolysis by acid and by various enzymes, followed by characterization of the resulting smaller fragments

Special attention was given to the peptides occupying *N*- and *C*-terminal positions in the polypeptide chain of cytochrome *c* The search was stimulated by the fact that numerous attempts to identify an amino-acid carrying a free α-amino group had been unsuccessful (Paléus, S , and Tuppy, H , unpublished work) Earlier reports claiming a histidine[8] or an arginine residue[9] to be *N*-terminal could not be confirmed.

In the tryptic digest of horse heart cytochrome *c*, a peptide (*T*-20) was observed which gave a very weak ninhydrin colour and which was devoid of a free α-amino group Cleavage by pepsin resulted in a tripeptide, Val-Glu-Lys, and an acidic fragment which failed to react with ninhydrin The latter, on total acid hydrolysis, gave glycine and aspartic acid, whereas partial hydrolysis with 2 *N* hydrochloric acid (40 min , 100°) yielded the dipeptide Gly Asp, heating at *p*H 2 (90 min , 105°) produced free aspartic acid and a substance chromatographically indistinguishable from acetylglycine, hydrazinolysis gave aspartic acid and two hydrazides behaving like glycyl-

Table 1 PEPTIDES FROM TRYPTIC HYDROLYSATES OF HORSE CYTOCHROME *c*

	Amino-acids in the hydrolysate*	Proposed structure †	Remarks
T-20	Asp,Glu,Gly,Lys,Val	Acetyl-Gly-Asp-Val-Glu-Lys	*a*
T-2	*Gly*,Lys	Gly-Lys-Lys	*b*
T-4	*Gly*,Lys	Gly-Lys	
T-12	Glu,*Ileu*,Lys,Phe,Val	Ileu-Phe-Val-GluN-Lys	*c*
Hæm peptide	Ala,*Cys*,Cys,Glu,His,Lys,Thr,Val	Cys-Ala-GluN-Cys-His-Thr-Val-Glu-Lys (18)	
T-6*a*	*Gly*,Gly,Lys	Gly-Gly-Lys	*d*
T-2*a*	*His*,Lys	His-Lys	*b*
T-10*a*	Arg,Asp,Gly,His,Leu,Phe,Pro,*Thr*	Thr-Gly-Pro-AspN-Leu-His-Gly-Leu-Phe-Gly-Arg	*e*
T-17	Ala,Asp,Glu,Gly,Lys,Phe,Pro,*Thr*,Thr,Tyr	Thr-(Ala,GluN,Gly,Pro)-Phe-Thr-Tyr-Thr-(Ala,Asp)-Lys	*f*
T-6*b*	*Asp*,Lys	AspN-Lys	*d*
T-11*a*	*Gly*,Ileu,Lys,Thr,Try	Gly-Ileu-Thr-Try-Lys	*g*
T-19	Asp,*Glu*,Glu,Leu,Lys,Met,Pro,Thr,Tyr	Glu-(Glu,Thr,Leu)-(Glu,Met)-Tyr-Leu-Glu-AspN-Pro-Lys	*h*
T-11	Gly,Ileu,Lys,Pro,Thr,*Tyr*	Tyr-Ileu-(Pro,Gly)-Thr-Lys	*i*
T-12*b*	Ala,Gly,Ileu,Lys,*Met*,Phe	Met-Ileu-Phe-Ala-Gly-Ileu-Lys	*k*
T-1	*Lys*,Lys	Lys-Lys	
T-8*a*	Arg,Glu,*Lys*,Lys,Thr	Lys-Lys-Thr-Glu-Arg	*l*
T-8	Arg,Glu,*Lys*,Thr	Lys-Thr-Glu-Arg	
T-15	Arg,Glu,*Thr*	Thr-Glu-Arg	
T-19*a*	Ala,Asp,*Glu*,Ileu,Leu,Lys,Tyr	Glu-Asp-Leu-Ileu-Ala-Tyr-Leu-Lys	*m*
T-18	*Ala*,Asp,Glu,Thr	Ala-Thr-AspN-Glu	*n*

Peptides are designated by the prefix *T* to indicate their origin from a tryptic digest

* The *N*-terminal amino-acid determined by the dinitrophenol method is given in italics

† Where amino-acids have been enclosed in brackets, neither their order nor molar ratio has been determined.

(*a*) Pepsin treatment gave acetyl-Gly-Asp and Val-Glu-Lys

(*b*) The peptides *T*-2 and *T*-2*a* were separated by electrophoresis at *p*H 6 4

(*c*) Chymotrypsin, pepsin and subtilisin produced the same fragments (Ileu,Phe) and Val-GluN-Lys On partial hydrolysis (Ileu,Phe,Val) was formed in good yield

(*d*) The mixture of *T*-6*a* and *T*-6*b* could be resolved by electrophoresis at *p*H 2

(*e*) Chymotrypsin split *T*-10*a* into two peptides Gly-Arg (*T*-5) and (Thr,Gly,Pro,AspN,Leu,His)-Phe (*T*-12*a*) Degradation with subtilisin gave Thr-(Gly,Pro,AspN), Leu-His, Gly-Leu, (Gly,Leu,Phe), free Phe and Gly-Arg *T*-12*a* was further degraded with pepsin yielding (Thr,Gly,Pro AspN,Leu) and (His,Gly,Leu,Phe) Partial hydrolysis of these two fragments gave (Thr,Gly), (Gly,Pro), (Gly,Pro,Asp,Leu), (Asp,Leu), (His,Gly) and (Leu,Phe)

(*f*) Chymotrypsin gave rise to Thr-(Ala,GluN,Gly,Pro)-Phe,Thr-Tyr and Thr-(Asp,Ala)-Lys In a peptic hydrolysate of *T*-17 the presence of (Tyr,Thr,Ala,Asp)-Lys was established

(*g*) Chymotrypsin split *T*-11*a* into Gly-(Ileu,Thr)-Try and free Lys. In a partial hydrolysate (Gly,Ileu) was found in addition to free amino-acids

(*h*) Chymotryptic cleavage gave (Glu,Thr,Leu,Met)-Tyr and (Leu,Glu,AspN,Pro)-Lys (*T*-14) Peptic cleavage gave (Glu$_2$,Thr,Leu), (Met, Glu), (Met,Glu,Tyr,Leu and (Glu,AspN,Pro)-Lys Partial hydrolysis of *T*-14 gave (Leu,Glu) and (Pro,Lys)

(*i*) Cleavage by subtilisin gave Tyr, (Ileu,Pro,Gly), (Ileu,Pro,Gly,Thr,Lys) and Thr-Lys (Tyr,Gly,Pro,Ileu), (Ileu,Pro,Gly) and Thr-Lys were identified in partial hydrolysates Tyr and Ileu were released from *T*-11 under the action of 'oxytocinase' (ref 16)

(*k*) Degradation with chymotrypsin yielded (Met,Ileu)-Phe and Ala-(Gly,Ileu)-Lys Partial hydrolysis of the latter gave (Ileu,Lys) and traces of (Gly,Ileu,Lys)

(*l*) Tryptic cleavage gave Lys-Lys (*T*-1), Thr-Glu-Arg (*T*-15), free Lys and (Lys,Thr,Glu)-Arg (*T*-8)

(*m*) Chymotrypsin cleavage yielded (Ala,Glu,Asp,Ileu,Leu)-Tyr (*T*-2I) and Leu-Lys (*T*-9) *T*-21 was further degraded with pepsin, the resulting two peptides being Glu-Asp-Leu and Ileu-Ala-Tyr

(*n*) Glu is *C*-terminal (hydrazinolysis) Subtilisin cleavage gave Ala-Thr and AspN-Glu Ala, (Thr,Asp,Glu) and (Thr,AspN,Glu) were found in partial hydrolysates

hydrazide and acetyl-hydrazide *T*-20 is, therefore, taken to be AcetylGly Asp Val Glu Lys and to represent the *N*-terminal portion of cytochrome *c* Thus, cytochrome *c* can be added to the growing list of proteins and polypeptides characterized by an acetylated *N*-terminal amino-acid residue, namely, the proteins of tobacco mosaic[10], cucumber 4 (ref 11) and turnip yellow mosaic virus[12], porcine α-melanocyte-stimulating hormone[13], and bovine fibrinogen[14].

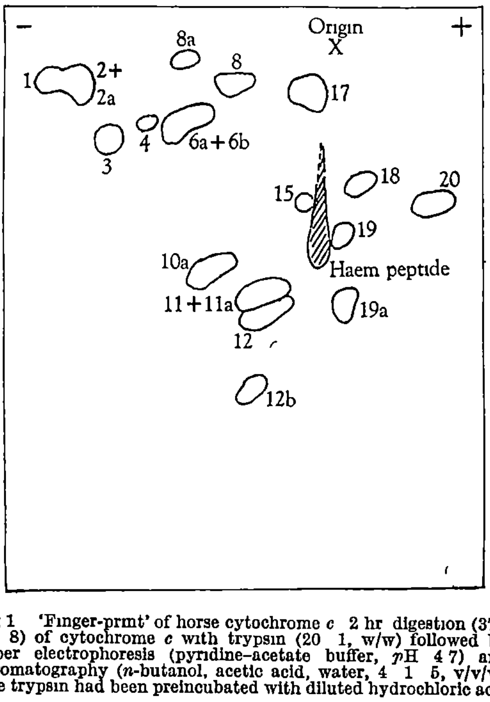

Fig 1 'Finger-print' of horse cytochrome *c* 2 hr digestion (37°, *p*H 8) of cytochrome *c* with trypsin (20 1, w/w) followed by paper electrophoresis (pyridine-acetate buffer, *p*H 4 7) and chromatography (*n*-butanol, acetic acid, water, 4 1 5, v/v/v) The trypsin had been preincubated with diluted hydrochloric acid

When trypsin virtually free of chymotryptic activity was used for the digestion of horse cytochrome *c*, all the fragments arising in good yield contained *C*-terminal lysine or arginine, except for one (*T*-18) the *C*-terminal residue of which, as determined by hydrazinolysis, proved to be glutamic acid Glutamic acid had previously been reported by Titani *et al* [15] to be in the *C*-terminal position in horse cytochrome *c*, a finding that we could readily confirm This obviously suggests that *T*-18, the structure of which has been determined to be Ala Thr AspN Glu, is derived from the *C*-terminus of the polypeptide chain of horse cytochrome *c*

While this work was in progress, we learned from Prof E. L Smith that an investigation of the chymotryptic peptides of cytochrome *c* was going on in his laboratory It is a pleasure to acknowledge the co-operative exchange of information which has led to simultaneous and joint publication of results Full details of this work will be published in *Monatshefte fur Chemie* We wish to thank the Rockefeller Foundation for a grant

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The Complete Amino-acid Sequence

FROM the work reported in the two preceding communications, it became possible to derive a unique amino-acid sequence for horse heart cytochrome *c* This sequence of 104 amino-acid residues, together with the major points of hydrolysis by chymotrypsin and trypsin, is given in Fig 1

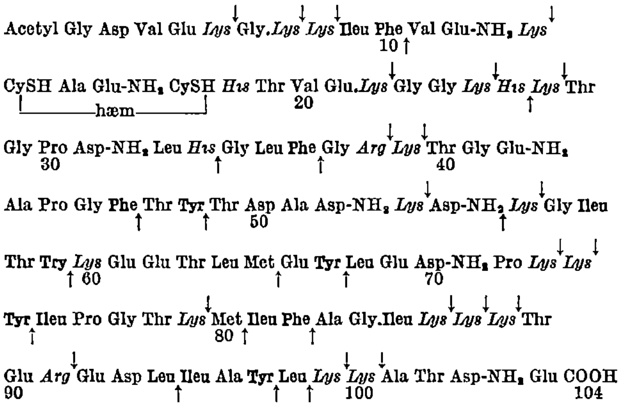
Acetyl Gly Asp Val Glu *Lys* Gly.*Lys* *Lys* **Ileu** **Phe** Val Glu-NH₂ *Lys*
10
CySH Ala Glu-NH₂ CySH *His* Thr Val Glu.*Lys* Gly Gly *Lys* *His* *Lys* Thr
hæm
20
Gly Pro Asp-NH₂ **Leu** *His* Gly **Leu** **Phe** Gly *Arg* *Lys* Thr Gly Glu-NH₂
30
40
Ala Pro Gly **Phe** Thr **Tyr** Thr Asp Ala Asp-NH₂ *Lys* Asp-NH₂ *Lys* Gly **Ileu**
50
Thr **Try** *Lys* Glu Glu Thr **Leu** **Met** Glu **Tyr** **Leu** Glu Asp-NH₂ Pro *Lys* *Lys*
60
70
Tyr **Ileu** Pro Gly Thr *Lys* **Met** **Ileu** **Phe** Ala Gly.**Ileu** *Lys* *Lys* *Lys* Thr
80
Glu *Arg* Glu Asp **Leu** **Ileu** Ala **Tyr** **Leu** *Lys* *Lys* Ala Thr Asp-NH₂ Glu COOH
90
100
104

Fig 1 Amino-acid sequences of horse heart cytochrome *c* The arrows above and below the line indicate the points of hydrolysis by trypsin and chymotrypsin, respectively The basic residues are in italics, the hydrophobic residues are in bold-faced type

In the following discussion the numbers used to identify the peptides recovered from cytochrome *c* after digestion with chymotrypsin and trypsin will be preceded by the letters *C* and *T*, respectively The assignment of the sequence, as shown in Fig 1, is based on the 13 largest peptides obtained from the chymotryptic digest[1] and the 20 peptides separated from the tryptic digest[2] as follows

Peptides *C*-XIX*a* and *T*-20 both contain the *N*-terminal sequence of the protein, since the *N*-terminal α-amino group of both these peptides is acetylated Peptides *T*-20, *T*-2 and *T*-4 are portions of the amino-acid sequence of *C*-XIX*a*, whereas *T*-12, Ileu Phe - Val Glu-NH$_2$ Lys, contains the *C*-terminal two residues of *C*-XIX*a*, Ileu Phe and three additional residues Indeed, the only other peptide from the chymotryptic digest containing an Ileu Phe sequence is the dipeptide *C*-XIV*a*, obtained in 53 per cent yield, if *C*-XIV*a* were to represent the *N*-terminal portion of *T*-12 it would have to be preceded by a residue which affords a bond highly sensitive to both chymotrypsin and trypsin, which is an unlikely possibility Moreover, *C*-XIV*a* was afterwards located in the interior of *T*-12*b* in the sequence Met Ileu Phe, which explains the release of Ileu Phe in good yield by chymotrypsin

The protein contains three valine residues, all of which are present in a Val Glu Lys sequence Two of these sequences are in the hæm peptides obtained from chymotryptic and peptic digests (ref 1 and preceding communication), whereas the third is in the *N*-terminal sequence present in *C*-XIX*a* Since *T*-12 continues *C*-XIX*a*, the latter is necessarily followed by the hæm peptide resulting from digestion with chymotrypsin, which like the hæm peptide from peptic digests has the *N*-terminal sequence Val Glu-NH$_2$ Lys Of the three Val Glu Lys sequences, the *N*-terminal sequence of the hæm peptide is the only one which contains a glutaminyl rather than a glutamyl residue, further strengthening this conclusion It should be noted that peptide *T*-6*a* with the unique sequence Gly Gly Lys is part of the *C*-terminal portion of the chymotryptic hæm peptide The above argument thus establishes the sequence of the protein from the *N*-terminal end to residue 26

The dipeptide *T*-2*a*, His Lys, contains the histidyl residue *C*-terminal in the chymotryptic hæm peptide, since of the three histidyl residues in the protein the other two are in the interior of the hæm peptide in a His Thr sequence and in *C*-XXV*a* or *T*-10*a* in a His Gly sequence The peptide following the chymotryptic hæm peptide must, therefore, have an *N*-terminal lysyl residue Three such peptides were isolated from the chymotryptic digest, *C*-XXV*a*, *C*-XVIII*b* and *C*-XXIII*a* Peptide *C*-XXIII*a*, Lys Lys Ala Thr Asp-NH$_2$ Glu, is the only peptide obtained from the chymotryptic digest that does not have a *C*-terminal residue at which chymotrypsin could hydrolyse a peptide bond and for which it was not possible to show that it is followed in the overall amino-acid sequence by another peptide It was therefore assigned to the *C*-terminal position in the protein (preceding communication by E M and E L S) Similarly, the corresponding *C*-terminal peptide *T*-18, Ala Thr Asp-NH$_2$ Glu, was obtained from the tryptic digest (preceding communication by G K and H T) *C*-XXIII*a* can therefore be eliminated as a possible candidate for continuation of the chymotryptic hæm peptide On the other hand, from the *C*-terminal end of the protein, peptide *C*-XVIII*b* follows the single tryptophan-containing peptide, *C*-XX*a*, because of the series of overlapping unique sections of chymotryptic and tryptic peptides discussed later and thus is located in the second half of the overall amino-acid sequence Hence, *C*-XXV*a*, Lys Thr Gly Pro Asp-NH$_2$ Leu His Gly - Leu Phe, is the only peptide in the chymotryptic digest which can represent the sequence immediately following the chymotryptic hæm peptide This conclusion is supported by the finding that, from a partial chymotryptic digest, a hæm peptide was isolated which contains, in addition to the minimal chymotryptic hæm peptide listed in Table 1 of the preceding communication (by E M and E L S.), also the amino-acid sequence of *C*-XXV*a*

The entire amino-acid sequence of *C*-XXV*a*, except for the *N*-terminal lysyl residue, is present in *T*-10*a*, which also contains a Gly Arg sequence at its *C*-terminal end This Gly Arg sequence is unique in the entire protein and *C*-XXVI*a* possesses Gly Arg at its *N*-terminal end Thus, *T*-10*a* provides a definitive overlap for placing *C*-XXVI*a* immediately after *C*-XXV*a*

The unique composition and the *N*-terminal residue of the first half of peptide *T*-17, Thr(Ala,Glu-NH$_2$,-Gly,Pro)Phe Thr Tyr Thr Asp (Ala,Asp)Lys shows that the *N*-terminal portion of this peptide contains the same sequence as the *C*-terminal part of *C*-XXVI*a*, the complete sequence of which is Gly Arg.Lys Thr - Gly Glu-NH$_2$ Ala Pro Gly Phe. Moreover, the *C*-terminal portion of the sequence of *T*-17 clearly contains the dipeptide *C*-XIII*a*, Thr Tyr, as well as the first five residues of *C*-XX*a*, Thr Asp Ala Asp-NH$_2$ Lys Asp-NH$_2$ Lys Gly.Ileu Thr Try. Hence the derived sequence of chymotryptic peptides is *C*-XXV*a* *C*-XXVI*a* *C*-XIII*a* *C*-XX*a*, and the sequence of tryptic peptides is *T*-2 *T*-10*a*.*T*-3 *T*-17, extending the overall amino-acid sequence to the 59th residue.

Peptide *T*-11*a* includes the *C*-terminal sequence of *C*-XX*a*, as well as a *C*-terminal lysine residue, thus showing that the chymotryptic peptide following *C*-XX*a* is one of the three having an *N*-terminal lysyl residue (*C*-XXV*a*, *C*-XVIII*b* or *C*-XXIII*a*). The same argument applies in this case as for the demonstration of the adjacent positions of the hæm peptide and *C*-XXV*a* already discussed above. Peptide *C*-XXIII*a* cannot follow *C*-XX*a* as it represents the *C*-terminal sequence of the protein. Since the remaining part of the amino-acid sequence of the protein can readily be established from the *C*-terminal end of the overall sequence to peptide *C*-XVIII*b*, *C*-XVIII*b* is the only possible continuation of *C*-XX*a*.

The *N*-terminal half of peptide *T*-19, Glu(Glu,Leu,-Thr)(Glu,Met) Tyr Leu Glu Asp-NH$_2$ Pro Lys, clearly contains the same sequence as *C*-XVIII*b*, Lys Glu - Glu Thr Leu Met Glu Tyr, except for the *N*-terminal lysyl residue. On the other hand, the *C*-terminal portion of *T*-19 contains the sequence of the first five residues of *C*-XXIV*a*, Leu Glu Asp-NH$_2$ Pro - Lys Lys Tyr, showing that *C*-XXIV*a* immediately follows *C*-XVIII*b*.

Since the *C*-terminal sequence of *C*-XXIV*a* is Lys Lys Tyr , the following tryptic peptide must be one having an *N*-terminal tyrosyl residue. Only one such tryptic peptide was found, namely, *T*-11, with the sequence Tyr Ileu(Pro,Gly)Thr Lys. The other three tyrosyl residues in the protein are present in the interior of tryptic peptides *T*-17, *T*-19 and *T*-19*a*, which contain the *C*-terminal tyrosyl residues of *C*-XII*a*, *C*-XVIII*b* and *C*-XXVIII*a*, respectively. The *C*-terminal sequence of *T*-11 is unique and corresponds to the first five residues of *C*-XVIII*a*, Ileu Pro Gly Thr Lys Met. Thus, *C*-XVIII*a* follows *C*-XXIV*a* and the overall sequence is established to the 80th residue.

The *C*-terminal sequence of *C*-XVIII*a* is Lys Met , hence the following tryptic peptide must contain an *N*-terminal methionyl residue. The only such peptide is *T*-12*b*, Met Ileu Phe Ala Gly Ileu Lys. The only other methionyl residue in the protein is present in the interior of *C*-XVIII*b* and *T*-19. Peptide *T*-12*b* contains, in addition to the second methionine in the protein, the dipeptide *C*-XIV*a*, Ileu Phe, and the first four residues of *C*-XXVIII*a*. This unique sequence, Ala Gly Ileu Lys, constitutes the four *C*-terminal residues of *T*-12*b* and the four *N*-terminal residues of the fifteen-residue chymotryptic peptide *C*-XXVIII*a*, Ala Gly Ileu Lys Lys Lys Thr Gly Arg - Glu Asp Leu Ileu Ala Tyr , this demonstrates that *C*-XXVIII*a* follows *C*-XIV*a*, which itself follows *C*-XVIII*a*. It should be noted that *C*-XXVIII*a* contains the sequences of the tryptic peptides *T*-8, *T*-8*a* and *T*-19*a*, so that the correct sequence of the tryptic peptides is *T*11 *T*-12*b* *T*-8*a* T-19*a*.

The six residues at the *N*-terminal portion of the octapeptide *T*-19*a*, Glu Asp Leu Ileu Ala Tyr Leu - Lys , are in the same sequence as the six residues at the *C*-terminal part of peptide *C*-XXVIII*a*, showing that the last two residues of *T*-19*a*, Leu Lys, follow the *C*-terminal tyrosyl residue of *C*-XXVIII*a*, thereby placing leucine (*C*-IV*a*) the only free amino-acid recovered in the chymotryptic digest. The chymotryptic peptide following *C*-IV*a* must contain *N*-terminal lysine and is, therefore, *C*-XXIII*a*, Lys Lys Ala Thr Asp-NH$_2$ Glu, the only chymotryptic peptide with *N*-terminal lysine not yet allocated and which represents the *C*-terminal sequence of the protein. It should be recalled (preceding communications) that cytochrome *c* possesses a *C*-terminal glutamic acid residue. Such a residue would not be expected to be *C*-terminal in peptides derived from chymotryptic or tryptic digests.

Discussion

The amino-acid sequence of horse heart cytochrome *c* given in Fig. 1 accounts for the entire composition and amide distribution of the protein. From this sequence the calculated molecular weight of the protein (Margoliash, E., Hill, R. L., Kimmel, J. R., and Schmidt, W. R., in preparation) is 12,398, corresponding to an iron content of 0.45 per cent. These values agree well with those previously determined from the iron content of the chromatographically purified[2] or the crystalline protein[3], on the basis of a content of one atom of iron per mole of protein.

Horse heart cytochrome *c* does not show any obvious relationship in its amino-acid sequence to either the α- or β-chains of human hæmoglobin *A* (ref. 4) or to that of sperm whale myoglobin (ref. 5), although there are distinct similarities between the two hæmoglobin chains and myoglobin (ref. 6). In horse heart cytochrome *c* the hæm is bonded by thiol ether linkages to residues 14 and 17, a position which is much nearer the *N*-terminal end of the chain than the location of the non-covalently bound hæm of either hæmoglobin or myoglobin[7,8]. This lack of similarity is not unexpected in view of the different functions of cytochrome *c* and the oxygen-carrying hæm proteins.

Some noteworthy features of the amino-acid sequence of cytochrome *c* are as follows:

(1) The *N*-terminal α-amino group is acetylated, a situation which has also been shown to occur in several other cases[9], already cited in the preceding communications. Acylated *N*-terminal residues may be expected to occur in other situations, particularly if one considers that *N*-terminal amino-acids have not been found in a number of other proteins by chemical methods.

(2) Those acidic residues which are not present as amides appear to have some tendency to be found in groups. Thus there are Asp Val Glu, Glu Glu , Glu Tyr Leu Glu and Glu Arg Glu Asp sequences. However, two glutamyl and one aspartyl residues are not in these areas.

(3) On the other hand, the basic amino-acids of cytochrome *c* are mainly grouped in small regions of the protein chain. These residues are shown in italics in Fig. 1. Of the nineteen lysyl, two arginyl and three histidyl residues, there are Lys Gly Lys Lys, Lys Gly - Gly Lys His Lys, Arg Lys, Lys Asp-NH$_2$ Lys, Lys - Lys Lys and two Lys Lys sequences. Only three lysyl, one arginyl and two histidyl residues are not included in these sequences.

(4) There is an even more remarkable grouping of large hydrophobic residues (Leu Ileu,Phe,Tyr,Try)

which are marked in bold-faced letters in Fig 1 The chain includes the following sequences of such residues Ileu Phe, Leu His Gly Leu Phe, Phe Thr Tyr, Ileu Thr Try, Leu Met, Glu Tyr Leu, Tyr Ileu, Ileu.Phe Ala.Gly Ileu, and Leu Ileu Ala - Tyr Leu.

Cytochromes *c* of the mammalian type, defined as those cytochromes *c* that can readily interact with the appropriate enzyme systems of the terminal oxidation chain obtained from mammalian sources, have been isolated from a large variety of organisms These include mammals, birds, fish and plant cells At least twelve of these cytochromes *c* have been crystallized to date All have molecular weights of about 12,000, a basic isoelectric point and an oxidation reduction potential of about + 0 250 V It will be of interest to ascertain whether this similarity of function and physical properties is expressed in amino-acid sequences that show the same striking features as the protein from horse heart A comparison of the amino-acid sequences of cytochromes *c* of the mammalian type should yield a picture of those portions of the pattern which are essential for the formation of the specific native three-dimensional structure that constitutes cytochrome *c*, and of those parts of the amino-acid sequence which can vary within the limits set by the structural requirements of the molecule Thus far, such a comparison has been limited to a study of sequences adjoining the hæm group in certain cytochromes[10]

We wish to acknowledge the aid furnished by grants from the National Institutes of Health, U S Public Health Service (E L S) and the Rockefeller Foundation (H T)

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OBITUARIES

Prof. M. W Travers, F.R.S.

MORRIS WILLIAM TRAVERS, emeritus professor of chemistry in the University of Bristol and the senior Fellow (by date of election) of the Royal Society, died on August 25 at the age of eighty-nine

Travers was born into a medical family on January 24, 1872 He was educated at Blundell's School, Tiverton, and University College, London After a period at the University of Nancy, he returned to University College as a demonstrator in 1894, and became assistant professor in 1898 The ten years from 1894 to 1904 were among the most fruitful in Travers's career, for he returned to London in time to play an active part in Ramsay's brilliant work which led to the discovery and examination of the rare gases The excitement of this period lived vividly with Travers during the next sixty years, and he never tired of recalling the steps which led to these discoveries

In 1904, shortly after his election to the Royal Society, Travers was appointed to the chair of chemistry at University College, Bristol He brought with him the hydrogen liquefier which he had just perfected at London, and during his time at Bristol his research was concerned largely with low temperatures His main interests, however, centred on the movement for the foundation of a University of Bristol He wrote a pamphlet on the need for a University and the principles to be observed in its constitution; this pamphlet had wide circulation, and Travers enlisted both local and national support for his proposals His enthusiasm was in no small measure responsible for the movement which culminated in the offer by H O Wills in 1908 of £100,000 towards the endowment of a University, but by this time Travers had left Bristol

In 1907 he became director of the Indian Institute of Science at Bangalore, a post which he held until his return to the United Kingdom in 1914 On the outbreak of war it was soon realized that Great Britain would suffer a serious shortage of chemical glassware, which until then had all been imported from the Continent Travers set to work to devise suitable glasses for chemical use and took his processes to a small glassworks in Walthamstow The enthusiasm with which he had carried through his researches in the laboratory was equally effective in the factory, and very soon 'Duroglass' was in successful production

After the First World War, Travers was concerned with furnace technology, he patented a furnace and for a while ran a factory to produce it In 1922 he was elected president of the Society of Glass Technology He returned to Bristol in 1927 as honorary professor, Fellow and Nash lecturer in chemistry He now resumed academic research and staged a come-back on a topic new to him—the study of gaseous and heterogeneous reactions In this field he brought to bear his superb experimental skill, and was ahead of his time in insisting on a complete and careful analysis of all the products of a reaction His return to academic life culminated in his presidency of the Faraday Society in 1936 He did not, however, sever all links with industry, he became a consultant to the National Smelting Co , Ltd , in 1931, and continued to work in this capacity, apart from a break during 1940–45, either on a full- or part-time basis up to his death In 1937 he retired from the University of Bristol and was accorded the title of emeritus professor

At the start of the Second World War Travers offered his services to the Royal Ordnance Factories, and, as a consultant, threw himself whole-heartedly into the diverse problems which had to be solved He made many contributions, particularly in connexion with the application of silicon iron in sulphuric acid manufacture, and in the development of rocket cordite In his dealings with technological problems he had a remarkable facility for putting his finger on the source of trouble It was amazing to see a man, who was for most of the War more than seventy years of age, travel many miles, usually at night, to face the problems in the widely scattered ordnance factories In this period he was a wonderful example to the younger scientists and technologists in his untiring enthusiasm for work

Soon after the end of the War, Travers moved to Stroud and began what was for him one of his greatest and most congenial tasks, the production of a full biography of Sir William Ramsay This work, which Travers had began in 1937, was taken up again in 1948, and the book was written between Travers's eighty-first and eighty-third birthdays, it is one of the great biographies of our time and it will surely remain as a monument to Sir William Ramsay and a fitting memorial to Travers

Prof Travers is survived by his wife, formerly Miss Dorothy Gray, a pianist of outstanding ability, whom he married in 1909, by their daughter and by their son, who is now professor of educational psychology at the University of Utah

I am indebted to Mr S W G. Morgan, Dr W G Hiscock and Mr J Lumsden, of the Imperial Smelting Corporation, for providing information about Travers's industrial career D H Everett

Prof. R. H Dastur, O.B E.

India has lost a distinguished plant physiologist and agronomist by the death of Prof R H. Dastur, which occurred at Bombay on October 1, 1961, at the age of sixty-five years

Rustom Hormusji Dastur was born on March 7, 1896 After graduating as bachelor of science in 1918 at the Gujarat College, Ahmedabad, he was appointed a demonstrator in botany at the same College and obtained the M Sc degree in botany, by research, in 1921 His association at the Gujarat College with the noted botanist Prof W T Saxton gave young Dastur a good grounding in systematic botany and ecology Dastur's interests, however, turned very early in his career towards plant physiology, despite the fact that he had little regular training in plant physiological research, except perhaps for a year (1924–25) he spent at the University of Reading, England, with Prof W Stiles There he experimentally tested his earlier ideas on the relation of water content to photosynthesis (*Ann Bot*, 38, 779, 1924) and made a new departure by measuring photosynthesis in a leaf attached to the plant (*Ann Bot*, 39, 769; 1925)

Dastur was appointed assistant lecturer in botany at the Royal Institute of Science, Bombay, in 1921, and continued there as professor and head of the Department of Botany during 1929–1935 During this period of about half a decade he established a very active school of plant physiology, and his investigations, in collaboration with his pupils, related to the influence on photosynthesis of factors such as water and chlorophyll content, and quality and polarization of light, physiology and anatomy of tendrils, respiration, and physiology of the rice plant The results of these investigations were published in the *Annals of Botany*, the *Journal of the Indian Botanical Society* and the *Indian Journal of Agricultural Science*.

In the year 1935 Dastur's interest shifted from the academic to the applied side of plant physiology, and work on the cotton crop claimed his entire interest for the next 26 years and constituted the *magnum opus* of his scientific output

In 1919 the crop of the Panjab-American cotton, hitherto the main standby of the cultivator in the canal colonies of the Panjab, suffered a serious setback when it was attacked by a disease called 'tirak' By 1935, another seven failures of the crop, partial or complete, occurred. Various theories were advanced to explain this condition but none satisfactorily accounted for and solved the mystery of the occasional failures of the crop Prof Dastur was then invited by the Indian Central Cotton Committee to undertake investigation into this problem, and it was indeed a far-sighted and bold step on the part of the Committee and Mr H. R (later Sir Herbert) Stewart, the then director of agriculture, Panjab, to pin their faith on one who until he undertook the investigation had no previous contact with or knowledge of the cotton plant This investigation, carried out from 1935 until 1943 and described in several scientific papers and eventually in a monograph, was reviewed in *Nature* (158, 524, 1946) Sir Herbert Stewart summed it up in the words "The story which it unfolds is a striking example of how investigations into the realm of pure science can be turned to practical account in everyday agricultural practice"

Dastur retired from service in 1951 on attaining the age of fifty-five years The Indian Central Cotton Committee, however, retained his services in an honorary capacity until the time of his death for directing and supervising plant physiological and agronomic research schemes on cotton

During 1944–61, Prof Dastur was engaged in investigations on (1) causes of bad opening of bolls in Sind, (2) causes of the low yield of sea-island and Egyptian cottons in the various cotton-growing tracts, (3) causes of red-leaf disease and several other related aspects, and obtained a number of important and useful results These results were compiled in two other monographs which were published by the Indian Central Cotton Committee in 1959 and 1960 Prof Dastur was in the process of constructing a theory for the application of fertilizers on the basis of a balance between nitrogen, phosphorus and potassium contents of the plant, and it is indeed unfortunate that death claimed him before he could complete this interesting and useful concept

Dastur had a reserved, but gentle and kindly disposition and shunned publicity, he virtually lived for his work and had little interest in social activities and scientific conferences which would take him away from it For his work on cotton in the Panjab he was made O B E. in 1946 He was a Foundation Fellow of the National Institute of Sciences of India and was elected president of the Botany Section of the Indian Science Congress in 1933 and of the Indian Society for Plant Physiology in 1959

He leaves behind a widow, a daughter, a son and a large circle of pupils and colleagues who will cherish his memory for a long time to come

R D Asana

NEWS and VIEWS

Post Office Radio Services Department:

Mr. T. A. Davies, O.B.E.

MR. T. A. DAVIES, inspector of wireless telegraphy in the Wireless Telegraph Section of the Radio Services Department of the Post Office, is to retire at the end of the year. Mr. Davies took a love of the sea to his job as head of the Wireless Telegraphy Section in 1948. This was to prove useful in his later work. After five years apprenticeship in the Royal Dockyard in his home city of Portsmouth, he obtained a first-class honours degree in engineering and joined the Post Office in 1925. While working in the Radio Branch of the Engineering Department he was able to use his knowledge of shipping in providing radio communications to ships and safety services for them while at sea. As inspector of wireless telegraphy he has been responsible for control and operation of the ship–shore services, inspection of ships' radio equipment, and the examinations for candidates for the Postmaster-General's Certificate. Twelve radio stations are administered from the headquarters in London.

Mr. R. Billington

MR. REG BILLINGTON has been appointed inspector of wireless telegraphy in the Wireless Telegraph Section of the Radio Services Department of the Post Office to succeed Mr. Davies. After obtaining an honours degree in the University of London, Mr. Billington worked in industry for two years before joining the Post Office Engineering Department in 1936. For a period during the Second World War he was seconded to the Iraq Government as chief engineer and returned to the Post Office in 1945, where he soon became an assistant inspector of wireless telegraphy. In 1948 he became deputy inspector.

Applied Electromagnetic Theory Section at the U.S. National Bureau of Standards

Mr. J. R. Johler

MR. J. R. JOHLER has been appointed chief of the new Applied Electromagnetic Theory Section, National Bureau of Standards, U.S. Department of Commerce. The section will be located in the Radio Systems Division at the Laboratories of the National Bureau of Standards at Boulder, Colorado. Mr. Johler's Section will be responsible for developing techniques for the solution of electromagnetic theory problems in terrestrial radio-wave propagation by numerical analysis, analytic and asymptotic series techniques, and will direct its work to electronic computer solutions. Much of the Section's work will be devoted to programmes of other Sections and Divisions in providing theoretical computations of propagation in various media. Since 1953, Mr. Johler has worked principally in the field of radio navigation systems evaluation, in low and very low frequency radio-wave propagation, and sferics (radio noise caused by lightning flashes). During 1953–55 he was responsible for the theoretical prediction of the performance of certain pulse-type radio navigation systems. Before joining the National Bureau of Standards in 1946, Mr. Johler had worked on the computational and engineering details of exterior ballistics at Aberdeen Proving Ground, Aberdeen, Md.

Atlantic Regional Laboratory, National Research Council, Canada:

Dr. A. C. Neish

DR. A. C. NEISH has been appointed director of the Atlantic Regional Laboratory of the National Research Council of Canada, at Halifax, Nova Scotia, in succession to Dr. E. Gordon Young. Dr. Neish studied at Nova Scotia Agricultural College, he obtained his B.Sc. (agriculture) from Macdonald College, majoring in agricultural chemistry, and his M.Sc. and Ph.D. (chemistry) from McGill University. His experience ranges from soils analysis and soils surveys at the Canada Department of Agriculture, and sessional lecturer, Department of Chemistry, McGill University (1942–43), to his research work with the National Research Council, first in the Division of Applied Biology (1943–48) and then with the Prairie Regional Laboratory at Saskatoon, Sask., where, in 1956, he was appointed head of the Physiology and Biochemistry of Plants Section.

Royal Society Expedition to Tristan da Cunha

THE Royal Society is to send a scientific expedition to examine the recent volcanic activity on the island of Tristan da Cunha in the South Atlantic. This activity is of considerable interest to geologists in Great Britain and the proposal is to mount an expedition which will include four or five geologists, leaving Cape Town some time during January 1962, to spend 2–3 months on the island. It is also proposed to include a zoologist and a botanist in the expedition to study any effects which the evacuation of the population of the island may have had on the flora and fauna. The expedition will be concerned not only with a detailed examination of the present volcanic activity but also to determine as accurately as possible the past geological history of the island. The scientific direction of the expedition is under the control of Prof. W. Q. Kennedy, professor of geology in the University of Leeds, with Prof. C. F. A. Pantin, professor of zoology in the University of Cambridge, as biological adviser, and with the co-operation of the Nature Conservancy.

H.M.S. *Jaguar* will be visiting Tristan da Cunha briefly later this month and advantage is being taken of this to send two geologists aboard the ship to make a preliminary investigation prior to the departure of the main expedition. These two geologists are Dr. R. W. Le Maitre, of the British Museum (Natural History), and Dr. P. G. Harris, of the Department of Geology, University of Leeds.

Science in Parliament

THE Parliamentary Secretary for Science, Mr. D. Freeth, dealt with a series of questions on science on December 5, ranging from nuclear energy, red deer stags on the Island of Rhum, engineering designers, strontium research, radioactivity, medical research on animals, the emigration of scientists, space exploration, the employment of scientists and the report on the long-term demand for scientific manpower. Mr. Freeth said that the Minister for Science is not satisfied with the present supply of engineering designers in Britain and he is in close touch with the President of the Board of Trade and trying to find ways to ameliorate the position The Department of Scientific and Industrial Research at present employs 170 scientists in Scotland, and it is intended to increase this number, subject to assessments of changing priorities and the resources available There are about 22,000 members of research associations, of whom a few belong to more than one association British plans for space exploration do not include projects for sending men into space

U K. Expenditure on Science, Technology and Education

In a written answer in the House of Commons on November 28, the Parliamentary Secretary for Science, Mr Denzil Freeth, said that the greater part of the expenditure on scientific and technological research in universities, estimated at £23 3 million in 1958–59 and £25 9 million in 1959–60, was met from the recurrent grants made by the Treasury on the recommendation of the University Grants Committee and was not separately identified Research Council grants to universities and to research units and institutes associated with universities amounted to £3 1 million in 1957–58, £3 5 million in 1958–59, £4 5 million in 1959–60, £6 million in 1960–61 and £8 2 million in 1961–62 In figures circulated for the Research Councils, with the exception of the Overseas Research Council, the grants from the Department of Scientific and Industrial Research are given as £1 315,000 in studentships and fellowships and £2,600,000 in research grants for 1961–62, the corresponding figures for 1960–61 being £1,050,000 and £1,490,000 For the Agricultural Research Council in 1961–62, studentships and fellowships amounted to £31,000, research grants to £157,000, expenditure of research units within universities £273,000 and grants to research units or institutes associated with universities, £1,011,000, for the period 1960–61, the figures are £24,000, £104,000, £299,000, and £931,000 For the Medical Research Council the corresponding figures are £115,000, £811,000, £1,526,000, and £317,000 in 1961–62, and £100,000, £620,000, £1,060,000 and £285,000 in 1960–61 For the Nature Conservancy, they are £21,000, £20,000, £11,000 under the first three headings in 1961–62, and £18,000, £11,000 and £7,000 in 1960–61

Replying to a question in the House of Commons on November 30, the Minister of Education, Sir David Eccles, said that the Ministry's present grant to the National Foundation for Educational Research was £7,000 and local education authorities were contributing £29,000 as members of the Foundation The Ministry was at present in discussion with the Foundation, and Sir David undertook to see that the availability of information was included

Toxic Chemicals in Agriculture

On December 5, the Joint Parliamentary Secretary to the Ministry of Agriculture, Fisheries and Food, Earl Waldegrave, announced publication of the report of the Research Study Group on Toxic Chemicals in Agriculture and Food Storage He said that the report concluded that protective measures now in force are generally successful, but affirmed the need for more fundamental and applied research in this field Earl Waldegrave said that the Government accepted the conclusions and intends to implement the Group's recommendations to the full extent of the additional resources which can be made available The Minister, Mr C Soames, made a similar statement in a written answer in the House of Commons on the same day, but, when pressed, neither he nor Earl Waldegrave would add to that statement In reply to further questions on December 7, Mr Soames said that he would be replying next week to the Select Committee on Estimates, which, in its Sixth Report of June 28, had recommended an inquiry into the effect on agriculture, public health and the ecology of Britain of all chemicals used in agriculture, and that he hoped that the restrictions on the use of cereal seed dressings containing aldrin, dieldrin and heptachlor which he had announced on July 3 would prevent casualties in future among wild birds and mammals His Department would intensify its survey of wild-life casualties as recommended by the Research Study Group, and consider after next session, with the interests concerned, whether any further action is necessary

The Commonwealth Scholarship Commission in the United Kingdom

The Commonwealth Scholarship Commission was constituted in December 1959 under the Commonwealth Scholarships Act, and in accordance with the proposed arrangements for administering in the United Kingdom the Commonwealth Scholarship and Fellowship Plan, recommended by the Commonwealth Education Conference in July 1959 and set forth in the White Paper presented to Parliament Pending the introduction of the relevant legislation and to facilitate the speedy implementation of the Plan, the Secretary of State for Commonwealth Relations had already appointed an interim committee, with Sir Eric Ashby as chairman, which met three times before the Commission held its first meeting on February 23, 1960, under the Earl of Scarborough as chairman, Sir Eric Ashby being then elected deputy chairman Confirming the preliminary arrangements made by the interim committee, the Commission endorsed the view that in the first year attention should be concentrated on the selection of Commonwealth Scholars and invitation for senior Commonwealth Fellowships deferred for a year. According to the first annual report of the Commission, up to the end of April 1960, 452 applicants were nominated by overseas agencies from some 5,177 applications received, and from these a Selection Committee recommended 219 candidates to the Commission, of whom only 178 accepted the offer of scholarships (Cmnd 1541 Pp 26. London H M S O, 1961 1*s* 6*d* net) Of these, 37 were from India, 17 each from Pakistan and Canada, 16 from Nigeria, 15 from Australia, 10 each from New Zealand and South Africa, and 8 from Hong Kong. Of these again, 74 were in the humanities, 50 in science, 33 in technology and 21 in medicine The chief considerations in selection were intellectual merit and a realistic and practicable plan of study, but selections were also made so as to ensure that the various parts of the Commonwealth are appropriately represented Applications have also been invited from candidates in the United Kingdom for awards offered by Canada, Australia, New Zealand, South Africa, Malaya, East Africa and Hong Kong, and scholarships have been offered by Canada to 13 candidates nominated

Exchange of Students for Technical Experience

The fourteenth annual report of the International Association for the Exchange of Students for Technical Experience reports continued growth in 1961, with three new members—Argentina, Luxembourg and the United Arab Republic—7,478 students exchanged, compared with 6,430 in 1960, and 3,265 employers offering training, an increase of 229. Since its foundation the Association has provided 59,769 students with technical training abroad. Exchanges of civil engineering and forestry students have increased considerably, but the exchange is still concentrated in a few European countries, and Germany, which received 1,757 students, with Great Britain and Sweden, represents more than 50 per cent of the training possibilities. Some 569 universities and colleges are now participating and 45 faculties are covered. These institutions and the participating firms are listed in the report by countries.

The corresponding report of the United Kingdom Association, which was established as an independent organization as from October 1, 1960, covers the year ending September 1961. During the summer vacation of 1961, 894 students were sent abroad for experience and 965 overseas students received and placed in British industry and commerce with the help of 389 British firms and organizations, compared with 883 and 908, respectively, in 1960. Of the students coming to Britain, Sweden (225), Germany (108), Netherlands (86), France (79) and Canada (57) sent the largest numbers, and of those received Sweden took 178, Germany 139 and France 117 (Fourteenth Annual Report, 1961. Pp. 79. Report of IAESTE in the United Kingdom for year ending September 1961. Pp. 14. London: International Association for the Exchange of Students for Technical Experience (U.K.), 1961).

History of Science and Technology at the Imperial College, London

Much thought is being given to-day, by those responsible for the education of scientists and technologists, to the best means of broadening their education and preventing excessive specialization. The Imperial College of Science and Technology is attacking this problem in various ways. A new development is now being added to those already in existence. A chair is being instituted in the history of science and technology which will result in a department for research and study of this subject in its own right, no exactly similar chair exists in a British university, although there are chairs elsewhere in the history and philosophy of science. The field of study, which is intentionally being made wide, will it is hoped embrace both the genesis of basic ideas and tools and the recent history of science and technology, the latter particularly because of the great acceleration in applying scientific discovery with important social and political results. The aim will be to teach scientists and engineers, rather than students of history, so that they become familiar with the background of major discoveries and inventions. To this end, the Department will start by providing postgraduate courses, both full-time and part-time. Later, it is hoped, optional undergraduate courses will be introduced in the third year of some degree courses. A generous contribution towards the cost of maintaining the new Department has been made by Imperial Chemical Industries, Ltd., which has recently supported the study of the history of technology by subsidizing some valuable publications. The establishment of the new Department will present both scientists and historians with a challenge, the first holder of the chair will have the opportunity to bridge one of the gaps between the humanities and the sciences. The results of this development will be watched with interest.

United Kingdom Fire Statistics, 1960

Since the Department of Scientific and Industrial Research and Fire Offices' Committee Joint Fire Research Organization began its statistical record in 1946, the number of fires in buildings occurring annually in the United Kingdom has almost doubled and, in 1960, was more than 62,000. The total number of fires of all kinds reached a peak in 1959 when, due to an exceptionally dry summer, a very large number of outbreaks occurred in the open. However, the figure for 1960, 133,484, is second only to that for 1959. These figures are revealed in *United Kingdom Fire Statistics 1960: Statistical Analysis of Report of Fires Attended by Fire Brigades in the United Kingdom during 1960*, which has recently been published as the first of an annual series made available to the general public (Pp. 106. London: H.M.S.O., 1961. 12*s*. 6*d*. net). This volume presents tables of figures relating to the origin and extinction of fires and to resultant casualties. Separate series are given for England and Wales, Scotland and Northern Ireland, and summary tables for the United Kingdom as a whole afford comparisons with previous years back to 1956. In the last-named section graphs are given of the annual and monthly incidence of fires, and there is a special graphical analysis of fires associated with space heating during the period 1949–60, this being a major cause of fire, particularly in dwellings. A marked downward trend in occurrences due to fires in open grates has been offset by a sharp rise in fires caused by oil-burning heaters, but the latter have been checked in the last year of the period under review. It is encouraging to note that the Fire Brigades are apparently relying increasingly on hose reel equipment for the first attack on fires and that this is proving adequate in a large proportion of cases. This should result in a continued reduction in water damage, although it is not possible to obtain figures to illustrate this point.

Dairy Research in Australia

In the small space of twelve short pages the Dairy Section of the Australian Commonwealth Scientific and Industrial Research Organization, led by Mr. G. Loftus Hills, presents a succinct account of its work for the year 1960–61 (Annual Report for the year ending June 30, 1961. Pp. 18. Melbourne: Commonwealth Scientific and Industrial Research Organization, Dairy Research Section, 1961). With a staff of about fifty people, of whom some twenty are graduates, the scope and value of the work now being done by the Section are very considerable. The topics under investigation include the mechanization of cheese-making, the surface deterioration of butter resulting from oxidative effects of the copper present in the parchment in which it is wrapped, and problems encountered in the manufacture of casein. The main buyers of Australian casein are paper manufacturers in the United States and Japan, where it is used for coating art paper, but this outlet for Australian casein is threatened by competition from soya bean proteins and from synthetic substances which give solutions of lower viscosity and which therefore allow the coating process to be done more rapidly than is at present possible with casein solutions. In an endeavour to solve this problem a detailed study is being made of the factors that affect the viscosity of casein solutions. A series of investigations, extremely important not only in dairying but also to many other branches of the food industry, is that concerned with flavour chemistry. The object of this work is to determine what carbonyl and other compounds are responsible for the various off-flavours, such as oily, tallowy, fishy and cardboardy, that can occur in certain circumstances in dairy products, and, if possible, to isolate and characterize the substances concerned. Marked progress has already been made in that direction. The report ends with a list of twenty-nine papers published during the year, some in the British *Journal of Dairy Research*, but most of them in the *Australian Journal of Dairy Technology*. Judging from this impressive list of publications, the Dairy Section of the Commonwealth Scientific and Industrial Research Organization can certainly claim that it had a satisfactorily fruitful year in 1960–61.

Strangers' Hall, Norwich

During the past year, four rooms on the street front of Strangers' Hall have, with the help of a Carnegie Grant, been converted into period rooms. On the ground floor is a kitchen with furnishings of about 1830 and a Georgian sitting-room. These rooms are seen from the street, but visitors do not go into them. On the first floor is a Victorian night nursery of about 1880 and a Victorian sitting-room of about 1860. Visitors walk along a roped pathway through these rooms.

Field Studies Council: Courses for 1962

The programme of courses for 1962, organized under the auspices of the Field Studies Council, has recently been issued. Such courses vary from those suitable for pupils below sixth-form level to those for university students, teachers and amateurs. Most natural-history subjects are covered. They are held at the following Field Centres: Slapton Ley, Kingsbridge, Devonshire, Preston Montford, near Shrewsbury, Malham Tarn, near Settle, Yorkshire, Juniper Hall, Dorking, Surrey, Flatford Mill, East Bergholt, near Colchester. Further information can be obtained from the wardens of these Field Centres or from the Publicity Secretary, Field Studies Council, Ravensmead, Keston, Kent.

University News: Glasgow

The following have been appointed to lectureships: Adam Fleck (biochemistry), N J McCorkindale, I C McNeill, K C Campbell and A L Porte (chemistry); W T. Morton (experimental physics), and D L Pursey (theoretical physics). A J Baker has been appointed Glaxo Fellow in chemistry, other fellowships have been awarded to G L le Bouvier, June Dixon and K R Dronamraju (genetics), John Cumming, A W Hendry and J G Lynch (nuclear physics), Robert McCrindle, M B Meyers and R D H Murray (organic chemistry), R M Turnbull (physics), and J. D. Roycroft (solid-state physics).

An Associated Electrical Industries (*RS*2) high-resolution nuclear magnetic resonance spectrometer (the cost of which was defrayed by the Department of Scientific and Industrial Research) is now in operation in the Chemistry Department.

For the present session, 392 students (292 men and 100 women) have been admitted to the science departments compared with 396 in 1960. The total of matriculated students in the University as a whole now stands at 6,765, compared with 6,600 in 1960.

London

Prof. Michael Abercrombie, professor of embryology in University College, has been appointed to the Jodrell chair of zoology and embryology at that College from October 1, 1962, in succession to Prof. P B Medawar (*Nature*, 187, 991, 1960).

The following appointments to readerships have been announced: Dr J R Butler (pure geochemistry) at the Imperial College of Science and Technology, Mr J. C G Coleridge (physiology) at the Royal Free Hospital School of Medicine, Dr S J. Taylor (mathematics) at Westfield College, Mr S. B Watkins (chemical engineering) at King's College, Dr. F W O'Grady (bacteriology) at St Bartholomew's Hospital Medical College, Dr. J. C Anderson (electrical engineering) in the Imperial College of Science and Technology.

The title of professor has been conferred on Mr J K T L Nash (civil engineering), in respect of his post at King's College, Dr H E Rose (powder technology), in respect of his post at King's College, Dr J H Westcott (electrical engineering), in respect of his post at the Imperial College of Science and Technology. Titles of reader have been conferred on Dr K W Gruenberg (pure mathematics) in respect of his post at Queen Mary College, Dr. J. M Zarek (engineering science) in respect of his post at King's College.

Announcements

The French Minister of Agriculture has conferred on Sir John Russell, formerly director of Rothamsted Experimental Station, the honour of Commander of the Order of Agricultural Merit on the occasion of the bicentenary of the French Academy of Agriculture.

Sir Charles Dodds, director of the Courtauld Institute of Biochemistry at the Middlesex Hospital Medical School, London, and a governor of the Lister Institute of Preventive Medicine since 1956, has been appointed chairman of the Governing Body of the Lister Institute, in succession to Sir Henry Dale, who has resigned after occupying that office for twenty years.

The Bradford Chemical Society and the Department of Chemical Technology of the Bradford Institute of Technology, in conjunction with the Yorkshire Council for Further Education, are organizing a symposium on "Antibiotics and Newer Synthetic Drugs", to be held at the Bradford Institute of Technology on February 10. Further information can be obtained from Dr R P. Sheldon, Department of Chemical Technology, Bradford Institute of Technology, Bradford.

The sixteenth annual symposium on fundamental cancer research, entitled "Conceptual Advances in Immunology and Oncology", is to be held in the University of Texas during March 1–3. Subjects under discussion will include theories of antibody production, control mechanisms of antibody synthesis, the nature of the antigen–antibody reaction, transplantation and immunological tolerance, and cancer specific antigens. Further information can be obtained from the Editorial Office, M D Anderson Hospital and Tumor Institute, University of Texas, Houston 25, Texas.

Erratum. In the communication entitled "Riometer Observations during a Solar Eclipse", by M A Gordon, in *Nature* of October 21, p 249, the legend to Fig 1 requires amendment: for "27 5 Mc /sec. radio wave" read "27 5 Mc /sec. (day) radio wave".

THE NEW MATHEMATICS AND OCEANOGRAPHY BUILDING OF THE UNIVERSITY OF LIVERPOOL

A FEW weeks ago the University of Liverpool took over from the building contractors (Messrs. Gilbert-Ash, Ltd) an almost completed Mathematics and Oceanography Building, the architects of which are Bryan and Norman Westwood and Partners, of London The building, which will cost about £260,000, has three wings, one on each of three sides of a grass-covered central courtyard, the remaining side of the courtyard will, at some later time, be open to one of the pathways of what will then be the University Precinct

The main wing is a gracious six-storeyed structure of glass, black concrete pillars, and white concrete panels of special design On the flat roof there is a 'wood and glass' penthouse in which the transcendental verities may be contemplated in relative comfort Below the building is a basement for the usual services, and farther below still there is the area boiler house

On the ground floor of the main wing, which is well set back from the road, and which is approached along a covered pathway traversing the courtyard, there is a spacious white-walled, airy, bright, central hall, the floor of which is made of attractive grey-green slate On one of the walls of the hall there is a five-panel mural inspired by the users, designed by John McCarthy, and executed by him in red terossa ferrata. This mural represents, in various groups, the history and development of mathematics, and many of the important branches of human activity in which pure and applied mathematics play, or have played, a significant part In addition, the hall contains a tall black-concrete abstract mural designed and executed by Eric Peskett Leading off the central hall there will be, when completed, the two principal lecture rooms of the new building The rooms will be large enough to hold audiences of 110 and 145, respectively, and they will be named after A. R Forsyth and J Proudman, the first professors of pure mathematics and applied mathematics of the University of Liverpool Proudman was afterwards transferred to the University chair of oceanography, and did much to give the Department of that subject its high reputation, it is therefore a particularly happy arrangement whereby the Departments of Mathematics and Oceanography are now housed in the same building

The first and second floors contain lecture rooms, the third floor contains more lecture rooms as well as the Sub-Department of Mathematical Statistics The fourth floor contains private rooms for the applied mathematics staff, and the fifth floor has similar rooms for the pure mathematics staff, as well as rooms for research students

At ground-floor level, at right-angles to the main block, there is a single-storey wing containing the Oceanography Laboratories, these are used for research and for instruction for honours under-

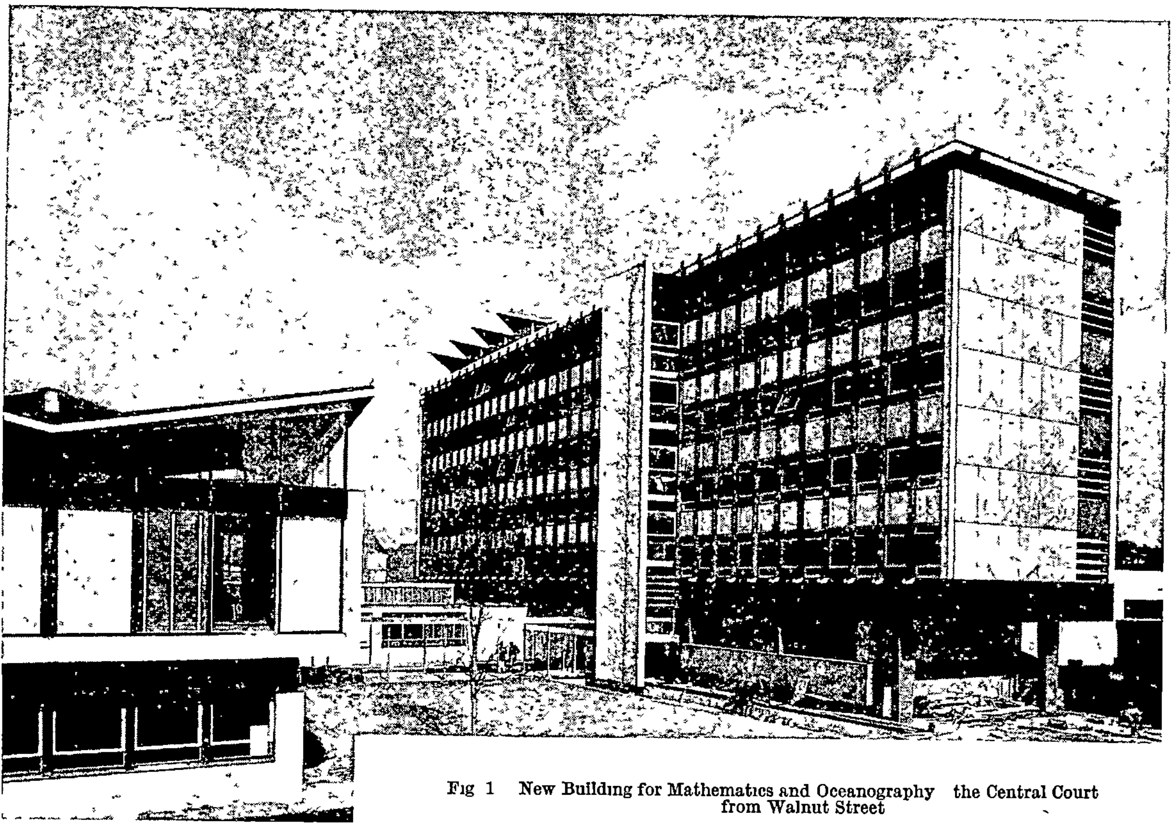

Fig 1 New Building for Mathematics and Oceanography the Central Court from Walnut Street

graduates. There are four main laboratories, two for physical work and two for chemical. Other rooms, such as a spectrographic room, balance room, dark room, workshop and store, are provided for special purposes. The laboratory accommodation and equipment offer facilities, on the chemical side, for work on the analysis of sea-water, particularly of trace elements by spectrophotometric methods, and on the composition of marine sediments. On the physical side, there are facilities for developing and testing apparatus for measuring, for example, the temperature and electrical conductivity of sea-water, and the velocity and turbulent fluctuations of currents.

At right angles to the single-storey wing, parallel to the main wing, there is a two-storey block containing, at one end, staff rooms and a combined library and chartroom for oceanography. The remainder of this block is occupied by the Sub-Department of Numerical Analysis, which possesses, in addition to rooms for its staff, others which house an electronic computer and its ancillary equipment, and a mathematical laboratory.

The building was designed to cater for the estimated number of science and engineering students, and the appropriate staff, likely to study mathematics in one or more of its aspects, or oceanography, within a total student community of 5,000. The erection of the building had scarcely been begun, however, when, under the stimulus of revised estimates of national requirements, the University agreed to plan its development on the basis of an ultimate student population of about 7,000. Large as it appears to be, therefore, the building will probably not be big enough to meet the demands likely to be made on it towards the end of the 'sixties.

The research carried out by the staff and graduate students of the Department of Pure Mathematics (Prof. A. G. Walker), the Department of Applied Mathematics (Prof. L. Rosenhead), with its Sub-Department of Mathematical Statistics (R. L. Plackett, reader in mathematical statistics), and its Sub-Department of Numerical Analysis (Dr. A. Young, reader in numerical analysis), and the Department of Oceanography (Prof. K. F. Bowden) covers a very wide range. The work that is going on in oceanography has been mentioned earlier; in pure mathematics research interests include classical analysis, group theory, algebraic and differential geometry and topology; and in applied mathematics work is being done on fluid motion, acoustics, quantum theory, nuclear physics, theoretical chemistry, astronomy, geophysics, numerical analysis and mathematical statistics.

The facilities of this well-co-ordinated group of teachers and research workers will be increased within the next few years when, with the aid of the grant recently promised by the University Grants Committee, an English Electric *KDF*9 Computer will be obtained to replace the existing *Deuce* of the Computer Laboratory, which was bought with funds provided by local industrialists and the University Council.

The University skyline at the top of Brownlow Hill is changing significantly—new buildings for physics, chemistry and veterinary science have been erected; still more buildings are going up for chemistry—and the foundations of engineering buildings as well as those of an extension of the Students' Union are now being laid. Within this complex of building activity the white-walled Mathematics and Oceanography Building stands out as something rather different and aloof, but the building will, we hope, serve as a university focus of mathematical thinking, teaching and research, and a centre from which mathematical service will flow to the University as a whole—as well as a place where important work on oceanography will be carried out.

L. ROSENHEAD

OXIDATION PROCESSES IN CHEMICAL MANUFACTURE

THE two-day symposium on "Oxidation Processes in Chemical Manufacture" organized by the London Section of the Society of Chemical Industry, was held in the William Beveridge Hall of the University of London during September 28–29, and attracted an audience of some 250 scientists from industry and the universities—with many overseas members.

The remarkable development in industrial oxidation processes over the past decade is perhaps best illustrated by the fact that while six comparatively new processes were discussed in some detail by the speakers, an equal number were mentioned only in passing. Among the latter were the direct oxidation of ethylene to ethylene oxide, the oxidation of propylene to acrolein, the subsequent oxidation of acrolein to glycerol, the direct oxidation of propylene in the presence of ammonia to acrylonitrile, and the production of hydrogen peroxide by the peroxidation of isopropyl alcohol. The processes chosen for discussion were the direct oxidation of ethylene to acetaldehyde, the vapour phase oxidation of acetaldehyde to peracetic acid, the uses of hydrogen peroxide in industrial oxidations with particular reference to epoxide formation, recent developments in the oxidation of aromatic hydrocarbons and the recovery of chlorine by oxidation of hydrogen chloride. Theoretical work on liquid-phase oxidation of organic compounds, vapour-phase oxidation of hydrocarbons and liquid phase oxidation catalysed by soluble metal salts was also discussed.

The direct oxidation of ethylene to acetaldehyde in the presence of a palladium chloride–copper chloride catalyst was described by Dr. Smidt (Consortium für Elektrochemische Industrie G.m.b.H.) as a single-stage reaction possibly applicable to other olefines. The mechanism of the reaction in the case of ethylene involves the formation of a complex between ethylene and palladium chloride. The resulting complex decomposes to give acetaldehyde, the palladium metal being precipitated. Since the decomposition of the complex is inhibited by acids, it is suggested that the first step in the reaction is the addition of a hydroxyl ion to the complex-bound olefine. In the second stage the π electron pair of the olefine passes over completely to the palladium with simultaneous migration of an hydride ion from carbon 1 to carbon 2. A double bond is then formed between carbon atom 1 and the oxygen of the hydroxyl group with expulsion of proton. On this hypothesis the oxygen required for the oxidation of the olefine is supplied by the water, and only the oxygen atom from the water passes over to the olefine during the reaction, all four hydrogen atoms originally present in the ethylene remaining in the aldehyde This is in contrast to the suggested mechanism discussed by Moiseev and co-workers Unpublished work of Dr Kreckeler of Farbwerke Hoechst has, however, shown that decomposition of the dinuclear ethylene–palladium chloride complex by heavy-water yields acetaldehyde which does not show any deuteration in mass spectrographic examination

The commercial development of the reaction to a continuous process required the precipitated palladium metal to be re-oxidized after reaction 1

$$C_2H_4 + PdCl_2 + H_2O = CH_3CHO + Pd + 2HCl \quad (1)$$

$$Pd + \tfrac{1}{2}O_2 + 2HCl = PdCl_2 + H_2O \quad (2)$$

The addition of cupric chloride enables the second step of the process to be carried out since cupric chloride readily oxidizes the finely divided palladium and the cuprous chloride so formed is quickly re-oxidized to the cupric state The overall reaction may then be written

$$C_2H_4 + 2Cu^{2+} + 4Cl^- + H_2O \xrightarrow{Pd\,Cl_2} CH_3CHO + 2H^+ + 2(CuCl_2)^-$$

the cuprous chloride being reoxidized by air

In the commercial process, as outlined by Dr Smidt, ethylene reacts with the catalyst solution, under moderate pressure and medium temperatures, in a flow reactor to 99 per cent conversion After releasing the pressure of the catalyst solution the acetaldehyde formed distils off (the reaction heat being sufficient to accomplish this), and the reduced catalyst solution is pumped to the oxidation reactor when it is regenerated by air and returns to the ethylene reactor Further details of this elegant process will be awaited with great interest

The development of a commercial process for the manufacture of peracetic acid by the vapour phase oxidation of acetaldehyde was described by J A John and F J Weymouth, of Courtaulds, Ltd , and British Celanese The major problem associated with this development was the explosion hazard associated with the concentration of peracetic acid Experimental work established that concentrations of peracetic acid in acetic acid below 40 per cent peracetic acid were stable and that in the vapour phase excess acetaldehyde reduced the hazard to safe conditions Three pilot plants, each of increasing capacity, had been successfully operated and the process was now considered suitable for commercial exploitation The major industrial usage of peracetic acid developed in recent years is as an epoxidizing agent for the production of epoxy soya bean oil, epoxy tall oil esters, di*cyclo*pentadiene dioxide and the diepoxide from vinyl *cyclo*hexene

The production of hydrogen peroxide by the barium process or by electrolytic methods has been virtually replaced in Britain by the method based on the cyclic oxidation and reduction of 2-ethyl-anthraquinol The development of an alternative method of production by direct oxidation of isopropylalcohol in the United States will lead to increased availability of this material T D Manly of Laporte Chemicals, Ltd gave an account of the wide variety of industrially important chemical processes using hydrogen peroxide Supplied in tank-car quantities stabilized against chance impurities the modern product can be used at all strengths under many different conditions without suffering detectable loss by decomposition

The recent developments in the production of a variety of chemicals using aromatic hydrocarbons from petroleum or coal tar were outlined by R Landau and R W Simon The processes discussed included the manufacture of phthalic anhydride from naphthalene or *ortho*-xylene, maleic anhydride from benzene—or from butene although a direct comparison of these two processes was not given—liquid-phase oxidation of mixed xylenes to yield isophthalic, phthalic, terephthalic acids and the recently announced oxidation of pseudocumene to give trimellitic anhydride Brief mention was also made of the many processes now available for the production of phenol from benzene and of newer processes using *cyclo*hexane and *cyclo*hexanol obtained by hydrogenation of benzene and phenol respectively, for the production of adipic acid

An account of recent developments in the oxidative recovery of chlorine from hydrochloric acid by W F Engel, S Muller and M J Wade of the Shell Laboratory, Amsterdam, introduced an inorganic oxidation process—the Deacon process—into the discussion The increasing amounts of by-product hydrochloric acid produced in conjunction with the manufacture of chlorinated organic compounds coupled with the probability of an increase in the price of chlorine as the demand for chlorine increases beyond the so-called caustic-chlorine balance—has stimulated development of processes for the recovery of chlorine from the acid Three such processes have been announced recently, the De Nova electrolysis of hydrochloric acid operated by Monsanto, the oxidation by mixed nitric/sulphuric acid developed by the Institut Français de Petrole and the modified Deacon process described by Shell Several companies have suggested the use of a fluidized-bed technique using modified cupric chloride catalysts The Shell process employs a modified cupric chloride catalyst supported on silica in a fluidized bed at temperatures between 350° and 360° C using air or oxygen Conversions of 76–77 per cent per pass are claimed On the figures presented the economics of the process are attractive

Theoretical aspects of oxidation process were reviewed in three papers Liquid-phase oxidation by molecular oxygen was reviewed by G H Twigg with particular reference to the oxidation of hydrocarbons and the reactions involved in the oxidation of cumene to phenol and acetone The extension of the general reaction mechanism to oxidation of aldehydes, ketones, alcohols and olefines while generally accepted does not entirely satisfy, and further work in this field is desirable Recent work on the oxidation of binary mixtures is likely to be of considerable theoretical interest The gaseous oxidation of organic compounds was discussed by C F Cullis with particular reference to the effects of temperature, pressure, surface catalysis and mixture composition on the course of oxidation of a particular starting material Investigations such as those reported on the vapour-phase oxidation of acetaldehyde to peracetic acid indicate that by careful choice of reaction conditions the reaction may be controlled to give, as final products, compounds which normally would occur only as intermediates in a more general oxidation It is, however, doubtful if the knowledge of low-temperature vapour-phase oxidation processes is sufficiently advanced to enable prediction of reaction conditions in particular cases to be made on the basis of generalized theory

The oxidation of organic compounds by metal ions and complexes was reviewed by H R Bacon with particular reference to complexes of silver and copper Recent work using argentic piccolinates to catalyse the oxidation of primary and secondary amines has indicated that the effectiveness of the metal ion may be modified by the nature of the ligand Comparison of reactions catalysed by cobalt, silver and copper complexes may lead to a better understanding of the reaction mechanism

The conference concluded with a general discussion of the relative merits of fluidized-bed versus fixed-bed reactors for oxidation reactions For those reactions, such as the modified Deacon process, in which thermal balance is readily achieved, the fluidized process is preferable In reactions liable to rapid heat evolution, leading to possible explosion hazard, fixed-bed reactors may be preferred although sufficient data on the successful application of the fluidized technique to such reactions are not yet available. F Morton

FAST REACTIONS IN PHYSICAL CHEMISTRY

THE first of the triennial Cambridge Physical Chemistry Summer Schools was held in August 1947 but the sequence was broken after 1956 by a gap of five years

Each school has been organized by Prof R G W Norrish and his colleagues in the Department of Physical Chemistry in Cambridge and this year Dr A B Callear and Dr B P Levitt acted as organizers. The topics were selected to form a composite pattern of ideas related to current researches in the Department Certain lecturers belonged to the Department, others were guest lecturers

The intention has always been to correlate facts and notions within the pattern chosen, to stimulate discussion, to invite, even to provoke, in a friendly way controversy, rather than to instruct by presenting the facts, figures and theory as finished uninspiring blocks of knowledge which could well be posted in cyclostyled form The pattern for the 1961 School was "The Study of Fast Reactions"

Dr T M. Sugden (Cambridge) dealt effectively with the recent developments in the collision and transition state theories, a matter of constant concern with so many chemists in industry and college alike In his energetic, elusively 'off the cuff' manner, he analysed the processes of energy exchange with special reference to fast reactions, unimolecular decomposition of polyatomic molecules and the recombination of atoms It may be well imagined that there was much animated discussion during the half-hour coffee-break.

Dr Sugden described the photometric investigation of flames He referred to experimental techniques for the measurements of H-atom, O-atom and OH— group concentrations in flames Significances of the method included the determination of bond strengths in molecules such as M—Cl, M = Mn, Ni, etc Trends into the future were not neglected. In another talk, Dr Sugden dealt with chemical reactions in the upper atmosphere. Methods of investigation of temperature, density, pressure, and composition gradients and the reactions occurring in different strata are bound to attract quickly growing interest in coming years

Reactions in shock waves were discussed by Dr A G Gaydon (Imperial College of Science and Technology), who outlined experimental methods of observing the emission spectra and temperatures of shock-heated gases Vibrational relaxation times of nitrogen and carbon monoxide, and the measurement of dissociation-rates of oxygen, hydrogen and carbon dioxide were other points of lively interest to the non-expert as well as to the expert listener In another lecture, Dr Gaydon discussed the advantages of low-pressure flames for the study of reaction zones, referring to the identification of reactive intermediates by emission spectra of flames, to the measurement of temperature by study of rotational structure of bands, the reversal of spectral lines and to reactions in hydrocarbon flames

Chain reactions in gases, especially those leading to the ignition of gases, were discussed by Dr P G Ashmore (Cambridge), who has taken part in a number of previous Schools Theories of ignition limits and of induction periods were discussed with reference to non-branching and branching chain reactions

Ultra-violet spectroscopy and fluorescence was dealt with by Dr A B Callear (Cambridge), who opened with a helpful reminder on the use of term symbols and mode of coupling of angular momenta in atoms Information on spin-orbit relaxation can be gained from examination of the quenching of the fluorescence of sodium and mercury Rate of vibrational relaxation of electronically excited I_2, S_2 and NO was referred to

Evidence for ions in flames was presented by Dr D R Jenkins (Cambridge), who outlined experimental methods for the determination of distribution of ions by means of Langmuir probes, microwave and radiowave attenuation and mass spectroscopy

Relaxation in liquids was the title of a lecture by Prof. J Lamb (Imperial College of Science and Technology), who outlined the fundamental theory of acoustic relaxation to what must have been many new attentive ears. The potentialities of the method were amply illustrated in a very convincing way.

Techniques for the measurements of fast reactions in solution were considered by Dr R H Prince (Cambridge), who gave a lucid survey of mechanism and reactivity revealed by a study of fast reactions in solution Substitution reactions in organometallic and co-ordination compounds, redox reactions, rapid isotope exchange were mentioned in particular

Kinetics of fast reactions in solution was the title of the lecture by Dr E A Moelwyn-Hughes (Cambridge), who, in his inimitable and stimulating way, brought freshness to the ever-difficult problems of rate data and transition-state characteristics Interpretation of rate data as a function of temperature, certain limitations of the transition state formulation and the derivation of equations used in the interpretation of relaxation of ionic equilibria were critically commented on

The lecture on fast electrode reactions by Dr G C Barker (Atomic Energy Research Establishment, Harwell) was a very helpful contribution. He considered the theoretical basis of the measurement of rapid redox reactions which occur at the electrolyte/electrode interface and remarked in useful detail on experimental methods involving single-step square-wave pulses and Faradaic rectification.

Not quite in the pattern but timely and very helpful was the talk on high-speed gas chromatography by Dr. J H. Purnell (Cambridge) Factors determining the correlation between the speed of a gas chromatographic analysis and the efficiency of the process were examined in a cursive and conciliatory way, appropriate in dealing with a technique which is still much of an art. If necessary, remarkably short analysis times, less than 1 sec, could be achieved, and are attractive in the study of gas reactions

Work on isothermal reactions of atoms and radicals by flash photolysis was discussed by Prof R G W Norrish, who illustrated the applicability of the techniques of flash photolysis in the detailed scrutiny of very fast reactions in gases In the last lecture of the School, he went into the matter of the chemistry of combustion as revealed by kinetic spectroscopy, showing the applicability of flash photolysis in the investigation of adiabatic processes Anti-knock action of tetraethyl lead was related to the appearance and disappearance of lead oxide and lead during explosion

Time for discussion was allowed at the end of each lecture and indeed there were some astute points put. There was animated and valuable discussion during the long coffee-break in the morning and during the afternoons, which were in the main devoted to visits to the laboratories and to a lecture at 5–6 p m. There were social meetings when easy exchange of ideas was stimulated

An outstanding feature was the well-organized social life of the School, and in this Mrs Norrish and other ladies had doubtless been influential There were some 120 members from industry and colleges and quite a number were from overseas

W. GERRARD

METALLURGY OF BERYLLIUM

BERYLLIUM has only emerged as a metal of industrial significance during the past few years Even now, its use is not securely established and is faced by a number of real difficulties The following assessment of its present position is based on seventy papers presented at an international conference arranged by the Nuclear Energy Committee of the Institute of Metals and held in London during October 16–18

The potential applications of beryllium at present lie in two main fields—aircraft, missiles and spacecraft on the one hand, and atomic reactors on the other. In the first, the advantageous properties of beryllium are its high-strength-to-weight ratio, high elastic modulus, specific heat and thermal conductivity and moderately low coefficient of expansion. With a density of 1 85 and a tensile strength of 50–70,000 lb./sq in, beryllium has a superior strength/weight ratio to all other metals up to about 350° C, although some of the recently developed high-strength stainless steels are not far behind. However, this is not beryllium's greatest attraction. Elastic stiffness and resistance to buckling under compression rather than tensile properties are the limiting factors in many design problems in this aeronautical and spacecraft field, and it is in this that beryllium's combination of low density and high modulus of elasticity ($E = 43 \times 10^6$ lb /sq in) is outstanding Thus, for example, a box beam designed in beryllium to resist a buckling load at up to 450° C is only about half as heavy as one in titanium alloy and about one-third as heavy as one in a high-strength stainless steel In addition, the high specific heat and thermal conductivity and the low coefficient of expansion are important in minimizing the effects of aerodynamic heating With these properties, "beryllium holds one of the keys to a successful flight to the moon" (I Perlmutter, Wright-Patterson Air Force Base)

For atomic reactors, on the other hand, the attraction of beryllium lies in its low neutron adsorption, and at present there is wide investigation of its possible use as the fuel element can in high-temperature, gas-cooled reactors In addition, the Australian Atomic Energy Commission is examining the use of beryllium, instead of graphite, as a moderator in low-power reactors on the grounds that this would permit smaller reactor cores and possibly reduce over-all cost It is also considering fuel elements in which the fuel, for example $(U,Th)Be_{13}$, is dispersed in beryllium

Unfortunately, these attractive properties are accompanied by two immediate disadvantages Beryllium is very costly, billets are about £20 per lb The United Kingdom is not a producer; supplies come from Pechiney and from the United States Secondly, there is a severe health hazard. Inhalation of the metal or its compounds produces acute chemical pneumonitis, or, at lower doses, a chronic lung disease, berylliosis. To keep the beryllium content of the air at safe levels, special workshops are required with air extraction and filtration, and total enclosure of some machines Monitoring of the beryllium-levels and health checks are necessary. However, the applications suggested for beryllium are those that can withstand a fairly high cost and, once a beryllium-handling facility has been established, the health-hazard is not too troublesome. There remains a third disadvantage, that of brittleness, and at present this is the major problem in the metallurgy of beryllium Practically every paper read at the conference in London was to some extent concerned with brittleness

Beryllium is quite ductile at normal hot-working temperatures of 1,000°–1,050° C, so hot-fabrication processes do not present any great difficulty However, below about 200° C there is a fairly sharp transition to a low ductility This not only means that fabrication by cold-work is difficult or impossible, but also, and more important, that in service high stresses, perhaps purely local, that might be relieved by plastic distortion, lead instead to fracture

This transition to brittleness is accompanied by a transition in fracture mode from so-called 'ductile fracture' to a process of separation along definite crystallographic planes, the cleavage planes Thus, the brittleness of beryllium appears to correspond to the classical brittle fracture of mild steel that has caused so much concern and investigation since the 'Liberty' ship failures during the War.

Investigations of mild steel have brought out the importance of two stages in the development of cleavage First, the initiation of a crack by some process such as the coalescence of the dislocations in a slip plane through compression against a grain boundary, or by the coalescence of dislocations through interaction with others on an intersecting slip plane or by twinning. Secondly, the breakout of this dislocation crack across a cleavage plane in the manner of a Griffith crack propagated by the externally applied stress. It is considered that the transition to cleavage occurs when the criterion for propagation is fulfilled.

This criterion involves three factors. First, the size of the dislocation crack depends on the number of dislocations forming the crack and therefore on the grain size. The smaller this size, the smaller the dislocation crack and the more difficult is cleavage. Secondly, the effective surface energy associated with crack growth represents partly the true surface energy and partly the plastic work arising from the operation of dislocation sources in the region of stress-concentration surrounding the growing crack. The easier dislocation movement, the greater the amount of plastic work, the shorter the initial dislocation crack and the more difficult its propagation. Thus, a high effective surface energy arising from easy dislocation movement favours ductility. Thirdly, the applied stress available for crack propagation is determined by the flow-stress at the instant of fracture. The reason why low temperature favours cleavage is that the flow-stress is then higher and the effective surface energy lower because dislocation movement is more difficult. The papers at the conference showed that the conclusions from the work on steel have now been applied at least qualitatively to beryllium.

The attempt to overcome brittleness by a fine-grain size at present divides beryllium work into two categories, powder and ingot. Present industrial producers of beryllium components start from powder, and the oxide derived from the coat on the particles inhibits the growth of grains during sintering and annealing. In this way, the grain size can be kept to about 50μ. Others, notably the group headed by G. C. Ellis, A. J. Martin and A. Moore at Aldermaston, argue that powder methods yield an intrinsically poorer material with reduced high-temperature ductility and resistance to corrosion and they have put their effort into an attempt to obtain fine grain size in wrought material derived from an ingot. The difficulty lies in the easy growth of grains of this material on re-crystallization. So far, the finest grains, 40μ, have been obtained by 'flash annealing', that is, exposure for a few seconds to a temperature of 900°–1,000° C., well above the normal re-crystallization temperature, so that many nuclei are active. Such treatment is only possible with thin sections. However, even by normal re-crystallization it has now proved possible to keep the grain size at 60–80μ in material that has been worked as far as sheet. A full assessment of the mechanical properties of the products derived from ingot has not yet been obtained.

Another possible approach to reduced brittleness is through purification. This may be beneficial because of a reduction in the flow-stress available for crack propagation and an increase in the effective surface energy through easier dislocation movement. The beryllium obtainable commercially is not particularly pure; it contains a few hundred p.p.m. of various metals, mainly iron, aluminium, silicon and of non-metals, oxygen, carbon and nitrogen. A number of laboratory-scale attempts at purification by distillation and zone-refining were reported at the conference. A. R. Kaufmann *et al.*, for example, reduced the contents of the metallic impurities to the 1–5 p.p.m. range. There appeared to be a reduction in flow-stress and increase in ductility. However, the purification is difficult and consolidation of the product to a uniform, fine-grained polycrystalline state has not yet been achieved. Thus, at the moment there does not seem to be an immediate easy path to ductility through purification. Perhaps in any event it may be unsafe to expect too much from this approach in view of the thin-film electron microscope work of H. G. F. Wilsdorf and F. Wilhelm that showed strict confinement of the dislocations to crystallographic directions, suggesting that the intrinsic lattice resistance to dislocation motion (the Peierls–Nabarro stress) is already high, independent of impurity hardening.

Although the brittleness of beryllium seems to be essentially similar in principle to that of mild steel, it emphasizes a point that is not important with steel. Beryllium is close-packed hexagonal and suffers from a dearth of slip systems. At room temperature, slip on the single basal plane is by far the easiest; (10$\bar{1}$0) slip is 4–5 times as difficult. Neither of these contributes any component in the *c* direction. Thus, any one grain in a polycrystal experiences difficulty in deforming crystallographically in conformity with the matrix. Consequently, as brought out by J. Sawkill and by B. Allen and A. Moore, there is bending and kinking of the slip planes and this supplies, at least in some cases, the fracture nucleation mechanism. This is the one suggested a number of years ago by Orowan and by Stroh in which the discontinuity in displacement when slip crosses a bend plane produces cracking down the slip plane. A second effect of the dearth of slip planes is that wrought products such as sheet or extruded bar, which have received highly directional working, are strongly textured with the basal planes parallel to the direction of working. This has a marked effect on ductility. For example, cross-rolled sheet can show 30–40 per cent elongation in the plane of the sheet even at room temperature, but there is practically no ductility in the third dimension and such sheet readily fractures in forming processes. Thus, the control of texture is an important aspect of the brittleness problem. Some success in achieving a more random orientation in sheet has been obtained by the press-forging of beryllium encased in a steel container and afterwards rolling the flat produced (R. L. Craik and D. A. Barrow).

Another interesting approach to the problem of brittleness has been attempted at the Armour Research Foundation, Chicago. This is to use 'liquid-phase sintering' as in the common tungsten carbide–cobalt tools. The beryllium is dispersed in a continuous plastic matrix of silver–aluminium–germanium that is liquid at the sintering temperature and spreads around the beryllium particles. The product is quite ductile at room temperature, but Young's modulus is reduced to 22 × 10^6 lb./sq. in.

In addition to low-temperature cleavage, beryllium also shows a dip in ductility around 600° C. due to the occurrence of intercrystalline fracture. This is not so important as cleavage, and in any event it has now been shown at Aldermaston and other centres that annealing about 800° C. followed by slow cooling removes the brittleness produced at 600° C. The heat-treatment appears to over-age impurity precipitates and restores a proper balance between the ease of deformation within a grain and at a grain boundary.

Apart from this brittleness problem, two other problems appear when beryllium is used in a reactor In the carbon dioxide–water coolant mixtures, breakaway, that is, accelerating, oxidation can occur The papers at the conference did not add a great deal on this topic, although the Australian Atomic Energy Commission reported an inhibition of breakaway by oxidation of the beryllium powder at 800° C in oxygen prior to sintering Irradiation damage also occurs in a reactor The normal displacement of atoms by neutrons that is responsible for the damage in steel and copper has little effect in beryllium, and damage only appears at doses above about 10^{19} neutrons cm^{-2} when it is due to the helium generated by neutron reaction with beryllium. R S Barnes and others have shown that when present as small bubbles the helium leads to hardening and loss of ductility Larger bubbles accumulate at the grain boundaries and these promote fracture in creep They can also give grain boundary permeability, which is, of course, highly undesirable in a fuel can.

It is perhaps clear that there is no lack of beryllium problems However, considering the advances that have been achieved in the quite short period of really active interest, the future is not without hope

N J Petch

CHANGES OF CLIMATE

As was entirely appropriate for a symposium sponsored by the Unesco major project on scientific research in arid lands, an effort was made on the occasion of the recent meeting in Rome (October 2–7), arranged by Unesco and the World Meteorological Organization, to restrict the interest to climatic variations which have occurred since the latest glaciation, with particular attention to the period of the meteorological record The reason behind this decision was no doubt the wish to talk about something which might conceivably have relevance to the nature and trends of the arid lands of to-day—relevance on the scale of economic planning, say, for a hundred years At the same time this emphasis on what, after all, is micrometric on the geological scale served the purpose of almost eliminating the endless speculations on the causes of ancient climatic change, fascinating though they undoubtedly are Most geological, geophysical and astronomical happenings which are potential climatic controls, such as mountain building and denudation, drifting of the continents, changes in solar constant and a score of other possible processes, are eliminated when the time-scale is a mere 100 or 1,000 years

It should not be supposed, of course, that examination of such recent changes will throw much light on events on the longer time-scale The odds are that variability on each different scale of time presents an essentially different problem with an essentially different theoretical explanation But it is good to keep a symposium within bounds, and the short-period problem is the most tangible, the most suitable for the attention of the theoretical meteorologist, and the most important economically

More than forty papers were presented, divided into four sessions each of one day and treating respectively the period of the meteorological record (chairman, R G Veryard), the late geological and early historical period (chairman, K W Butzer), theories of recent climatic change (chairman, R C Sutcliffe) and significance of changes of climate (chairman, R O Whyte) A summing-up by C C Wallén on the last day was particularly well received

The first session was much helped by the chairman's fine survey of the literature, and the papers presented did not seem to take us very much further with the facts, although substantiating the main features now becoming generally familiar, in particular the warming of extratropical latitudes over the century ending about 1940 J M Mitchell's world-wide view of the evidence will be found particularly useful, most of the other contributions being local studies An effort was made to give attention to the statistical treatment of time series on the basis of a paper by R Sneyers, it being well known that a good proportion of the published literature on climatic change suffers from uncritical and undefined statistical standards The general technique of power spectrum analysis received mention but was not pursued. It was, however, evident that those in the field were very conscious of the problems

The second session fell mostly to the non-meteorological disciplines, geology, geomorphology, archæology and dendrochronology being represented Apart from the chairman's valuable introduction, the most persuasive paper was by R W Fairbridge on mean sea-level during the past 20,000 years It is remarkable and a little puzzling that such clear evidence should be obtainable from data which one would have thought to be confusing

Under the heading of "Theories of Changes of Climate", the papers were entirely by meteorologists, some of whom were new to the field This was a good feature, as a weakness of the subject is its failure to attract expert meteorologists aware of the complex behaviour of the atmosphere The chairman directed attention to the possibilities of variability through feed-back contained within the Earth–ocean system without necessarily recruiting the aid of extraneous factors, radiation, dust, carbon dioxide and the like, and other papers developed the theme both J Bjerknes and J Namias were on this side On the other hand, the most substantial paper was perhaps that by E B Kraus, giving the results of a heavy calculation nobly carried out on the effects of a radiation change on the general circulation The paper was more an illustration of the mathematical approach than a practical solution, but was important as being one of very few attempts that have yet been published

In the final session, L P Smith's paper on "The Significance of Climate Variations in Britain" was very much to the point, and anyone who previously had doubted whether the subject had any economic significance must have given way at this point

The broad impression created by the meeting is that, for the period of the instrumental record, the subject, which has got to a certain descriptive stage by pure empiricism is in danger of languishing for want of ideas Perhaps the symposium marks a turning point, and the attention which climatologists are giving to statistical techniques and which theoretical meteorologists are giving to the general circulation are pointers to the future For the longer record the interest must continue to rest on techniques of exploration and interpretation of evidence, and here there is no slackening of interest, for most of the Earth's surface is almost unexplored

The facilities provided by the Food and Agriculture Organization and the Italian authorities were admirable After several experiences of large meetings, one looks forward to those where headphones and simultaneous translations are provided To be able always to hear is a wonderful asset, even if the language presents no difficulty It is understood that the complete papers are to be published by Unesco and this will be worth while A good proportion of the material had been heard or read before, but there would be no sense in insisting on complete novelty for an interdisciplinary meeting where the object is to exchange ideas, not to be mutually incomprehensible R. C SUTCLIFFE

CHEMICAL WARFARE ON PLANT PESTS AND DISEASES

THE first British Insecticide and Fungicide Conference organized by the Association of British Manufacturers of Agricultural Chemicals was held at Brighton during November 7–9 under the presidency of Dr H. G Sanders, chief scientific adviser (agriculture) to the Ministry of Agriculture, Fisheries and Food It was truly an international gathering, for of the 470 delegates attending from official research stations, Government departments and manufacturing firms, nearly one-quarter came from fourteen countries overseas

The conference consisted of ten successive sessions devoted to important present-day problems in plant pathology and their possible solution by chemical means During the first two days each session began with one or more introductory papers by authorities on the subject under discussion, and these were followed by brief reports of relevant research in progress at various centres The morning of the third day was given over to research reports on new insecticides, fungicides and acaricides, and on novel formulations as well as new methods of application In the final session attention was appropriately directed to the problems involved in translating research into practice and in introducing insecticides and fungicides into agricultural practice both in Britain and overseas. In all, more than fifty papers or reports were presented from the research departments of official agricultural institutes and industrial firms, including a number from Holland, Germany and Italy

On introducing the president, Mr H C Mellor, the chairman of the Manufacturers' Association and of the conference, said that the industry is at present under fire, though much of the criticism levelled at it is uninformed. The members of his Association are as anxious as anyone to find safer chemicals, and are most careful to honour the agreements made on their behalf with the Ministry of Agriculture, as well as to satisfy the requirements of the Notification Scheme under which all new chemicals are notified to the Ministry for clearance before marketing.

In his opening address Dr Sanders referred to the increasingly important part played by chemicals in farming Unfortunately, some of them are toxic to mammals and wild-life, and this has led to alarm in the public mind. He believed that much of this concern is due to ignorance of the many hurdles new chemicals have to surmount before they are cleared for marketing by Government departments of health and agriculture. The present conference was mainly a scientific one, but the delegates included many whose functions were to translate science into practice, and it should therefore provide a good opportunity for getting things, including toxic hazards, into proper perspective Dr Sanders also commented on the close co-operation now existing between industry, research workers and Government officials, and expressed appreciation to the manufacturers for disclosing to him highly confidential information which has fully satisfied him that during the past five years there has been a very substantial reduction in the acreage sprayed with the more toxic chemicals, even though the total acreage sprayed has doubled during that period

The first session was concerned with soil pests and diseases of agricultural crops, and included a review by Dr Hubert Martin (until recently director of the Pesticide Research Institute, London, Ontario) of a symposium called privately, in February 1961, to discuss the effects of chlorinated hydrocarbons and other chemicals on the ecology of the soil fauna and flora. Dealing with the eelworm problem, Mr F. G W. Jones (Rothamsted Experimental Station) said that a first-class soil nematicide is still awaited Only three—bromomethane, dibromoethane and dichloropropene—have stood the test of time, though some dithiocarbamates show promise He doubted whether soil treatments were the right approach Apart from difficulties of mixing and dispersion, at least 1,000 tons of soil per acre has to be treated Eradication of eelworms is impossible and unnecessary, and he looked forward to a systemic nematicide moving from foliage to roots, suppressing the cyst-hatching factor, and perhaps passing out into the soil to exert a repellant effect Dr Mary Noble (Department of Agriculture for Scotland) considered that seed is not the best launching vehicle for a chemical intended to control soil-borne fungus diseases, while Mr M J Way (Imperial College of Science and Technology) did much to restore a balance in the campaign against insecticidal seed dressings by insisting that the minute quantities used are effective and well placed and do not cause off-flavours in crops, injure beneficial insects or encourage resistance in the pests attacked The problem of deaths of birds after eating dressed seed should be met by more circumspect use of seed dressings and not by restrictive legislation, a view that received much support

The use of systemic insecticides for virus control in potatoes and sugar beet was considered at a second session Hitherto, potato virus diseases have been controlled through crop certification schemes, a method that has become traditional Dr L Broadbent (Glasshouse Crops Research Institute) and others have shown that similar ends may be achieved by using systemic insecticides, though few farmers have yet attempted to take advantage of this With sugar beet there has been no tradition of certification, and farmers responded quickly to a spray-warning scheme begun in 1957 to deal with virus yellows. Dr R Hull

(Rothamsted Experimental Station) disclosed that 95 per cent of the crop was sprayed in 1959 and suggested that spraying had converted 1961 from a 'severe' to a 'marginal' yellows year. Other reports about this disease dealt with the value of menazon and dimethoate as sprays, and with the application of granular formulations of phorate and thiodemeton to the soil or foliage.

In a session on forecasting pest and disease outbreaks great interest was shown in the pioneering work of Mr. G. H. Brenchley (Ministry of Agriculture, Fisheries and Food) on the use of aerial infra-red photography to detect the primary foci of potato blight and to study patterns of its early natural spread. Dr. E. Evans (Fisons Pest Control, Ltd.) reported on difficulties in evaluating materials for blight control and suggested that the performance of copper products and dithiocarbamates differs according to the nature of the epidemic.

Three sittings were devoted respectively to apple mildew, fruit tree pests and the control of *Botrytis* diseases. It was obvious some years ago that apple mildew would again become the problem it now is, and older delegates listening to the seven speakers no doubt recalled similar discussions held thirty years and more ago. In the meantime, mildew has been adequately controlled under a sulphur programme for apples, but it became resurgent as captan and organo-mercury sprays replaced sulphur for scab control and as the renewal system of pruning evolved. Much of the discussion revolved around the difficulty of dealing with continuous conidial inoculum and preventing bud infection, which can evidently be fully accomplished only by weekly or even more frequent application of dinocap or other appropriate fungicides. On fruit tree pests, Dr. G. H. Dicker (East Malling Research Station) and others emphasized the importance of a knowledge of the biology of red spider mite, codling moth and tortrix in the effective timing of fruit tree sprays during the flowering period. No specific methods emerged for dealing with species of *Botrytis*, notably *B. cinerea*, which causes so much destruction to many crops, and the prospect was not made brighter by the ease with which Dr. R. K. S. Wood (Imperial College of Science and Technology) has been able to produce *in vitro* strains of *Botrytis* highly resistant to the fairly effective chlorinated nitrobenzenes. To protect strawberry and raspberry fruits, Dr. W. R. Jarvis (Scottish Horticultural Research Institute) felt that the only real solution would be in a systemic fungicide applied before the flowering stage.

In a session on soil sterilants, Dr. W. Madel (CELA, G.m.b.H.) described experience in Germany with metham-sodium against harmful soil organisms, and Dr. L. P. Flipse (Ligtermoet and Zoon N.V.) showed how valuable this fumigant and herbicide is to Dutch horticulture, particularly in controlling soil-borne fungus diseases of bulbs, carnations and tomatoes. But though practical results continue to accumulate, little is yet known about the mode of action of soil sterilants.

Comment on much of the work discussed at the conference must perforce await publication in the *Proceedings*. Other items included progress reports on a number of new aphicides and acaricides, on the potentialities of organic tin compounds and polybutene formulations, and on special formulations for low-volume spraying. In all the developments outlined it was not difficult to perceive a general desire to find effective chemicals of low mammalian toxicity (if possible, systemic in action) having prolonged effects, and preferably applied in a form that reduces hazards to man, animal and wild-life to the absolute minimum. It was also encouraging to learn from the Laboratory of the Government Chemist of the progress being made in solving the problems which face the analyst examining foodstuffs for pesticide residues.

Speaking as an adviser concerned with the translation of research into practice in Great Britain, Dr. M. Cohen (Ministry of Agriculture, Fisheries and Food) emphasized the difficulty that faces everyone confronted with the vast number of new materials produced during the past fifteen years, and referred to the apparently insoluble problem posed by the confusing combination of endless chemical, common and proprietary names for insecticides and fungicides on the market. Finally, and in an absorbing talk that was delightfully refreshing at the end of three days of "sharing the benefits of new knowledge", Mr. G. Ordish (Department of Technical Co-operation, Tropical Products Institute) dealt with the introduction of insecticides and fungicides into agricultural practice overseas. Little had been said at the conference about economics, though in his view this was perhaps the most important factor of all. In his closing remarks the president mentioned some of the highlights of the discussions and expressed the hope that the conference would be but the first of a biennial series.

W. C. MOORE

THE SOVIET HYDROMETEOROLOGICAL SERVICE

A DECREE, signed by Lenin in June 1921, was the virtual foundation of the modern Soviet Hydrometeorological Service. In a recent issue of *Meteorologiya i Gidrologiya* (No. 6, 1961) the present chief director, A. A. Zolotuhin, traces the history of the Service over the ensuing forty years.

There had been a small meteorological service in Imperial Russia, but owing to foreign and civil war it was in a state of complete decline by 1921. Progress, however, was slow and inadequate during the 'twenties, and by 1929 there were twelve meteorological services in the U.S.S.R. which arose from departments forming their own services, because the main service could not meet their demands. The U.S.S.R. has for long undoubtedly regarded a soundly administered and technically efficient hydrometeorological service as essential to its national economy and to its defence. So in the early 'thirties a unified service was recreated and thereafter progress was rapid. For example, it is claimed that the world's first radiosonde was released in January 1930, and that a network of sounding stations was immediately commenced. By 1941, at the outbreak of War, forty radiosonde stations were in being. Advances in other directions were also substantial.

This was, of course, a period of great expansion for many national meteorological services owing to the development of the science itself and to the increasing demands of military and civil aviation. The U.S.S.R. was clearly no exception.

Since the War, the Hydrometeorological Service has been greatly expanded further. Separate specialist branches have been set up for particular branches of the economy. Emphasis is placed on activities of special importance to the economy of the Union, for example, civil aviation, the hydrology of rivers and watersheds, agricultural meteorology. Nevertheless, the confession is made that the rate of improvement in the accuracy of the forecasts has not really been adequate to satisfy its needs.

Dr. Zolotuhin, however, makes the point that it is the level of scientific research which determines the quality of the service, and there are now a considerable number of meteorological, hydrological and oceanographical research institutions in the U.S.S.R., and the Hydrometeorological Service also has a number of specialist educational establishments for training its staff and potential staff.

From the complete decline of 1921, the past forty years have clearly been years of major expansion, an expansion which indicates the importance attached by the U.S.S.R. to the sciences of meteorology and hydrology. Indeed, with its 29 local and republic directorates and its 20,000 observing points, embracing one-sixth of the Earth's land surface, the Soviet Hydrometeorological Service must now surely be about the largest in the world.

R. F. Zobel

POPULATION TRENDS IN THE U.S.S.R. AND THE UNITED STATES

In terms of population, the Soviet Union and the United States are the third and fourth largest nations in the world. Their combined population of approximately 402 million is close to the population of India, but only slightly more than half that of China. The two nations are alike in many demographic respects. A recent survey entitled "Population Trends in the U.S.S.R.", issued by the Population Reference Bureau, Inc., compares the various population trends in the U.S.S.R. with those in the United States*.

In 1959, the Soviet census counted 209 million people. The birth-rate was 25, the death-rate 7.6 per thousand. In 1959, the United States population was 176 million, and the birth-rate 24.1 and the death-rate 9.4 per thousand. The age-specific death-rates in the U.S.S.R. were higher than those of the United States below age forty, and lower above that age. In both countries, the populations are made up of many ethnic groups, illiteracy is virtually non-existent, and great numbers of people are receiving more education. Only 1.5 per cent of Russians aged 9–49 were reported by the census to be illiterate, but there are urban–rural differences at every school-level. The educational pattern is very different in the two countries.

Migratory trends are similar. With the growth of industrialization, the people of both nations have been on the move, mainly from the agricultural areas to the urban areas. The force which motivates this migration is mainly the desire for advancement.

In 1926, 82 per cent of the population in the U.S.S.R. lived in rural areas and 18 per cent lived in cities. By 1959 the proportion had changed to 52 per cent rural and 48 per cent urban. Russians have been moving east in response to a Government-planned effort to colonize the 'new lands' where industrial complexes have been established.

In contrast, Americans have been going west and mainly into urban areas in a wholly unplanned, casual manner because they desire to live and work there. In 1900, 60 per cent of the population of the United States was rural and 40 per cent was urban. By 1960, the proportion had changed, 37 per cent being rural and 63 per cent urban.

There is a vast difference in the kinds of occupation in the two countries. In 1959, almost 39 per cent of the total labour force in the U.S.S.R. was engaged in agriculture. In 1960 less than 9 per cent of the labour force in the United States was so employed.

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* Population Reference Bureau, Inc. *Population Bulletin*, Vol. 17, No. 6 (October 1961). Population Trends in the U.S.S.R. Pp. v+76. (Washington, D.C.: Government Printing Office, 1961.) 45 cents.

NEW SYNTHETIC DETERGENTS

Although the biologically 'soft' detergent which has been developed by the manufacturers, and has been widely distributed in the south of England, has eased the difficulties which have been caused by the discharge of detergent residues to surface waters, the new material does not afford a complete solution to the problems arising from the widespread use of synthetic detergents, and further improvement of this material or the development of new types of surface-active materials is necessary. This is the gist of the Fourth Progress Report of the Standing Technical Committee on Synthetic Detergents*. The new 'soft' detergent, originally supplied to the Luton area, has now been distributed to a large part of southern England, and increased removal of detergents at sewage treatment plants has been observed in many districts. No similar change has taken place in the north, where the type of detergent on sale has not been altered.

One of the difficulties encountered in experimental work on the breakdown of synthetic detergents is the lack of analytical techniques which can be used to study the breakdown products. The analytical methods used are specific for molecules of a given range of size and structure, and it is possible that during sewage treatment some molecules may be only partly degraded or otherwise changed in structure, and may then be undetected in the effluent. It is therefore emphasized in the report that figures given for percentage removal do not necessarily imply that the material has been fully degraded. An extensive programme to be undertaken by various members of the Committee will include investigations of the biological breakdown of single substances, in order to throw light on this part of the problem.

Nora H. Johnson

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* Ministry of Housing and Local Government. *Fourth Progress Report of the Standing Technical Committee on Synthetic Detergents*. Pp. 19. (London: H.M.S.O., 1961.) 1s. 6d. net.

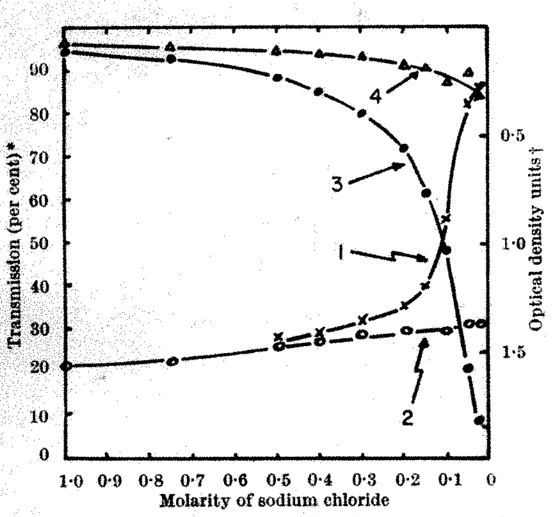

Fig. 1. Effect of concentration of sodium chloride on the turbidity of suspensions of a marine bacterium and on the absorption at 259 mμ of the supernates obtained from these suspensions in the presence and absence of Mg^{2+}. Curve 1, percentage transmission of bacterial suspensions in the absence of magnesium chloride; curve 2, percentage transmission in the presence of 0·05 *M* magnesium chloride; curve 3, optical density units of supernates from suspensions in the absence of magnesium chloride; curve 4, optical density units of supernates from suspensions in the presence of magnesium chloride

* 655 mμ filter, 1 *M* sodium chloride = 100.

† Calculated as optical density per ml. of supernate from the absorption of a suitable dilution at 259 mμ.

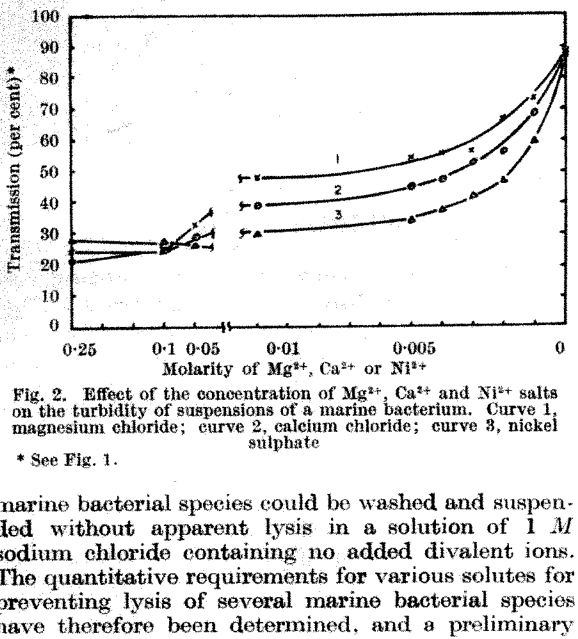

Fig. 2. Effect of the concentration of Mg^{2+}, Ca^{2+} and Ni^{2+} salts on the turbidity of suspensions of a marine bacterium. Curve 1, magnesium chloride; curve 2, calcium chloride; curve 3, nickel sulphate

* See Fig. 1.

marine bacterial species could be washed and suspended without apparent lysis in a solution of 1 *M* sodium chloride containing no added divalent ions. The quantitative requirements for various solutes for preventing lysis of several marine bacterial species have therefore been determined, and a preliminary report is presented here.

Organisms were grown in a nutrient broth–yeast extract medium containing salts previously established to be required for growth. Cells were collected by centrifugation at 4° C., washed twice in 1 *M* sodium chloride and finally suspended in a volume of the latter such that a 1 : 40 dilution gave about 20 per cent light transmission in a Coleman spectrophotometer equipped with a 655-mμ filter. A 0·25-ml. volume of cell suspension was then added to each of a series of tubes containing solutions of sodium chloride or other solutes to give a final volume of 10 ml.

The results obtained with organism *B*-16, a Gram-negative, polarly flagellated rod, tentatively identified as a species of *Pseudomonas* or *Spirillum*, are given in Fig. 1. Curve 1 shows that there is a small decrease in turbidity of the cell suspension (as indicated by the change in percentage transmission) between 1·0 *M* and 0·2 *M* sodium chloride and a very marked drop below 0·15 *M* sodium chloride. That the drop in turbidity below 0·15 *M* is due to cell lysis is indicated by the fact that turbidity cannot be restored by raising the concentration of sodium chloride. The change in turbidity is thus not merely an optical effect[6]. Furthermore no whole cells can be detected below the 0·15 *M* sodium chloride level by phase contrast microscopy. The same concentrations of lithium chloride and sucrose as sodium chloride and two to three times as much potassium chloride and glucose were also found to be capable of preventing lysis of these cells. The presence of 0·05 *M* magnesium chloride in the suspensions (Fig. 1, curve 2) prevented cell lysis at all concentrations of sodium chloride tested. When the suspensions at the various salt concentrations were centrifuged clear and tested for absorptive capacity at 259 mμ it was found (Fig. 1, curve 3) that the marked decrease in turbidity of the suspensions corresponded to a sharp increase in optical density of the supernatant solutions, indicating the release of nucleic acid material at low salt concentrations. The presence of 0·05 *M* Mg^{2+} in the suspending solutions largely prevented this release (Fig. 1, curve 4).

When the quantitative requirements for divalent cations for preventing cell lysis were examined it was found (Fig. 2) that as little as 0·001 *M* of such ions has a measurable capacity to prevent lysis while above this concentration the turbidity of the suspension was proportional to the concentration of ion added up to about 0·05 *M*. As Fig. 2 shows, Ca^{2+} and Ni^{2+} were more effective than Mg^{2+} in preventing cell lysis, while Mn^{2+} and Co^{2+} activities (not shown) lay between those of the latter. In general the order of activity of the divalent cations in preventing cell lysis appeared to be similar to that of their capacity to form chelate complexes.

Four other marine bacterial species have also been examined: another *Pseudomonas*, a *Flavobacterium* and two strains of *Photobacteria* (kindly made available by Dr. W. D. McElroy). Considerable species variation has been observed both in the capacity of cells to lyse at low sodium chloride concentration and in the ability of divalent cations to prevent it. It is possible to conclude in the case of all species examined, however, that Mg^{2+} and Ca^{2+}, the two divalent ions most prevalent in sea-water at their concentrations in the sea (0·05 *M* and 0·01 *M* respectively), would in all cases reduce and in some cases eliminate the requirement for other solutes such as Na^+ salts for preventing cell lysis. Complete details of these experiments will be published shortly.

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with the largest specimens found by Fauvel from Cam Ranh.

I am indebted to E. and C. Berkeley for directing my attention to Fauvel's paper.

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Oil Pollution of Marine Organisms

Although much publicity has been given to the harmful effects of oil on bird life, little evidence is available to show how marine organisms are influenced by oil pollution. Recent accidents involving the release of crude oil in Milford Haven afforded an opportunity to commence an assessment of the effects of this substance on the commonest intertidal animals and plants of a rocky coast.

It was found that even when the surface of the water is covered by a thick layer of oil, only a very thin film of oil is deposited on littoral animals and plants as the tide recedes, and much of this is washed off as the tide returns. Oil is, however, deposited at high-tide mark in the form of a band the width of which varies according to the degree of wave action on the shore at the time, and successive high tides may leave a series of bands of oil at slightly different levels. On very sheltered shores, each band may be only a few inches broad but on exposed shores the oil may be spread over several vertical feet of rock face. Clearly, therefore, the intertidal organisms most likely to be affected by oil pollution are those which occur between the lowest high water level of neap tides and the extreme high water level of springs, and include the acorn barnacles, *Elminius modestus*, *Balanus balanoides* and *Chthamalus stellatus*, the limpet, *Patella vulgata*, and on sheltered shores the algae *Fucus spiralis* and *Pelvetia canaliculata*.

Once deposition of crude oil has occurred, the lighter fractions gradually evaporate to leave a tarry residue which seems to be resistant to leaching by rain and moderate wave action. Erosion by water-borne particles (corrasion) probably occurs, but this is likely to be a very slow process except on shores subjected to intense wave action.

Examination of contaminated shores shortly after oil had been deposited showed that those acorn barnacles and limpets the shells of which had been covered by oil appeared to be unharmed and no barnacles were found in which the opercular plates had been glued together by the tarry residues. Examination a fortnight after deposition showed that the amount of oil remaining on surfaces in the immediate vicinity of limpets had been much reduced and that residual patches of oil situated a short distance from these animals exhibited the characteristic imprints of limpet radulas. It appeared likely that these molluscs were continuing to browse over the nearby barnacles and areas of bare rock in order to obtain food, but that in doing so they were inadvertently removing the layer of deposited oil. Since there was no decrease in the limpet population in the contaminated area, it seemed probable that the oil was non-toxic and that it was being passed through the alimentary canal and voided with the other fæcal material.

Some confirmation that limpets are at least partially responsible for removing oil from contaminated shores was obtained during an examination of the latter six months after the accident. All traces of oil had been removed from the areas which support a population of limpets, but on one shore where oil had been deposited to a level above that of the uppermost limpets the tarry residue remained. Examination of barnacles which had been thickly smeared with oil showed that not only had all of it been removed but also that they had suffered no unusual mortality. Plants of *Pelvetia* and *F. spiralis* were also apparently unaffected and had continued to grow at a normal rate.

During the past two or three years, industrial emulsifiers have been increasingly used to clean up oil-polluted shores and they have been found to give effective results on sand and shingle beaches where the amenity value is of importance. Emulsifiers have also been used to clean up contaminated rocky shores, but it has been noticed that their use is followed by a severe mortality in intertidal organisms. Subsequent observations, as yet unpublished, have shown that the type of emulsifier most commonly used destroys all the limpets on a shore and 80–95 per cent of the acorn barnacles, and is extremely toxic to all other forms of intertidal life. It is suggested that in view of the fact that oil films are fairly quickly removed from rocky shores by natural processes, it is both wasteful and unnecessary to use emulsifiers on such shores.

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MICROBIOLOGY

Solute Requirements for preventing Lysis of Some Marine Bacteria

Previous work has revealed a specific requirement of a number of marine bacteria for sodium for growth[1,2] and oxidative metabolism[3,4]. For optimum rate and extent of growth, 0·2–0·3 *M* Na^+ was required though limited growth could occur after a sufficiently long incubation period at as low as 0·03 *M* Na^+ (ref. 2). Since sucrose and K^+ exerted only a slight sparing action on the Na^+ requirement of the organisms examined it was concluded that the maintenance of an appropriate osmotic pressure in the medium was by no means the sole, or even the primary, function of Na^+ in the growth of the cells. Nevertheless, cells of many of the species lysed quickly when washed in distilled water, and in the case of one of the species examined in more detail, lysis occurred also when cells were suspended in solutions containing sodium chloride and potassium chloride at concentrations of these salts that were optimum for growth. Lysis, however, did not occur when 0·05 Mg^{2+} or Mn^{2+} was included in the suspending medium[3].

Since Mg^{2+}, and in some cases Ca^{2+} as well, are required for growth by all the species examined[5], the presence of these ions in the growth media used could possibly explain why no apparent osmotic requirements could be demonstrated during growth of the cells. Support for this conclusion was obtained when it was observed that cells of a number of mixed culture and that it was a factor in promoting both growth and nitrogen fixation.

We have also found that treatment with sodium dodecyl sulphate[3] to extract extracellular deoxyribonucleic acid (DNA) resulted in the appearance of DNA in the slime substances. Further experiments to clarify the nature of these phenomena are being performed.

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Seasonal Variation in Alginic Acid, Mannitol, Laminarin and Fucoidin in the Brown Alga, *Ecklonia radiata*

The brown alga *Ecklonia radiata* (Ag.) J. Ag. which occurs abundantly in the sub-littoral zone of the southern coasts of Australia, has been found to contain alginic acid, mannitol, laminarin and fucoidin, in conformity with observations on other species of the Laminariales from South Africa and the northern hemisphere. The above constituents were isolated from the seaweed and characterized by physical constants, chromatography and infra-red spectra[1]. An examination has now been made of the seasonal variation in the content of these carbohydrates, which is of interest in connexion with their biochemical function and possible utilization.

Samples were collected at Point Lonsdale, Victoria, each month during December, 1959–January, 1961. Each sample consisted of about twelve plants, which were dissected into laminæ, midrib and stipe. The 'midrib' was taken as that portion of the frond, comprising the elongation of the stipe, from which the 'laminæ' had been removed. The separate portions of the plant were milled through breaker plates in a Bauer defibrator and dried by azeotropic distillation with benzene. They were then ground in a Wiley mill to pass through a 100 mesh/in. sieve, and analysed according to the methods of Cameron, Ross and Percival[2]. In the case of the alginic acid determination, however, much more extensive acid washing was required than suggested by these workers in order to remove calcium ions from the alginate.

The maximum and minimum contents of the various components are shown in Table 1, with their time of occurrence. Marked variations were observed in the contents of laminarin, mannitol and alginic acid, the differences between the maximum and minimum contents decreasing in that order; however, the variations are less than those observed by Black and Dewar[3] for British *Laminaria* species. The fucoidin content is low and less variable relative to the total dry matter. It will be observed also that the contents of laminarin, and to a lesser extent mannitol, decrease from lamina to stipe, whereas the alginic acid content increases.

Table 1. Seasonal Variation in Composition of *E. radiata*

	Maximum			Minimum		
	Lamina	Mid-rib	Stipe	Lamina	Midrib	Stipe
Alginic acid (per cent)*	24·5 April	28·4 April	30·2 April	17·0 September	19·0 September	19·8 September
Mannitol (per cent)*	16·6 February	16·7 April	13·6 June	8·5 July	7·4 August	6·7 October
Laminarin (per cent)*	9·8 May	7·0 May	1·1 May	1·0 August	0·0 August	0·0 August
Fucoidin (per cent)*	1·3 July	1·4 June	1·1 April	0·4 —	0·8 —	0·7 —
Dry matter (per cent)†	22 January	24 June	41 June	17 August	15 August	13 September

* Based on total dry material.
† Based on original wet weight.

Laminarin is regarded by some investigators as a reserve carbohydrate. It is certainly stored in the frond, its content increasing to a maximum in summer and autumn, and decreasing rapidly to zero in mid-winter. The rapid decrease may be associated partly with the shedding of the lamina as well as utilization of reserve carbohydrate. The laminarin content may vary more widely in British species because of a slightly greater range in sea temperatures (45°–57° F. as against 54°–65° F.) and the considerably lower minimum temperature. In the case of laminarin and of alginic acid the seasonal fluctuations are similar for all parts of the plant. However, for mannitol the maximum and minimum contents are reached earlier in the laminæ than in the stipes, which seems to be more in accordance with the behaviour of a reserve carbohydrate than the variations observed for laminarin. The alginic acid content of *E. radiata* appears to be at a minimum in spring, at which time a maximum is reached in the British species.

From the point of view of possible utilization, *E. radiata* should be a useful source of alginic acid and mannitol and may yield appreciable quantities of laminarin, provided that due attention is paid to the seasonal variation.

We wish to thank Mr. K. J. Harrington for helping with the collection and preparation of samples, and Miss Glenys Chong for assistance in their examination.

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Occurrence of *Phyllochaetopterus claparedii* McIntosh in the North-east Pacific

The waters near the Friday Harbor Laboratories of the University of Washington have been extensively dredged in recent years. It was of some interest, therefore, that a solitary *Phyllochaetopterus* of relatively large size was brought up from 22 fathoms over a muddy bottom just south of Orcas Island on August 26, 1960, with Forster's anchor dredge[1]. The specimen proved to be identical with the description of *Phyllochaetopterus claparedii* by McIntosh[2] found off Kobe, Japan. It has been recorded since only by Fauvel[3] from Cam Ranh and Tourane Annam (Vietnam). Treadwell's[4] specimens from Porto Rico are only doubtfully attributed to this species. The specimen from Friday Harbor had a pale yellow colour when alive apart from the grey-green gut, and was found in a transparent horny tube about 1 m. in length which was only faintly ringed (not jointed) distally and about 4 mm. across, comparable in size

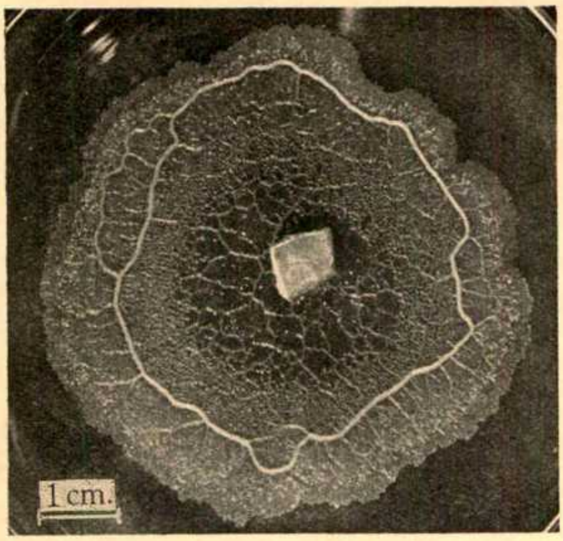

Fig. 1

direction, either clockwise or anti-clockwise. At other times various distributions of streaming in opposite directions in different parts of the main 'vein' are observed. We have observed no systematic correlation between streaming in the main 'vein' and streaming in the branches from it, nor between streaming at any point in the main 'vein' and any other point.

The existence of unidirectional streaming in such a continuous closed circuit, in the absence of any discernible valve- or ratchet-like mechanism, vitiates any hypothesis based on a spatial gradient of physical or chemical factors. On the positive side, this evidence seems to us to offer rather striking support for the notion that the mechanism which generates motive force must be distributed throughout the system and probably arises in the streaming material itself, not merely in the containing walls.

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Production of Slime Substance in Mixed Cultures of *Rhodopseudomonas capsulatus* and *Azotobacter vinelandii*

WE have reported[1] that a symbiotic relationship exists between *Rhodopseudomonas capsulatus* (photosynthetic bacteria) and *Azotobacter vinelandii*. When they were mixed, the combination fixed three times more molecular nitrogen than did either pure culture. If the *p*H of the medium were controlled, the nitrogen fixation in the mixed culture was stimulated twelvefold[2].

Microscopically, the cells were surrounded by slime substances produced in the mixed culture after one month, but not observed in the pure culture. It seemed that these slime substances were produced by the interaction between the bacteria. Apparently the substances promoted nitrogen fixation in the mixed culture and also the growth of the mixed culture. Therefore, we extracted the slime substances and investigated them by paper electrophoresis.

van Niel's basal medium (without nitrogen and sodium bicarbonate) was used in this experiment, and 0·3 per cent sodium acetate, 0·2 per cent yeast extract and 0·2 per cent peptone were added to the medium. *R. capsulatus* and *Azotobacter vinelandii*, separately and in combination, were incubated in 3-l. flasks at 27° C. aerobically in the light (10,000 lux). After being cultured for 9 days the bacteria were collected, using a Sharples centrifuge, and washed twice with distilled water. The bacterial cells were stirred with 4 per cent sodium hydroxide solution for 2 hr. and then, after centrifugation, the supernatant was taken. This treatment did not appear to injure the bacterial cells because they remained very active, motile, and capable of good growth. Therefore, it was considered that the 4 per cent sodium hydroxide treatment exerted no effect other than to remove the slime substances from the outside of the cells. The slime substances were dialysed against distilled water at 2° C. for 7 days. The precipitate produced at that time was removed by centrifuging.

Fig. 1 shows the paper electrophoresis of the slime substances. It was found that a peak, labelled '*M*', was observed only in the mixed culture, and not found in either of the pure cultures of *R. capsulatus* and *Azotobacter vinelandii*. The slime substance was positive for ninhydrin, biuret, anthrone reactions and negative for the Benedict reaction. We conclude that the slime substance could be a kind of mucoprotein or mucopolysaccharide, produced specifically in the

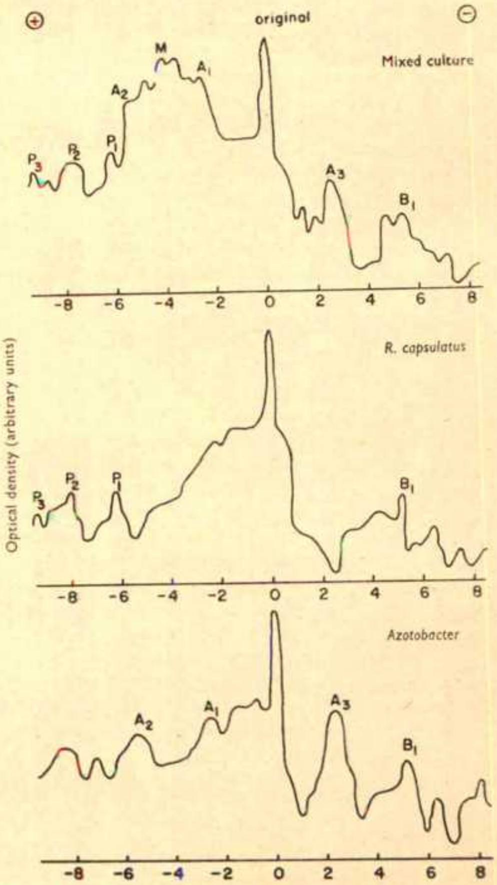

Fig. 1. Electrophoretic patterns of materials extracted from cell walls of *R. capsulatus* and *Azotobacter vinelandii* and their mixed cultures. Conditions: veronal buffer at *p*H 8·6; ionic strength, 0·05; Toyo No. 52 paper; 13 V./cm., 78 m.amp., 5 hr. Absorbancy measured with densitometer (Toyo-roshi Co.)

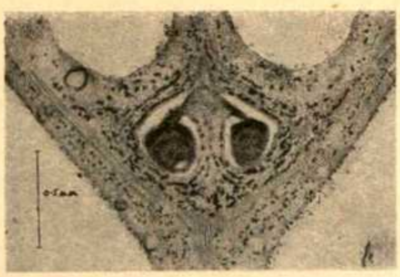

Fig. 1. Vertical section of barren receptacle

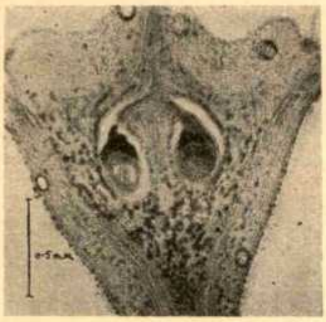

Fig. 2. Vertical section of bearing receptacle

biennial bearing. We have found consistent differences between barren and bearing trees, the flowers of the latter having fewer anthers. Anthers in five buds were counted from each of 30 trees in a random sample which was found to consist of 16 barren and 14 bearing trees. Barren and bearing trees had averages of 103·41 and 52·25 anthers per flower respectively.

Associated with high and low anther numbers were characteristic shapes of the receptacle. Figs. 1 and 2 show comparable sections through newly opened barren and bearing flowers. The former receptacle is seen to be relatively flat due to lack of development of tissue at the base of the style and the ovules are more nearly anatropous.

Flowers of barren trees were found almost always to fall after pollen shedding. Very occasionally one or two berries set but were in our experience invariably one-seeded. There was, however, an additional reason for non-abscission of barren flowers. Infestation by a gall midge (Cecidomyidae) caused destruction of ovules, but at the same time induced an irregular growth of the receptacle. Hitherto the gall midge has only been observed in the larval stage and a complete identification was not possible.

Pimento is a semi-wild crop, and the amount of deliberate planting is negligible. Apart from harvesting the only attention rendered in some groves is the removal of a few barren trees. Under such conditions, if barrenness were a genetic feature such as diœcy, the two types might be expected to approach a one to one ratio with perhaps a slight excess of bearing trees. Table 1 shows a series of samples taken to examine this possibility.

Table 1. FREQUENCY OF BARREN AND BEARING TREES IN SIX RANDOM SAMPLES

Estate	Barren	Bearing	Total	χ^2 P	Remarks
Bengal	14	16	30	0·9–0·8	No barren trees removed
Montpelier	14	16	30	0·9–0·8	Removal of barren trees very infrequent
Grierfield	15	15	30	0·9–0·8	
Greencastle 1	17	13	30	0·7–0·5	A small proportion of barren trees removed annually
2	11	19	30	0·3–0·2	
3	9	21	30	0·05–0·02	
Total	80	100	180		

It was noticed that the barren stigma tended to disintegrate a few hours after flower opening, probably thereby hindering its pollination. Moreover, while it was possible to fertilize bearing plants with pollen from barren trees, inter-fertilization between bearing trees was not obtained. Since examples of the self-fertilization of bearing trees were found it was thought possible that the viability of bearing pollen declined more rapidly. Such a conclusion was supported by a series of pollen-culture experiments.

The present work suggests that pimento although structurally hermaphrodite shows, between barren and bearing trees, quantitative differences in the directions of maleness and femaleness respectively. Such a condition was considered by Darwin[5] to precede the establishment of a complete separation of sexes. It seems probable, therefore, that pimento shows, within the framework of a structurally hermaphrodite flower, an incipient diœcism.

We wish to thank Mr. T. H. Farr, of the Institute of Jamaica, for assistance with the entomological aspect of this work.

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Circular Streaming Patterns in a Slime-Mould Plasmodium

IN a previous publication[1], we suggested that the classical 'squeezing from behind' mechanism for protoplasmic streaming is inadequate to explain the actual movements observed in slime-mould plasmodia. As a result of a recent change in culture conditions, remarkably simple and direct evidence to substantiate this suggestion has appeared. Fig. 1 is a photograph of a plasmodium of *Physarum polycephalum* after two days growth at 22° C. in the dark on a particular growth medium. This medium contains, per litre, 20 gm. agar; 5 gm. 'Bacto-tryptone'; 5 gm. dextrose; 0·75 gm. 'Bacto-yeast' extract; 5 mgm. hæmin; 0·8 gm. potassium dihydrogen phosphate; 0·2 gm. disodium hydrogen phosphate; 100 mgm. calcium chloride; 100 mgm. magnesium sulphate; 200 mgm. citric acid; 10 mgm. ferrous chloride; 14 mgm. manganese chloride and 6 mgm. zinc sulphate, adjusted to pH 6·2 and autoclaved for 30 min. at 15 lb./sq. in. (This is modified from the formula kindly supplied to us by Dr. H. P. Eusch, University of Wisconsin.)

The noteworthy feature of this culture is the continuous circular 'vein' which traverses the plasmodium. This feature is observed repeatedly under these culture conditions.

In such a 'vein', all the streaming protoplasm may at times be seen to move continuously in a single

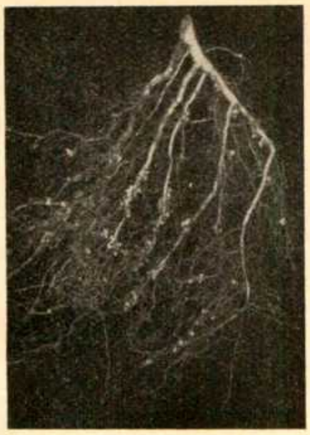

Fig. 2. Nodulation of a molybdenum-deficient alder plant showing many very small nodules uniformly scattered over the entire root system

much higher than in normal plants. Moreover, the same pattern of nodulation has been found in legumes inoculated with ineffective strains of *Rhizobium*.

Thanks are due to Prof. E. G. Mulder, who suggested this work, and to Dr. W. B. Deijs and Mr. O. C. Spoelstra for the chemical molybdenum analyses.

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Œstrogen Content of Plants as a Function of Conditions of Culture

IN the course of a broad investigation on the hormone content of plants, one of us[1], referring to the work of Alexander[2], stressed the important influence that conditions of culture could exert on the amount present in plants of substances having a hormonal action. Preliminary results of our investigation point to a definite influence in this respect.

Carrots cultivated over a period of several years on plots of soil that were left free from fertilizer and farm manure, or were treated either with a mixture of nitrogen, phosphorus and potassium, or with farm manure, were gathered and dried in the air at approximately 60° C., and tested for their œstrogen content, using immature female mice weighing 12–15 gm. The technique used was that described by Simon[3]. It is based on that used by Evans *et al.*[4], with the difference that both the carrot extract, and the diethylstilbœstrol used for the comparison, were given mixed in the diet. The diet was made up as follows: dextrine, 20; linseed meal, 13; barley, 10; wheat, 22; oats, 15; fish meal, 1·5; meat meal, 1·5; yeast, 0·5; mineral mixture, 2; gelatin, 2; dried milk, 12·5.

The food was prepared in the form of a biscuit of 0·5 cm. width.

The mice, divided into groups of six, were fed this diet during a 72-hr. period. They were then killed after 8-hr. fasting. The ovaries, Fallopian tubes and uteri were dissected out up to the cervices, stripped of all extraneous tissues, and weighed.

The findings are recorded in Table 1; they are given in equivalents of the doses of diethylstilbœstrol which served for comparative measurements in the control groups.

Table 1. CONTENT, IN EQUIVALENT OF DIETHYLSTILBŒSTROL, OF CARROTS GROWING ON SOIL CONTAINING VARIOUS FERTILIZERS

Type of fertilizer	Content in equivalent of diethylstilbœstrol (γ/100)	
	Fresh product	Dry product
Control with no fertilizer	0·12–0·16	0·6–0·8
With nitrogen, phosphorus and potassium mixture	0·24–0·26	1·2–1·3
With farm manure	0·40	2

It is readily seen that, depending on the conditions of culture, the titre of œstrogenous substances in carrots can differ by as much as two-fold. Using the same procedure with wheat seeds, our figures were approximately 0·32 γ/100 of dry product of equivalent of diethylstilbœstrol for wheat growing on plots treated with nitrogen, phosphorus and potassium, and about 0·65 γ/100 for wheat growing on plots treated with farm manure.

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Incipient Diœcy in Pimento

Pimenta dioica (Linn.) Merrill, known as allspice or pimento, is an important Jamaican crop and the island is the world's chief supplier. Unfortunately, improvement of the crop is hindered by a characteristic type of unfruitfulness among certain trees. The problem has long been recognized and was mentioned by P. Browne[1] in 1755.

The genus *Pimenta* (Myrtaceae) is mono-specific and is of tropical distribution. Its flowers have four sepals and petals, many anthers and an inferior bicarpellary ovary enclosed in a receptacle. The single style is curved, the plane of the curve coinciding with the septum between the carpels. The specific name alluding to diœcy is of Linnæan origin and was based on herbarium material[2]. Preserved specimens of barren plants differ from bearing ones in that the former have a relatively conspicuous development of anthers.

Previous workers have proposed different explanations of barrenness. Grisebach[3] assumed the plant to be diœcious, the barren plants functioning as males. Browne[1], and more recently J. F. Ward[4], on the basis of the hermaphrodite floral structure assumed barrenness was due to some physiological disorder.

The Jamaican Department of Agriculture has had a number of pimento trees under observation for several years, and it is known that barrenness is a property of certain trees and not a transient feature such as stronger effect on *Paramecium caudatum* than on *Trichomonas vaginalis*.

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BIOLOGY

Molybdenum and Symbiotic Nitrogen Fixation by Alder (*Alnus glutinosa* Gaertn.)

THE specific effect of molybdenum on the nitrogen fixation by *Azotobacter* spp.[1,2], *Clostridium pasteurianum*[3,4], some blue-green algae (*Nostoc* and *Anabaena*)[5,6] and the symbiotic nitrogen fixation by legumes[7-9] is well established. So far, it is unknown whether molybdenum is also essential for the symbiotic nitrogen fixation of a non-legume such as alder (*Alnus glutinosa*). The aim of the present investigation was to seek such an effect.

The distribution of molybdenum in the different parts of an alder tree growing under natural conditions in a normal acid soil is shown in Table 1. From these observations it will be seen that molybdenum is accumulated in the root nodules. Except the seeds, all other parts show far lower molybdenum-levels. This result points to a molybdenum requirement for symbiotic nitrogen fixation in alder, since the root nodules are the seat of the nitrogen-fixation process.

To obtain more evidence that molybdenum is essential for the symbiotic nitrogen fixation by alder, growth experiments were performed in Neubauer glass jars containing 500 gm. of molybdenum-deficient low-moor peat soil containing ironstone. One set of pots received no treatment except a basic dressing of phosphate and magnesium, whereas a second received in addition 150γ molybdenum per pot. After a growth period of 6 months when the experiment was concluded, the alder plants on the molybdenum-deficient soil showed a pronounced reduction in development as compared with plants growing in the same soil, but with molybdenum added (Fig. 1). The molybdenum-deficient plants had pale-yellow leaves in contrast to the dark green colour of the leaves of plants supplied with molybdenum. In addition, the lower leaves of the molybdenum-deficient plants showed marginal scorch.

Table 1. NITROGEN AND MOLYBDENUM CONTENTS OF DIFFERENT PARTS OF AN ALDER TREE GROWING IN THE FIELD* AT WAGENINGEN (MANSHOLTLAAN), THE NETHERLANDS

Part of plant	Nitrogen percentage in dry matter	Molybdenum p.p.m. in dry matter†
Leaves (young)	3·84	0·25
Stems and branches	0·92 (0·73–1·11)	0·18
Roots	1·76	0·28
Nodules	2·37	1·71
Seeds	3·69	2·10

* The soil was a sandy loam (pH = 5·3) containing approximately 0·2 p.p.m. available molybdenum (*Aspergillus niger* method) (ref. 10).

† Determined chemically with potassium rhodanide, after reduction of any hexavalent molybdenum by stannous chloride.

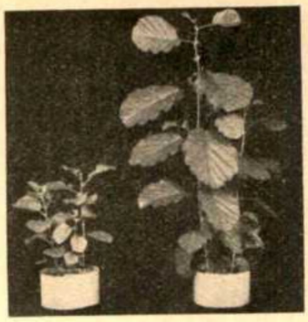

Fig. 1. Effect of added molybdenum on alder plants growing in molybdenum-deficient soil. Left, no molybdenum added; right, 150γ molybdenum as $Na_2MoO_4.2H_2O$ per pot

Obviously, the molybdenum-deficient plants suffered from nitrogen deficiency since yellow leaves and marginal scorch were found to be typical symptoms of nitrogen deficiency in non-nodulated alder plants growing in nitrogen-free molybdenum-containing nutrient solutions. Analyses showed that the molybdenum-deficient plants were considerably lower in nitrogen than those supplied with molybdenum (Table 2). The total amount of nitrogen in the tops of the molybdenum-treated plants was 370 per cent higher than that of control plants.

The molybdenum content of the various tissues of the molybdenum-deficient plants was extremely low (Table 3). Only in the root nodules was an appreciable amount of molybdenum found. The molybdenum-treated plants contained about 10–20 times more molybdenum in their tissues than molybdenum-deficient plants. The root nodules of the plants supplied with molybdenum contained 17·30 p.p.m. molybdenum in the dry matter, a value considerably higher than that known for legumes under comparable conditions[8].

The nodulation of the molybdenum-deficient plants was entirely different from that of plants grown in soil with an adequate molybdenum supply. In the latter plants mostly only one very big nodule (crown-nodule) was formed at the upper part of the root system, whereas in the molybdenum-deficient plants many small root nodules had developed which were uniformly scattered over the entire root system (Fig. 2). A similar effect has been observed by Mulder[9], who found that in molybdenum-deficient clover and lucerne plants the number of nodules was

Table 2. EFFECT OF MOLYBDENUM ON YIELD AND NITROGEN FIXATION BY ALDER PLANTS GROWN IN MOLYBDENUM-DEFICIENT SOIL

	Shoots				
Molybdenum* added (γ per pot)	Mean shoot length (cm.)	Fresh weight (gm. per pot)	Dry matter (gm. per pot)	Total nitrogen in shoots (mgm. per pot)	Nitrogen (percentage increase)
0	18·2	11·2 ± 2·0	2·38 ± 0·64	46·4 ± 8·8	—
150	33·7	34·4 ± 0·1	7·58 ± 0·25	218·3 ± 8·6	370

* Applied as $Na_2MoO_4.2H_2O$.

Table 3. MOLYBDENUM CONTENTS OF LEAVES, STEMS, ROOTS AND NODULES OF ALDER PLANTS GROWN IN MOLYBDENUM-DEFICIENT SOIL IN THE ABSENCE OR PRESENCE OF ADDED MOLYBDENUM

Plant tissue	Molybdenum content p.p.m. in dry matter I*	II†
Leaves	< 0·01	0·27
Stems	0·14	1·89
Roots	0·24	2·62
Nodules	2·00‡	17·30

The molybdenum-deficient soil (pH = 5·2) from Groningen contained approximately 0·006 p.p.m. available molybdenum (*Aspergillus niger* method) (ref. 10).

* I, No added molybdenum.

† II, 150γ molybdenum (as $Na_2MoO_4.2H_2O$) supplied per pot containing 500 gm. soil.

‡ Approximate value since the sample was very small.

Table 1. VIABLE UNIT COUNTS IN THE TISSUES AND THE DISTRIBUTION OF MICROSCOPIC LESIONS IN THE MESENTERIC LYMPH NODES OF HAMSTERS

Time after dosing months)	Dose (viable units)	Hamster No.	Counts expressed as $\log_{10}$ viable units* Spleen	Liver	Small intestine	Large intestine	Lesions in mesenteric lymph node
1	10^7	14	—	—	3·49	—	—
		13, 15–18	—	—	—	—	—
2	10^7	32	2·39	—	5·51	—	—
		33	3·09	3·39	—	—	—
		35	2·88	—	—	—	—
		31, 34, 36	—	—	—	—	—
1	10^8	7	4·22	3,92	—	—	—
		8	—	3·39	—	—	—
		9	—	—	3·79	—	—
		10	—	—	3·49	—	—
		11	5·35	6·08	4·35	3·49	+
		12	—	—	—	—	—
2	10^8	25	2·39	—	3·49	—	—
		26	4·66	3·65	4·49	4·54	+
		27	3·18	—	5·45	—	+
		28	4·08	2·88	5·74	—	+
		29	—	2·39	5·21	3·49	+
		30	4·54	—	—	4·58	+
1	10^9	1	—	2·39	4·49	4·45	—
		2	3·00	3·86	—	—	—
		3	5·29	5·04	4·98	4·20	+
		4	2·39	—	3·49	3·98	—
		5	3·95	—	3·98	4·10	—
		6	4·89	5·68	3·79	3·49	—
2	10^9	19	4·63	4·29	5·12	3·98	+
		20	4·32	3·70	4·88	—	+
		22	—	5·28	5·39	3·49	+
		23	5·18	4·43	4·54	3·79	+
		24	5·00	5·15	5·52	3·98	+

* The minimum detectable infections were $\log_{10}$ 2·39 viable units in livers and spleens and 3·49 viable units in intestines.

dosing lesions were present in the mesenteric lymph node of 1 hamster in each of the 2 groups given the higher doses. At 2 months, lesions were seen in 5 of 6 animals given 10^8 units and in all 5 hamsters given 10^9 units. No microscopic lesions were detected in the liver of any hamster.

Since there are already indications in sheep that vaccination may exert a beneficial effect by controlling the initial multiplication of *M. johnei* in the intestinal walls[2], the hamster is likely to prove a suitable experimental animal for the evaluation of vaccines against infection with *M. johnei.*

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Behaviour of Protozoa under the Influence of the Synergetic Action of Polyethylene Glycol and Dyes

INVESTIGATIONS on trichomoniasis and the search for a remedy which would effectively destroy *Trichomonas vaginalis* are still being pursued. It has been difficult to find a preparation which is toxic to *Trichomonas vaginalis* and at the same time harmless as regards the vaginal mucous membrane.

Biological examination of the properties of polyethylene glycol showed that it fulfils these requirements[1]. Polyethylene glycol is available in both liquid and solid form depending on its molecular weight. The toxicity of this polymer decreases with increase in its molecular weight. Polyethylene glycol of 1,500–4,000 mol. wt. administered to experimental animals orally and intravenously for 2 years caused no pathological changes[2]. Intravaginal application of the polymer to rabbits did not bring about any deviations from the normal conditions in the vaginal mucous membrane[3]. In 40 hospital patients infected with *Trichomonas vaginalis* treated with the polymer satisfactory results were achieved in about 80 per cent of the cases[4]. The best results are obtained with aqueous solutions in concentrations of 25–50 per cent. For large-scale administration these concentrations are uneconomical. We decided, therefore, to ascertain whether a combination of the polymer with diamond green and gentian violet would not be more effective. It is known that these dyes are to a certain extent toxic in their action on *Trichomonas vaginalis*[5].

The lethal mechanism after administering polyethylene glycol is almost the same for *Paramecium caudatum* as for *Trichomonas vaginalis*. Since it is easy to culture and is very mobile, *Paramecium caudatum* was found to be more suitable for experimental purposes.

All the experiments were carried out as follows: Cultures of *Paramecium caudatum* were placed in a medium of egg-yolk and were used for the experiments when the density of the animals reached approximately 1,000 per 1 ml. of culture. Next, sterile aqueous solutions of polyethylene glycol of mol. weight 4,000, in concentrations of 1, 5, 10, 15, 20, 25 and 30 per cent, were prepared in 4 rows of test-tubes. To each row we added equal parts of:

(1) 0·1 per cent aqueous solution of diamond green
(2) 0·01 ,, ,, ,, ,, ,, ,, ,,
(3) 0·1 ,, ,, ,, ,, ,, gentian violet
(4) 0·01 ,, ,, ,, ,, ,, ,, ,,

By means of a platinum loop, 1 drop of *Paramecium caudatum* culture and 1 drop of the appropriate polyethylene glycol solution with the dye, was placed on a concave glass slide. On mixing the liquids, the time when the *Paramecium* stopped moving was noted. This was taken to be the moment when the animals died, since afterwards they showed no sign of life even when replaced in the culture medium. Each experiment was repeated 5 times from which averages were calculated. Comparisons of these averages are given in Table 1. Investigations with the same solutions were carried out on *Trichomonas vaginalis* at the same time[6]. The results of this experiment are also given in Table 1.

Table 1. TIME REQUIRED FOR *Trichomonas vaginalis* AND *Paramecium caudatum* MOVEMENT TO CEASE AFTER THE ACTION OF POLYETHYLENE GLYCOL WITH GENTIAN VIOLET OR DIAMOND GREEN EXPRESSED IN HUNDREDTHS OF A MINUTE

	Concentration		Concentration of polyethylene glycol (per cent) 30	25	20	15	10	5	1
Trichomonas vaginalis	gentian violet	0·1	3·90	3·95	4·92	5·72	5·74	6·42	8·00
		0·01	7·32	9·74	11·12	17·30	21·50	35·20	38·80
	diamond green	0·1	4·47	4·74	4·82	5·76	8·26	11·63	13·72
		0·01	5·56	6·97	8·75	14·05	25·18	32·31	37·00
Paramecium caudatum	gentian violet	0·1	0·18	0·23	0·23	0·24	0·25	0·30	0·30
		0·01	0·34	0·39	0·41	0·54	1·70	2·90	3·18
	diamond green	0·1	0·23	0·25	0·26	0·28	0·29	0·31	0·32
		0·01	0·32	0·34	0·38	0·70	1·50	3·70	4·00

It may be concluded from our experiments that so far as these protozoa are concerned polyethylene glycol and the dyes have a synergetic action. On the grounds of our previous experiments and those described in this communication we consider that polyethylene glycol may be used for treating trichomoniasis. It is a non-toxic compound, cheap, and easy to apply. The addition of the dyes clearly has a more positive effect in the lower concentration of polyethylene glycol (1–20 per cent). This compound has a of 17 and 19 months), the 2 rats in the group treated with normal anti-sub-microsomal fraction sera died as the result of abdominal cancer. It is likely from the size and extension of the tumours that they had first appeared at a much earlier date. At 12 months after cessation of treatment, the rats injected with the 3′-DAB fraction appeared normal and were killed. No significant pathological lesions were found.

The first rat to die had a spherical tumour (3 cm in diameter) adherent to the hilum of the liver with minute white foci throughout the liver. The peritoneum was riddled with small metastatic tumour nodules. The histology of the tumour was that of a typical bile duct carcinoma. Its origin was presumably in a branch of the common bile duct in the liver hilum. The liver itself showed very severe centrilobular necrosis with a cellular reaction and early periportal regeneration, but with many cells in this area still lysing. Some bile ducts in unaffected portal tracts appeared cancerous. The second tumour was quite different. It was very large (6 cm × 5 cm) and sited centrally in the posterior and upper abdominal wall. Its origin was obscure, but at autopsy the possibility of an upper para-aortic lymph node was considered. The whole of the liver surface showed minute yellowish-white foci. The tumour was highly malignant, consisting of large polyhedral cells with no definite acinar formation. Incorporated in it were areas of normal pancreatic glandular tissue. The liver parenchyma in this case was only mildly damaged, but the tumour was invading the liver subcapsular channels extensively. Many cells contained prominent blobs but did not stain for mucus. The nature of the tumour is unknown, but the possibilities include an undifferentiated pancreatic carcinoma and a lymphoid or reticulum cell sarcoma.

If we assume, as there is every reason from long experience with this rat strain, that these tumours were induced and not spontaneous, this result, in spite of the small numbers involved, is highly interesting for the following reasons. They are the first malignant tumours to be produced by the injection of antiserum. A tissue-specific antiserum has in one instance resulted in a tumour of a component of the tissue concerned, which would seem extremely unlikely to be a chance event. Not knowing the nature of the other tumour, there is no point in speculating at this stage on the mechanism of induction, except that it was very probably related to the treatment. Equally important is the fact that the antiserum to the carcinogen-treated liver fraction produced no tumours and no liver damage. There appears to be a striking parallel here with the results obtained by Price[3] at Leeds. She showed that treatment of kidney cells in tissue culture with 3 4-benzopyrene for 10 days rendered them incapable of inducing an antiserum nephrotoxic to mice *in vivo*. The inference was that the tissue-specific antigens had been modified antigenically by the carcinogen. It would appear that the hepatocarcinogen has also modified by haptenic binding the liver tissue-specific antigens and so made the induced antibody innocuous to normal liver.

The normal antiserum produced extensive liver damage in one case, and the process was still continuing at death long after cessation of treatment. There is a possibility here that an auto-immune reaction following liver damage had been established.

This experiment, although now of course being repeated in a larger and more detailed way, would seem to have greater significance, since it was planned on a purely theoretical basis in the hope of achieving the end which, at least in part, resulted. The postulate was that repeated exposure to tissue-specific antiserum would induce adaptive loss of tissue-specific antigens with malignancy as an essential consequence. If the results are repeatable on a larger scale, it would demonstrate beyond doubt that an immunological approach to carcinogenesis is valid, and that the immunological theory of the nature of cancer (see Green[4] for references) may be basically sound.

I am much indebted to Dr. G. M. Bonser for reporting on the tumour sections.

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Susceptibility of Hamsters to the Oral Administration of *Mycobacterium johnei*

THE development of a method of counting viable units of bacilli in the tissues of sheep[1] has enabled us to re-examine the potentialities of laboratory animals as experimental hosts for *M. johnei*. The oral route of challenge was chosen because it is the most likely route of infection in naturally occurring Johne's disease. Progressive infection in the tissues after oral dosing was considered to be a better test of the true susceptibility of animals than the direct introduction of bacilli by parenteral methods.

Three groups of twelve hamsters of about 20 gm in weight were dosed orally on one occasion with 10^9, 10^8 and 10^7 viable units of *M. johnei* respectively. The strain of *M. johnei* was the sixth sub-culture from a clinical case of Johne's disease (bovine type) in a sheep. Five or six hamsters from each group were killed at 1 month and at 2 months after dosing. At these times the total viable unit counts were made of the spleen, the liver and the whole of the intestinal walls of each animal. The mesenteric lymph nodes and a piece of liver from each hamster were examined histologically. The results are shown in Table 1.

At 1 month after dosing, infections of the intestines were found in 1 of 6 hamsters given 10^7 viable units, in 3 of 6 given 10^8 units and in 5 of 6 hamsters given 10^9 units.

At 2 months the numbers of animals with intestinal infections were 1 out of 6, 6 out of 6 and 5 out of 5 at the 10^7, 10^8 and 10^9 dose-levels respectively. Where detectable infections were present in the small intestines the mean group counts at the 2-month examination were at least one $\log_{10}$ unit greater than those at the 1-month examination. This shows that progressive infections had been established. Infections were not progressive in the large intestines. *M. johnei* was recovered from the livers or spleens of all the hamsters given 10^8 and 10^9 viable units and examined at 2 months. The viable counts on these tissues were not significantly greater at the second examination compared with those obtained at the first. Whether the infection passed through the intestinal barrier at the time of dosing or as a result of secondary spread from primary progressive foci in the intestine is not yet known. At 1 month after

IMMUNOLOGY

Mast Cell Lytic Antibodies

It is known that mast cell damage by antigen invariably occurs in those circumstances in which an anaphylactic release of histamine is produced in actively sensitized rats[1], mice[2] and guinea pigs[3]. The observation that in passively sensitized guinea pigs the mast cell reaction to antigen was rather uniform and graded with the dose of antibody[4] suggested that the large variation observed in actively sensitized guinea pigs could be due to variation in the relatively small amount of antibodies produced by them. Alternatively, some variation in the quality of the antibody was considered, for example, a difference between precipitating and non-precipitating antibody. However, early work[5,6] showed that precipitating and non-precipitating antibodies were equally efficient for passive sensitizing of guinea pigs.

I was led to the same conclusion by work on the behaviour of guinea pig mast cells in passive anaphylaxis[4]. However, some experiments with rats have suggested that in this species precipitating antibodies are not responsible for the mast cell damage and histamine release occurring in anaphylaxis, but that some other type of antibody might be responsible for these phenomena. For example, while on one hand mast cell damage and histamine release are very much increased in anaphylaxis of pertussis-treated rats[7] which produce very little, if any, precipitating antibodies, on the other both these phenomena may be much reduced in rats sensitized with Freund's adjuvant having considerable amounts of circulating precipitating antibodies. Actually, rats sensitized with Freund's adjuvant seem to be able to produce both types of antibodies since some of these animals present marked mast cell damage and histamine release when challenged with antigen, while others do not. However, this seems to bear no relation to the amount of precipitating antibodies produced by the animal. Thus, there is a difference between the amount of precipitating antibodies produced by immunized rats and the induction of mast cell damage by antigen in anaphylaxis.

Furthermore, I have not succeeded in duplicating mast cell damage and histamine release in anaphylaxis of rats passively sensitized with rabbit or even homologous precipitating antibodies. This occurred also when pertussis-treated rats were passively sensitized and shocked[8]. Therefore, it seems reasonable to conclude that some particular factor or type of antibody is required for the production of mast cell damage in rat anaphylaxis and that the anaphylactic syndrome produced in rats after passive sensitization with rabbit or homologous precipitating antibodies duplicates only that part of anaphylaxis due to precipitating antibodies.

As a working hypothesis we think that rat anaphylaxis is brought about by two different antigen–antibody reactions: (*a*) the reaction of antigen with precipitating antibodies—that do not cause mast cell damage; (*b*) the reaction of antigen with 'mast cell lytic antibodies', which are responsible for the anaphylactic release of histamine. It seems that rats produce normally both types of antibodies and that, depending on the condition of immunization, one of these types of antibody would predominate over the other. Thus, rats sensitized with Freund's adjuvant would produce predominantly precipitating antibodies while rats injected with *B. pertussis* along with the sensitizing dose of antigen would produce predominantly 'mast cell lytic antibodies'. However, the 'mast cell lytic antibodies' would never attain a concentration in the serum adequate to produce passive sensitization. This condition is necessary for explaining the repeated failure of serum taken from pertussis-treated rats to passively sensitize rat tissues either *in vivo* or *in vitro*. Furthermore, since the pharmacologically active substances released from the mast cells cannot account for the severity of anaphylaxis in pertussis-treated rats, one has to consider that the 'mast cell lytic antibodies' are not necessarily specific to these cells but are also very active in causing damage to other cells. Further experimental work is needed on this point.

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PATHOLOGY

Apparent Induction of Malignant Tumours by Organ-Specific Antiserum

It was reported[1] that rats injected with 3′-methyl-4-dimethylaminoazobenzene (3′-DAB) showed firm binding of this hepatocarcinogen to the structural lipo-protein complex forming the cytoskeleton, that is, endoplasmic reticulum of the liver cell. The binding here in terms of protein nitrogen was higher than elsewhere. It was shown similarly that firm binding occurred in a sub-microsomal fraction shown by Vogt[2] to contain all, or nearly all, the tissue-specific antigens in the liver cell. This fraction contained the bulk of the phosphatide in the microsomes and was therefore thought to be derived from the endoplasmic reticulum. As with the structural lipo-protein, binding was higher here than in any other cell fraction.

Two rabbits were immunized with 0.1 ml. of the sub-microsomal fraction prepared from the livers of rats injected intraperitoneally 48 hr. previously with 20 mgm. of 3′-DAB in arachis oil and two with a similar fraction from rats injected with arachis oil only. After nine injections (intravenous), the first with 1 ml. of Freund's adjuvant (intramuscular), tube-precipitin tests were erratic in regard to specificity but revealed precipitins to both fractions in all the undiluted sera. Absorption tests indicated that normal liver homogenate absorbed the precipitin from the control but not from the antiserum to the fraction from rats treated with carcinogen.

At this stage, 2-month-old Wistar rats (2 in each group) were injected (intraperitoneally) with pooled rabbit antiserum, absorbed for 1 hr. at 37° C. with normal rat kidney, spleen and lung homogenates, at a dose of 1 ml. spread over 15 injections in 7 months. Immunization of the rabbits providing the sera was continued for the first 9 weeks of this period. At 8 and 10 months after treatment ceased (that is, at the age

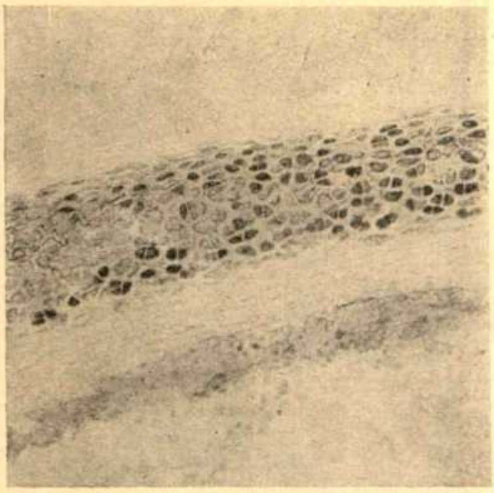

Fig. 1. Rib cartilage of mouse demonstrating marked uridine diphosphoglucose dehydrogenase activity of the subperichondrial cells. Perichondrium and intercostal muscle are negative. Incubation time, 60 min. (× 115)

(Figs. 1, 2). It was considerably reduced or absent in the more mature cartilage cells. The dye was deposited in a finely granular distribution throughout the cytoplasm; the nuclei were consistently negative. The controls, which lacked either substrate or diphosphopyridine nucleotide, were uniformly negative, thus excluding reduction of the ditetrazolium salt via oxidation of endogenous substrates or endogenous reductants. *p*-Chloromercuribenzoate (10^{-4} *M*) completely inhibited the reaction.

The mechanisms involved in the histochemical demonstration of oxidative enzyme activity by use of tetrazolium salts as indicators has been considered elsewhere[4]. In the system under discussion electrons are transferred from uridine diphosphoglucose to the coenzyme via a dehydrogenase and from the reduced coenzyme to the tetrazolium salt by a diaphorase. We are thus demonstrating the activity of a dehydrogenase–diaphorase system, both components of which must be acting to produce a positive result. The reduced tetrazolium salt forms a dark blue granular, insoluble, substantive diformazan at the site of enzyme activity. The rinsing preceding incubation as outlined in the method is important in that it removes endogenous substances capable of reducing the tetrazolium salt independently. We have been unsuccessful in developing a histochemical method to demonstrate this enzyme system in the liver. The extreme diffusibility of some component of the system does not permit the rinsing which excludes or sufficiently minimizes non-specific reduction of the tetrazolium salt, a prominent effect in this organ at *p*H 8·4 (ref. 5). The enzyme in cartilage does not diffuse appreciably and thus is suitable for demonstration by this histochemical technique.

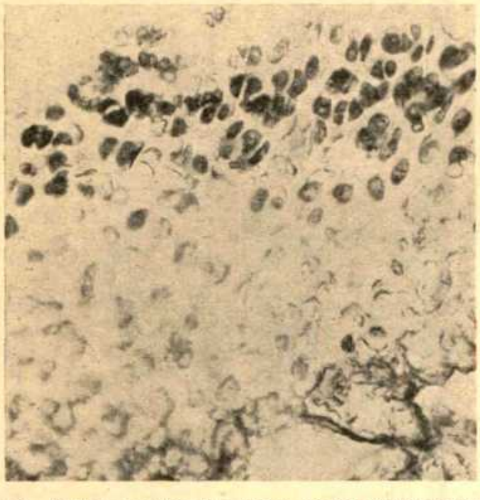

Fig. 2. Higher magnification shows marked intracytoplasmic diformazan deposition in the young chondrocytes (top). Enzyme activity gradually disappears in the deeper lying cartilage cells. Incubation time, 60 min. (× 220)

Glucuronic acid is important in intermediary metabolism, particularly in the conjugating mechanism of the liver (glucuronides), and as a component of mucopolysaccharides throughout the body[1,2]. There is evidence that the glucuronic acid available for these functions is synthesized from glucose via its uridine diphosphoglucose derivative[3,6]. Uridine diphosphoglucose is oxidized through a diphosphopyridine nucleotide-mediated dehydrogenase to uridine diphosphoglucuronic acid. The activity of this system in mammalian liver is well established[3]. Its role in the synthesis of hyaluronic acid in group *A Streptococcus* has been examined[7], and the presence of this system in murine mast cell tumour[8], and epiphyseal cartilage homogenates[9] from young rabbits, has been observed.

The distribution of uridine diphosphoglucose dehydrogenase in cartilage conforms to what we would expect in that it is most active in developing cells at the periphery which are presumably in the process of synthesizing new matrix. These cells would, therefore, require the greatest activity of an enzyme system which produces glucuronic acid for ultimate synthesis into the acid mucopolysaccharides of the matrix. The more mature central cells within well-developed ground substance may no longer require an active production of matrix components and show reduced or absent enzyme activity.

This method therefore demonstrates uridine diphosphoglucose dehydrogenase activity in the cytoplasm of murine cartilage cells from varying body sites. The synthesis of glucuronic acid available for incorporation into mucopolysaccharides probably takes place through the action of this enzyme system. The technique for its demonstration is based on the reduction of a tetrazolium salt.

This investigation was supported by a grant (*C*–3424) from the National Cancer Institute, U.S. Public Health Service.

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satisfactory kinetic analysis; (2) the inhibitory effects of various lots of diazonium salts; (3) the low solubility of the azo compounds. The finding that free 'Napthol *AS*' exhibited fluorescence which was abolished by esterification and restored on hydrolysis provided the basis for a simple assay of chymotrypsin. 'Napthol *AS*' fluoresces maximally at 515 mμ when excited at 310 mμ. The excitation maximum coincides with an absorption peak that is absent in the esters. Self-quenching restricts the range of concentration over which fluorescence intensity is linearly related to concentration of the fluor. Fortunately the range of concentration for which the relationship is linear (0·5–15 μ*M*) is appropriate for the assay of enzyme activity.

Crystalline α-chymotrypsin was tested with each of the esters utilizing the fluorescent assay; the phenylproprionoxy ester was hydrolysed at an initial rate 10 times faster than the phenylbutyroxy and 30 times faster than either the benzoxy or phenylacetoxy naphthoic acid anilides. The *p*H optimum of 8·5 for the hydrolysis of the phenylproprionoxy ester agreed with the value obtained using *N*-acetyl tyrosine ethyl ester.

Rat tissues were examined histochemically for the presence of enzymes which would hydrolyse any of the esters. The standard method was to fix tissues in cold neutral buffered formol, washed with gum-acacia sucrose solution[5], and sectioned in the Pearse cryostat. Lipid was removed from the sections with methanol, and the sections incubated in the reaction mixture containing substrate at a concentration of 40 μ*M* in 40 per cent methanol in *tris* buffer at *p*H 8·0. Fast garnet *GBC* was used as the coupler. Sections were incubated 15 and 30 min. at room temperature, and then washed and mounted in polyvinylpyrrolidone[6]. Under these conditions, mast cells were the principal cell type to exhibit enzymatic activity in a variety of tissues examined, including liver, kidney, bone marrow, thymus and myocardium. The mast cell enzyme activity was parallel to that of chymotrypsin; the phenylproprionoxy ester was hydrolysed rapidly, the phenylbutyroxy slowly and the phenylacetoxy and benzoxy esters not demonstrably in the histochemical system. The mast cells from one dog mastocytoma and one cat mastocytoma also contained an enzyme which hydrolysed the phenylproprionoxy derivative.

In rat stomach and intestine, a population of cells distinct from the usual connective tissue mast cells evidenced esterase activity with the phenylproprionoxy and phenylbutyroxy compounds. These cells were usually smaller than connective tissue mast cells, with sparser granules staining less intensely with toluidine blue. Many of these cells appeared in the lamina propria while others were insinuated between epithelial cells of the mucosa in small intestine and colon.

Pancreatic zymogen granules which are known to contain chymotrypsinogen did not hydrolyse the phenylproprionoxy naphthoic acid anilide without prior activation of the enzyme precursor. Sections of pancreas were therefore treated with 0·05 per cent crystalline trypsin at *p*H 6 in the presence of calcium ion for 15–60 min. at room temperature. After trypsin activation, enzyme activity was evident in zymogen granules, in acinar lumina, in intralobular and interlobular ducts. The enzyme in the ducts was better demonstrated when methanol followed activation, whereas staining of enzyme in zymogen granules was greater if methanol preceded activation.

Two types of specificity are demonstrated in this work. There is the specificity of chymotrypsin and the related enzyme of mast cells for the phenylproprionoxy ester of a series of homologous 'Napthol *AS*' derivatives, and there is the restriction of the enzyme hydrolysing the phenylproprionoxy naphthoic acid anilide to connective tissue mast cells, intra-epithelial mast cells of the gastrointestinal tract, and pancreatic zymogen granules and intraductal secretion. The digestive function of pancreatic chymotrypsin is established; the presence of a chymotrypsin-like enzyme in connective tissue mast cells and intra-epithelial mast cells of the gastro-intestinal tract is a contribution of enzyme histochemistry yet to be exploited in work on normal and pathological function.

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Histochemical Localization of Uridine Diphosphoglucose Dehydrogenase in Cartilage

THIS communication presents a method for the histochemical demonstration of uridine diphosphoglucose dehydrogenase in cartilage. This enzyme system is active in the pathway of synthesis of glucuronic acid[1,2].

Fifteen young adult albino mice on standard laboratory diet were killed by decapitation, and cartilage from various parts of the body, including larynx, trachea, ribs, joints and epiphyses were immediately removed. Fresh tissue blocks were frozen on dry ice. These were cut in a cryostat (−18° C.) with a rotary microtome at 16μ. The sections were placed on coverslips and rinsed for 5–10 min. in cold (4° C.) 0·1 *M* phosphate buffer at *p*H 8·4. They were then removed to an incubating mixture composed of 5 mgm. of the sodium salt of uridine diphosphoglucose, 2 mgm. diphosphopyridine nucleotide, and 2·5 mgm. of the paranitrophenyl substituted ditetrazolium salt ('Nitro-*BT*') in 5 ml. of 0·1 *M* phosphate buffer at *p*H 8·4. The incubation medium was warmed to 37° C. prior to introduction of the tissues, which were incubated for 30 min.–1 hr. The medium was then drained off and replaced by 10 ml. of 10 per cent neutral formalin at room temperature, in which the tissues were fixed for 1 hr. Sections were then mounted on slides in glycerogel and examined under the microscope.

In each experiment sections were also incubated in solutions which individually excluded substrate or coenzyme to control the specificity of the reaction. *p*-Chloromercuribenzoate (10^{-4} *M*) was used in some experiments as an inhibitor[1,3].

Uridine diphosphoglucose dehydrogenase activity was clearly demonstrated in cartilage cells from the larynx, trachea, ribs, joints and epiphyses. In all instances activity was most prominent in the cartilage cells located at the inner aspect of the perichondrium, and extended for only several cell layers in thickness potentiation was of long duration, often lasting for 30 min or more In this respect there was a difference between the catecholamines and the other smooth muscle stimulants since the effects of adrenaline and noradrenaline rarely exceeded 15 min and were often shorter A few experiments were conducted with substance *P*, which was also found to potentiate the response to hypogastric stimulation when doses of 0 2–0 6 unit/ml were added to the bath

In order to examine the influence of specific inhibitors on the potentiation described, some experiments were performed with atropine Atropine in doses of 0 1–1 μgm /ml abolished the effect of acetylcholine but not those of the catecholamines and histamine

I wish to thank Prof U S von Euler for help and valuable advice

This investigation was supported by a grant to Prof U S von Euler from the Thérèse and Johan Andersson Memorial Fund

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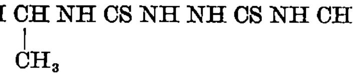
Non-steroid Inhibitors of Pituitary Gonadotrophic Function

WE have obtained striking changes in the reproductive system of animals of certain species treated with some dithiocarbamoylhydrazines (U K patent 878,177) We were prompted to make our experiments by an observation of our colleague, Dr J K Walley

Among the most active of the compounds we examined is 1-α-methylallylthiocarbamoyl-2-methylthiocarbamoylhydrazine (compound 33,828)



$$CH_2{=}\underset{\displaystyle CH_3}{CH}\,CH\,NH\,CS\,NH\,NH\,CS\,NH\,CH_3$$

Administration of this substance to rats of either sex results in selective and reversible inhibition of pituitary gonadotrophic function We have obtained evidence of similar action in dogs and monkeys, but not in mice, guinea pigs, rabbits or horses

This action is manifest in various ways Thus, when female rats with regular œstrus cycles are given daily oral doses of the compound at a level of 20 mgm or more per kgm , cyclic activity, as judged by vaginal smears, ceases within two or three days Smears of the diœstrus type persist for as long as dosing is continued when it is stopped the normal vaginal cycle is resumed within three or four days, even after several months dosing The ovaries lose weight progressively during administration of the compound and, in histological sections, show a decrease in the number of maturing follicles as compared with normally cycling control animals

Table 1 PERCENTAGE REDUCTION IN THE RELATIVE WEIGHT OF THE GONADS AND ACCESSORY ORGANS OF MALE RATS (INITIAL BODY-WEIGHT, *c* 110 GM) GIVEN DAILY ORAL DOSES OF COMPOUND 33,828

Daily dose of compound (mgm /kgm , p o)	Percentage reduction in relative weight					
	After 10 daily doses			After 24 daily doses		
	Seminal vesicles	Ventral prostate	Testes	Seminal vesicles	Ventral prostate	Testes
16	44	21	1	71	61	30
40	66	29	11	88	80	51
100	67	39	6	88	83	55
250	69	49	18	88	85	74

In male rats, daily dosing with the compound at similar levels results in progressive atrophy of the accessory sex glands and a slower loss in weight of the testes The latter change is associated with arrest of spermatogenesis at the stage of early spermatocytes The interstitial cells seem to be unaffected Table 1 shows the percentage reduction in the relative weight of the seminal vesicles, ventral prostate and testes of rats after 10 and 24 daily doses of the compound at various levels, as compared with the relative weight of these organs in control animals of the same age and starting weight Mean figures are given for groups each of six rats having a body-weight of about 110 gm at the start of experiment The treated animals were killed on the day after that on which the last dose was given

The adrenals and thyroid of rats treated with the compound show some increase in relative weight but no histological abnormality In the experiment cited the increase was greatest in the animals given 250 mgm per kgm for 24 days, and then amounted to 37 per cent for the adrenals and 17 per cent for the thyroid

The effect of the compound on pregnancy in the rat depends on the level and duration of dosage and the stage at which it is administered If it is given in sufficient dose (*c* 100 mgm per kgm orally) on any three consecutive days of pregnancy, no litters are obtained This is probably due, if treatment is given in the early stages of pregnancy, to inhibition of implantation, and if in the later stages, to fœtal death and resorption However, in animals given doses insufficient to interrupt pregnancy, the surviving offspring have appeared normal Moreover, the termination of pregnancy in rats by administration of the compound leaves their subsequent fertility unimpaired

Although the effects of the compound on the reproductive system of rats are apparent with daily doses as low as 16 mgm per kgm , daily doses of 350 mgm per kgm are tolerated for up to six months without serious effects on well-being or on the morphology of other internal organs Dosage at levels of 16 mgm per kgm a day and upward does, however, reduce the rate of gain in body-weight and causes, in some rats, patchy loss of hair The acute median lethal dose for rats is of the order of 1 gm per kgm (single oral dose), at which level perforation of the head of the duodenum is often the cause of death

The compound does not affect the increase in weight of the uterus of spayed female rats given œstradiol, nor that of the seminal vesicles or prostate of castrate males given testosterone Atrophic changes produced by its administration in the gonads and accessory organs of sexually mature rats can be prevented by the simultaneous administration of human chorionic gonadotrophin ('Pregnyl'), but the compound does not counteract, in immature animals of either sex, the effects of human chorionic gonadotrophin, pregnant mare serum gonadotrophin ('Gestyl') or human menopausal gonadotrophin These observations suggest that the action of the compound is central rather than peripheral and this is further supported by observations on the pituitary of treated animals Although administration of the compound does not significantly affect the cytology of the pituitary in intact rats, it largely prevents the appearance of castration cells in castrate males, when it is continued from the time of operation until the animals are killed one month later. We therefore conclude, provisionally, that the compound either directly interferes with the production of gonadotrophins by the pituitary, or else affects the presumed hypothalamic control of that function.

Atrophic changes have been detected in the testes and accessory sex organs of dogs given the compound, and in female rhesus monkeys inhibition of menstruation results from its daily administration. We have been unable to obtain any clear evidence of endocrine effects from the compound in mice, guinea pigs, rabbits or horses.

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Permeability of the Synovial Membrane to Glycoproteins

ELECTROPHORETIC investigations have indicated differences in glycoprotein pattern between serum and synovial fluid[1-3]. The most marked difference is that the relative amount of α_2-glycoproteins is considerably less in synovial fluid than in serum. It was recently demonstrated[4] that the low α_2-content of rheumatoid arthritic synovial fluid is mainly due to the low concentration of haptoglobin. The synovial fluid–serum ratio for this glycoprotein was significantly lower than for albumin or γ-globulin. The concentration of the small-molecular glycoprotein orosomucoid was, on the other hand, that expected from the total protein content, indicating a varied permeability of the blood-synovial fluid barrier to glycoproteins.

Table 1. SYNOVIAL FLUID – SERUM RATIOS OF VARIOUS PROTEIN FRACTIONS IN CASES WITH AND WITHOUT JOINT DISEASE

Group	No of cases	Vol of synovial fluid (ml.)*	Synovial fluid – serum ratios							
			Glycoproteins			Electrophoretic fractions				
			Haptoglobin	Orosomucoid	Prothrombin-proconvertin	Albumin	α_1	α_2	β	γ
I Cases without joint involvement										
(a) Without evidence of inflammatory disease	5	0.2–0.3	0.03	0.39	0.10	0.43	0.23	0.13	0.23	0.23
(b) With evidence of inflammatory disease (pyelonephritis)	2	0.2–0.3	0.18	0.72	0.27	0.65	0.53	0.35	0.30	0.48
II Osteoarthritis	6	0.5–10	0.24	0.64	0.18	0.46	0.45	0.21	0.32	0.33
III Rheumatoid arthritis										
Untreated	5	10–50	0.38	0.52	0.36	0.57	0.60	0.39	0.51	0.62
After hydrocortisone †	5	2–8	0.18	0.50	0.16	0.58	0.49	0.28	0.42	0.63

* As judged from the amount obtained on puncture.
† Puncture 4 days after intra-articular administration of 50 mgm. hydrocortisone acetate.

Therefore, it appeared of interest to extend the investigations to the glycoprotein permeability of the synovial membrane under normal conditions as well as in other cases with joint effusion. Since it has recently been demonstrated[5] that certain coagulation factors appear to be absent from normal bovine synovial fluid, determinations of the coagulation factors prothrombin and proconvertin, which are known to be glycoproteins, were also included in the present work.

Using previously described methods, synovial fluid-serum ratios for haptoglobin[6], orosomucoid[7], prothrombin and proconvertin[8], and certain electrophoretic protein fractions[3] were determined in cases without evidence of joint disease as well as in cases of osteoarthritis and rheumatoid arthritis. The effect of intra-articular administration of corticosteroids was also studied.

The results are summarized in Table 1. The synovial fluid–serum ratios for haptoglobin and prothrombin–proconvertin are seen to be extremely low in the cases without joint involvement (group I *a*) but increase moderately with increasing degree of synovial inflammation. Following intra-articular administration of hydrocortisone in rheumatoid arthritic cases the ratios for haptoglobin and prothrombin–proconvertin decrease markedly. The ratio for orosomucoid, on the other hand, is seen to be closely similar to that for albumin in the various groups.

The cases of group I *b* (Table 1) deserve special mention. In these cases the synovial fluid was of normal amount and appearance and there was no evidence of joint disease. However, in contrast to the cases collected in group I *a*, the patients of group I *b* presented abnormal serum protein patterns, markedly increased serum-level of haptoglobin and orosomucoid, as well as elevated erythrocyte sedimentation-rate. The changes in the synovial fluid–serum ratios for some of the glycoproteins observed in this group were of a degree found in mild synovitis and may possibly reflect a general change in connective tissue permeability to glycoproteins in infectious conditions.

The results indicate that under normal conditions the synovial membrane is very slightly permeable to certain glycoproteins. This semipermeability is obviously not related to the molecular size of the glycoprotein. Already slight synovial inflammation causes marked changes in glycoprotein permeability, which therefore should be a sensitive index of the degree of inflammation. The mechanism of the selective permeability remains unclarified.

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Dietary Citrate and Hypomagnesæmia in the Ruminant

THE sudden onset of clinical hypomagnesæmia in the lactating cow most commonly occurs soon after the change from stall feeding to grazing fresh spring grass, and in the absence of any marked reduction in intake of magnesium. This condition is probably brought about by reduced absorption of magnesium, but the reasons for this are not fully understood. Marked reductions in output of urinary magnesium on changing the diet from hay or grass nuts to fresh grass have been described for sheep[1] in spite of increased intakes of magnesium; this probably indicates reduced absorption. Care[2] recently calculated that a 50 per cent reduction in absorption could lead to the development of hypomagnesæmia within 7 days in sheep.

Mineral balance of herbage[3], low sodium intakes[4], and increased ruminal ammonia production[5] from the appreciable amounts of soluble nitrogenous constituents of spring herbage have all been suggested as precipitating factors, but none of these has been consistently implicated in clinical cases of the disease. In the course of an investigation into the effects of adding organic acids and their salts to the diet of ruminants, we have obtained evidence that dietary citrate levels may be related to the occurrence of hypomagnesæmia.

Eighteen 4–6 month old Ayrshire heifer calves were housed in individual pens and fed 3.5 lb. of a commercial concentrate, 0.5 lb. crushed barley and hay *ad lib.* to a maximum of 4 lb. daily. The calves were paired and allocated at random to treatments providing (*a*) 30 gm. sodium chloride or (*b*) 50 gm. granular sodium citrate in individual daily doses mixed with the barley. Each supplement provided 11.8 gm. sodium per day.

Intakes of food and gains in live weight were similar for both treatments over an 8-week experimental period. In the eighth week, blood samples for the determination of serum calcium, magnesium and inorganic phosphorus were taken on two occasions with the results shown in Table 1. Those calves which had received sodium citrate showed significantly lower levels of serum magnesium and appreciably lower levels of serum inorganic phosphorus than the calves which had received sodium chloride.

Table 1. EFFECTS OF SODIUM CITRATE UPON SERUM CALCIUM, PHOSPHORUS AND MAGNESIUM (MGM./100 ML. SERUM. MEAN OF TWO OBSERVATIONS PER CALF)

Treatment	Magnesium	Calcium	Phosphorus
(*a*) Sodium chloride	2.11	9.47	9.18
(*b*) Sodium citrate	1.86	9.73	8.43
Significant difference ($P = 0.05$)	0.18	0.52	0.79

The results suggest that the ingestion of small quantities of sodium citrate reduce levels in serum of magnesium and possibly inorganic phosphorus. Since skeletal magnesium is more readily available in the young calf than in the adult cow[6] any factor which interferes with magnesium absorption would be expected to take longer to reduce calf serum magnesium significantly.

The daily intake of citric acid (as sodium citrate) amounted to 1.0 per cent of the dietary dry matter; levels of 0.4–0.7 per cent were reported for fresh perennial ryegrass by Hirst and Ramstad[7]. The occurrence of hypomagnesæmia might thus be associated with increased intakes of citrate from herbage. Further experiments are in progress to extend these observations to the lactating cow and to determine more precisely the effect of citrate intake on magnesium metabolism in the ruminant.

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Difference in Metabolism of Labelled Thyroxine between Thyrotropic and Adrenotropic Mouse Pituitary Tumours

LITTLE is known about the mechanisms within the pituitary which are involved in the control of thyrotropin secretion exerted by circulating thyroid hormone. In the work reported here mouse pituitary tumours were used to examine this problem since each of these several tumour types reflects quite closely the behaviour of its prototype cell in the normal pituitary. The results suggest there may be a special thyrotropin-secreting cell with an unusual pattern of metabolism for thyroxine. Thus, mouse pituitary thyrotropic tumours were found to contain large amounts of labelled 3,5,3′-triiodothyronine (T_3*) 4 hr. after injection intraperitoneally of labelled thyroxine (T_4*), whereas mouse pituitary adrenotropic tumours and other tissues contained none.

Three strains of thyrotropic tumours in LAF_1 mice, originally made available by Furth[1], were used. Several transplants of an adrenotropic tumour in LAF_1 mice also were furnished by Dr. Furth. A spontaneous fibrosarcoma in LAF_1 mice, kidney, muscle and pituitary from thyroidectomized LAF_1 mice, and a carcinoma of the breast in Swiss mice from Dr. S. Graff were examined as control tissues.

With mouse pituitary tumours, valid conclusions generally may be drawn from groups of 4 mice each (Furth, J., personal communication). Nevertheless, all experiments were repeated for confirmation. Groups of 5 mice each were used for the thyrotropic tumours, and groups of 3 mice each with the other tumours due to a limited number of mice available.

Since two of the three thyrotropic strains grow only in thyroidectomized mice, the thyroid was destroyed in all thyrotropic tumour hosts[2]. The adrenotropic tumours were maintained both in intact and adrenalectomized mice given deoxycortisone subcutaneously once a week.

The biological methods[2] and the methods for extraction of serum[3] used by this laboratory have been detailed elsewhere. Chromatographic separation of the iodinated compounds in tissues was done by the methods of Roche *et al.*[4]. 10–20 μc. T_4* (Abbott) repurified, when necessary, by paper chromatography and containing 0.4–1 μgm. stable T_4, were injected intraperitoneally and autopsy performed 4 hr. later. All tissue samples were rapidly weighed, counted for radioactivity to 5 per cent accuracy in a well-type scintillation counter and then frozen and lyophilized.

After chromatography, further identification of the radioactive compounds was made by two-dimensional

Table 1. PRESENCE OF T_3* IN PAPER CHROMATOGRAMS PREPARED FROM THYROTROPIC MOUSE PITUITARY TUMOURS 4 HR. AFTER INJECTION SYSTEMICALLY OF T_4*, BUT NOT FROM OTHER TUMOURS OR CONTROL TISSUE

Mouse strain	Tissue	Radioactivity in tissue/serum ratio	Range of values for percentage distribution of iodinated compounds on chromatograms		
			I*	T_4*	T_3*
LAF_1†	L23 thyrotropic tumour (autonomous, non-responsive)	0.1–0.3	17–54	18–53	28–30
	L24 thyrotropic tumour (autonomous, semi-responsive)	0.1–0.2	27–30	18–40	22–52
	4183 thyrotropic tumour (dependent, non-responsive)	0.1–0.2	4–35	23–65	31–42
	Adrenotropic tumour	0.2–0.3	29–30	70–71	0
	Fibrosarcoma	0.2	27	73	0
Swiss†	Mammary adenocarcinoma 755	0.1–0.2	43–44‡	56–57	0
LAF_1	Kidney	0.3–0.5	25–49	35–55	0
	Muscle	0.05	21	79	0

† See text for numbers of animals used.

‡ Corey, K. D., and Gross, J., *Cancer Res.*, **20**, 476 (1960), have reported formation of radiation iodide from T_4* in experimental mammary tumours.

chromatography, radioautography, and then elution and chromatography of the various radioactive areas.

The results obtained are shown in Table 1. Chromatograms of all three strains of thyrotropic tumours contained T_3* in equal, and high, concentrations, as well as T_4*, iodide and traces of radioactive 3,5,3′,5′-tetraiodothyroacetic acid. The adrenotropic tumours, however, contained no T_3*, and no T_3* was present in control tissues or in serum. A small amount, presumably of 3,3′,5′-triiodothyronine* (reverse T_3*), was demonstrated in chromatograms of serum and of some of the control tissues.

A specific alteration in hormonal environment is required to induce each of the secretory types of experimental mouse pituitary tumour. The resultant tumours are histologically homogeneous and specific. With dependent tumour strains, the same specific end organ hormone is required to suppress growth and hormonal secretion as is normally required to suppress pituitary secretion of the corresponding tropic hormone.

These close similarities to normal pituitary function suggest that each of the secretory pituitary tumours may have originated from corresponding specific anterior pituitary cells and thus reflect their functional behaviour. Ezrin has already postulated[5] from histological evidence that cells secreting thyrotropin and adrenocorticotropin are distinct and can be distinguished from the cells secreting the other hormones of the gland.

Since all three thyrotropic tumours of the present experiment contained a high proportion of T_3* after intraperitoneal injection of T_4* whereas the adrenotropic tumours contained none, the interesting possibility is raised that not only may there be a specific thyrotropin-secreting cell but also that it has a unique metabolic pathway for thyroxine. The formation of large amounts of T_3* could well be related to the inhibitory action which thyroxine exerts on thyrotropin secretion. Unfortunately, both dependent and autonomous thyrotropic tumour strains contained equally high proportions of T_3* and so any relationship to suppression of growth and hence to tumour autonomy or dependency seems to be eliminated.

It is possible that T_4* was metabolized to T_3* elsewhere in the body and was simply concentrated by the thyrotropic tumours. Predilection of the thyrotropic tumour for T_3* as opposed to the adrenotropic tumour would be interesting enough. However, no T_3* was identified in the serum or the various control tissues studied, and T_3* did not accumulate disproportionately in the thyrotropic tumours compared with control tumours and other tissues after T_4* injection[6].

Another possibility, that all pituitary cells deiodinate T_4* to T_3* but lose this system in the formation of adrenotropic tumours, seems unlikely. It is the metabolic behaviour of the thyrotropic tumours which is unique. Similarly, the possibility that the enzyme system responsible for direct diodination to iodide is lost in the thyrotropic tumours so that T_3* is formed alternatively seems excluded by the fact that T_3* is formed by the normal pituitary in about the same relative amounts[8].

This work was aided by Contract *SA*-43*ph*-2402, Cancer Chemotherapy National Service Centre, National Cancer Institute, Public Health Service.

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PHARMACOLOGY

A Micromethod for the Determination of Bradykininogen in Blood Plasma

BRADYKININOGEN is the inactive precursor from which bradykinin, the hypotensive and smooth-muscle stimulating nonapeptide, can be released by the action of trypsin[1]. The concentration of the precursor in blood can be evaluated by the amount of bradykinin liberated by the action of trypsin on known volumes of plasma. The main difficulty found in a procedure for the determination of bradykininogen is the presence of trypsin inhibitors which interfere with the release of bradykinin, and the presence of enzymes which inactivate it in fresh plasma. This difficulty can be overcome if the plasma is previously heated in boiling water in the presence of diluted acetic acid, in order to obtain a denatured substrate. By this procedure trypsin inhibitors and bradykininolytic enzymes are inactivated[2,3].

After an examination of the conditions for the liberation of maximal amounts of bradykinin, a method for the determination of bradykininogen can be described as follows.

Venous blood is collected in a siliconed syringe moistened with 20 units of an anticoagulant ('Liquemine', Roche) and immediately transferred to a 15-ml. siliconed glass or polyethylene centrifuge tube. The blood is centrifuged and 0.2 ml. of plasma is transferred with a siliconed pipette to an 18 × 150 glass tube containing 1.8 ml. of 0.2 per cent acetic acid. The mixture is heated in a boiling water-bath for 30 min., cooled to room temperature in a tap-water bath, and is then adjusted with N sodium hydroxide (*c.* 0.06 ml.) to pH 7.30–7.80 (Merck Universal indicator paper) and buffered at pH 7 8 with 0 5 ml of 0 2 *M tris* buffer The suspension formed is incubated with 0 2 mgm of crystalline trypsin (Worthing Labs), for 30 min At the end of this period the enzyme in the clear digest is inactivated by adding 5 ml of boiled alcohol After immersion of the tubes for 5 min into a water-bath adjusted at 70° C the liquid is quantitatively transferred to a 125-ml. round-bottom flask and evaporated with reduced pressure to dryness The dry material is suspended in 4 ml of saline and assayed for bradykinin in a guinea pig ileum preparation using the 2 × 2 bioassay described by Rocha e Silva[4]

The bradykinin content does not change significantly if the blood is treated 2–4 hr after collecting or if the dry digest is assayed after keeping it in an evacuated desiccator 12–24 hr

The standard used for comparison was a preparation containing 3 units of bradykinin/mgm , one unit of this preparation was equivalent to 0 5 µgm of synthetic bradykinin (prepared by the Sandoz Labs) according to the procedure of Boissonnas *et al*[5]. One unit of bradykininogen is defined as the amount of the precursor able to liberate one unit of standard bradykinin or 0 5 µgm of synthetic bradykinin

Table 1 shows the concentration of bradykininogen found per ml of blood plasma of man and other mammals measured according to the method described above

Table 1 Bradykininogen (units/ml Plasma)

	Average	Range
Man	21 2	14 8–28 5
Ox	28 9	23 7–33 9
Horse	18 9	17 6–19 7
Sheep	12 7	12 0–13 8
Pig	8 1	5 5–11 6
Dog	12 3	12 0–13 0
Rabbit	20 1	17 5–22 7
Guinea pig	17 7	15 8–20 0
Rat	4 0	2 5– 5 0

Average of determinations made on the plasma of 10 men and 5 women (normals), other species, average of 3 animals

The method described is being used in this laboratory for the examination of conditions expected to alter the bradykininogen content of plasma in several types of experimental shock, surgical conditions and pregnancy

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Biological Spectrum of Some New Tuberculostatic 1,3,4-Oxadiazolones with Special Reference to Cross-Resistance and Rates of Emergence of Resistance

THE tuberculostatic activity of 5-(4-pyridyl)-1.3,4-oxadiazolone (*S* 57) has been repeatedly confirmed[1-3], and complete cross-resistance with isonicotinic acid hydrazid (isoniazid) in laboratory tests using *Mycobacterium tuberculosis* is reported by all workers in this field It was, therefore, a little surprising to find that, whereas isoniazid is practically inactive against human leprosy, but active against rat murine leprosy, *S* 57 is highly active against both animal and human leprosy[4-6]

Although isoniazid and *S* 57 show complete cross-resistance in laboratory tests, yet if *H* 37 *Rv* is cultured in the presence of a concentration of isoniazid just sufficient to reduce, but not completely inhibit, growth, and a concentration of *S* 57 one-tenth of that required for growth inhibition, the rate of emergence of resistance to isoniazid is substantially reduced, showing that some difference in the biological spectra of isoniazid and *S* 57 is probably present[7,8] This observation might have some bearing on the unexpected activity of *S* 57 in human leprosy, the more unexpected since it contains, in contrast to most leprostatics, no sulphur in the molecule

The low toxicity and low local irritant properties of *S* 57 led to the extension of the above observations to the salicylate and *p*-amino-salicylate class of substance which shows reduced therapeutic usefulness owing to local irritant properties[9] Conversion of the free carboxy group to the corresponding 1,3,4-oxadiazolones yields compounds possessing similar pharmacological spectra to the original salicylate and *p*-amino-salicylate, but showing, in fact as predicted, reduced toxicity and local irritant action Thus 5-(2′-hydroxyphenyl)-1,3,4-oxadiazolone is an active anti-inflammatory, anti-pyretic and analgesic agent of low irritant properties and toxicity 5-(2′-hydroxy-4′-aminophenyl)-1,3,4-oxadiazolone (*WS* 127) shows a similar tuberculostatic spectrum and order of activity as *p*-amino-salicylic acid, but is considerably more stable chemically Here again complete cross-resistance to various strains of *Mycobacterium tuberculosis* has been observed in laboratory tests using strains resistant to *p*-amino-salicylic acid Yet, on culturing *H* 37 *Rv* in the presence of both *p*-amino-salicylic acid and *WS* 127 at slightly subliminal concentrations the rate of emergence of resistance to both substances is substantially reduced compared with findings obtained using *p*-amino-salicylic acid or *WS* 127 individually on the same strain The same phenomenon has been noted using *p*-amino-salicylic acid and 5-(2′-hydroxy-4′amino-phenyl)-1,3,4-oxadiazol-2-thione

Thus, the laboratory cross-resistance tests commonly used apparently miss some of the finer details of activity, at least in this class of substance, which may be necessary to predict therapeutic effectiveness and rates of emergence of resistance both in animals and man

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HÆMATOLOGY

Isolation of an α_2-Globulin from Human Plasma

While examining a method for the isolation of haptoglobin by chromatography on *DEAE* cellulose[1] we noticed that several α_1- and α_2-globulins were adsorbed under the experimental conditions used One of these proteins, an α_2-glycoprotein, has now been obtained at about 90 per cent purity The protein adsorbed together with haptoglobin, cæruloplasmin, cholinesterase and some α_1-globulins at pH 5 0 from human plasma diluted to a freezing point of $-$ 0 15° is the last of these proteins to be eluted at the same pH but with increasing molarity of acetate buffer Indeed, haptoglobin is eluted at 0 1 *M* acetate buffer, cæruloplasmin together with some α_1-globulin at 0 2 *M* and the unknown protein at 0 3–0 4 *M*

When submitted to starch-gel electrophoresis[2], this protein migrates to the α,β-position Protein π, as it may be called, is an α_2-glycoprotein containing about 10 per cent hexoses Protein π is different from these three well-known α_2-globulins haptoglobin, cæruloplasmin and α_2-macroglobulin It differs from haptoglobin by its electrophoretic mobility on starch-gel, showing but a single band in the α,β-position (Fig. 1) and a somewhat faster migration on paper strip Protein π has no hæmoglobin-binding capacity and no immunological relationships with haptoglobin The new protein is precipitated by rivanol at pH 7, whereas haptoglobin is not The protein under consideration differs from cæruloplasmin in its position on starch-gel at pH 8 6 It is colourless and contains no copper and shows no oxidase activity There is no immunological relationship with ceruloplasmin as studied by the Ouchterlony technique and immunoelectrophoresis Finally, there is no immunological relationship between α_2-macroglobulin and protein π and again the position on starch-gel of the two proteins is quite different

The protein separated by chromatography on *DEAE* from whole plasma contains zinc in various amounts Work is in progress to establish whether zinc is a definite part of the native protein, or a fortuitous contaminant Protein π has been isolated under the same experimental conditions from fraction III as obtained during routine fractionation of human plasma following a slightly modified Nitschmann technique[3] Whole plasma contains about 0 2 gm /l of this protein and some total anti-human-serum gives a precipitation line with the purified protein A screening for specific enzymatic or clotting activity has not yet given a positive result

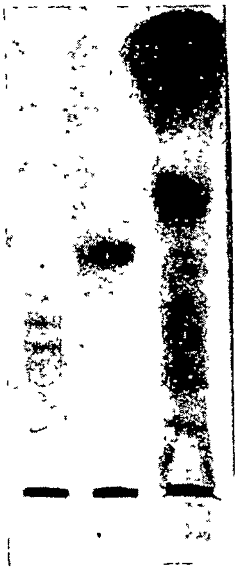

Fig 1 Position on starch-gel of protein π (in the centre), whole plasma (right), isolated haptoglobin (left)

We thank Dr Fine for immunoelectrophoretic analysis

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Quantitative Sub-Groups of the *B* Antigen in Man and their Occurrence in Three Racial Groups

Numerous authors[1–5] have described rare variants of the *B* blood group antigen which may differ qualitatively from other *B* antigens Formaggio[6] concluded from his examination of 250 samples of *B* agglutinogens that differences within the *B* group were quantitative rather than qualitative During the performance of 50 per cent end-point hæmagglutination assays of the *B*-anti-*B* system[7], it was noted that *B* cells from a Negroid individual were more strongly agglutinated than were those of Caucasoid origin The probability–log assay curves in Fig 1 show the difference observed with four doubling dilutions of anti-*B* serum in an equilibrium system with equal numbers of the two types of *B* cells of equal freshness The percentages of cells agglutinated were obtained by the free-cell counting technique described in an earlier paper[8] and are plotted against the logarithms of the antiserum concentrations Since the parallel assay curves indicated that these agglutinogens differed quantitatively rather than quali-

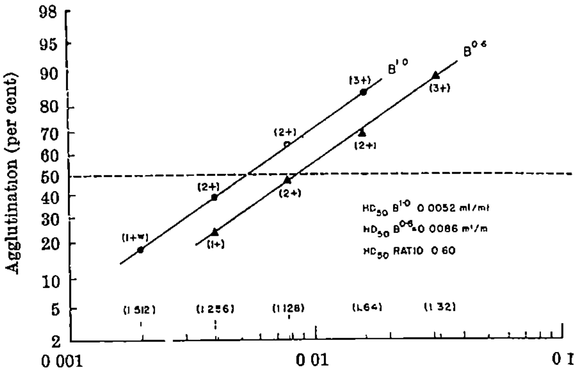

Fig 1 Hæmagglutination assay curves of red cells of two sub-groups of the *B* antigen.

The degree of gross agglutination is indicated in parentheses 3+, Large visible clumps of cells with only a few free cells, 2+, macroscopically many small clumps with more free cells, 1+, macroscopically no visible agglutination, microscopically many small clumps of 4–8 cells, 1+*w*, no visible agglutination macroscopically, microscopically small clumps of 2–4 cells

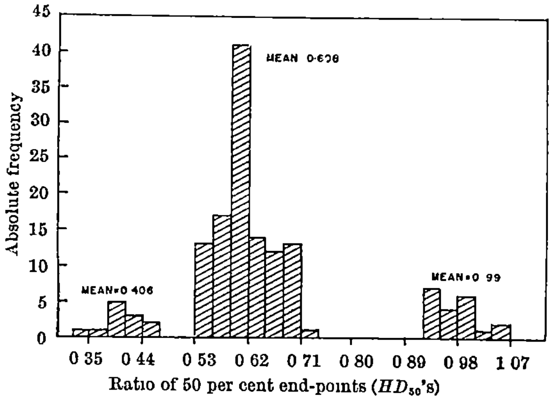

Fig 2 Distribution of HD_{50} ratios for red cells of 143 blood group B individuals

tatively, the relative 'strength' of the B cells could be ascertained from the 50 per cent hæmagglutination end-points or HD_{50}'s interpolated from the assay curves A ratio of the HD_{50}'s showed that the Caucasoid cell possessed only 60 per cent of the agglutinating strength of the Negroid cell This difference was observed with numerous samples from these same individuals over a period of two years, and thus appears to be an intrinsic property of the agglutinogen from a given individual The stronger cell was tentatively assigned a value of unity and provisionally designated $B^{1\cdot0}$ while the weaker Caucasoid cell was termed $B^{0\cdot6}$ to avoid confusion with the terminology B_{100}, B_{80}, B_{60}, etc , proposed by Hirszfeld[9,10] for the B_c (complete) antigen and its transition forms

In order to determine whether quantitative subtypes vary in ethnic groups, samples of group B blood from Caucasian, Negro and Mongolian individuals were collected in equal volumes of modified Alsever's solution at the Washington Red Cross Blood Donor Center The cells were compared with a standard $B^{1\cdot0}$ cell on the day of collection by the assay method Concentrations of standard and unknown test cells were carefully adjusted to 26,000 ± 800 cells/mm^3 by means of the Coulter counter. The frequency distribution of the HD_{50} ratio values for a population of 143 individuals of blood group B is shown in Fig 2 It is evident that a segregation into three distinct populations ('percentage grades') was possible The group of 12 samples which were more weakly reactive than the $B^{0\cdot6}$ group has been designated $B^{0\cdot4}$ since they exhibited approximately 40 per cent the agglutinability of the standard cell The results of the testing of 100 Caucasoid, 25 Negroid and 18 Mongoloid B antigens are presented in Table 1 It is noted that $B^{1\cdot0}$ occurs in half the American Negro population but in only 9 per cent of Caucasians and in none of the Mongolians The $B^{0\cdot6}$ predominates in Caucasians and Mongolians while $B^{0\cdot4}$ appears to be in highest frequency among Mongolian peoples The fact that the results do not accord with the distribution expected of homozygotes (BB) and heterozygotes (BO) suggests that the observed differences are not related to the ABO genotype of the individual In support of this, it was found that samples from one individual whose most probable genotype is BB as evidenced by family studies of three generations, and three from known BO individuals, fell within the same percentage grade ($B^{0\cdot6}$). Subjects were not available for examination of the heredity of these antigens, however, it is reasonable to assume that they are expressions of multiple allelomorphs at the B locus These sub-groups may, indeed, correspond to the pleiades B_m, B_r, B_j described by Hirszfeld and Amzel[10] On the other hand, $B^{1\cdot0}$ may be an exclusive character of the Negroid race since it is possible that the nine Caucasoid $B^{1\cdot0}$ antigens encountered are the result of race crossing Whether $B^{1\cdot0}$ is an exclusive Negroid character or occurs in all races in different frequencies can only be resolved by testing pure racial populations.

Table 1 Distribution of B Antigens in Three Racial Groups

Racial group	Types of B antigen $B^{1\cdot0}$	 $B^{0\cdot6}$	 $B^{0\cdot4}$
Caucasoid	8	87	9
Negroid	12	13	0
Mongoloid	0	15	3

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A Correlation between Surface Charge and Coagulant Action of Phospholipids

The property of water-insoluble amphipathic molecules to alter the electrokinetic charge of lipids has been exploited by Bangham and Dawson[1] in elucidating the charge requirements of certain enzyme/substrate interactions Their results show clearly the interdependence of both sign—positive or negative—and magnitude of enzyme and substrate interaction in any particular ionic environment Whereas variation of pH, uni- or di-valent ion concentrations may affect the charge on both enzyme and substrate simultaneously, the addition of a water-insoluble amphipathic molecule to the lipid ensured an alteration of the substrate exclusively

In the light of this experience and of the apparent anomalies in the ability of various phospholipid emulsions to accelerate the clotting of plasma[2-4], an attempt has been made to correlate the ζ potential of lecithin/amphipathic molecule mixtures with their effects on the clotting reaction promoted in plasma by Russel viper venom

To 1 5 µmole-aliquots of ovo-lecithin in chloroform (isolated from mixed egg phospholipids by chromatography on alumina and silicic acid columns) were added progressive volumes of synthetic di-cetyl-phosphoric acid (0 66 µmole/ml) in chloroform Each mixture was taken to dryness *in vacuo* and emulsified in 1 0 ml of a 0 045 *M tris* buffer in 0 1 *M* sodium chloride Electrophoietic mobilities of the emulsion particles were measured by direct observation in a horizontal microelectrophoresis cell of the type described by Bangham, Flemans, Heard and Seaman[5] The clotting system utilized fasting human plasma, obtained by centrifuging citrated blood

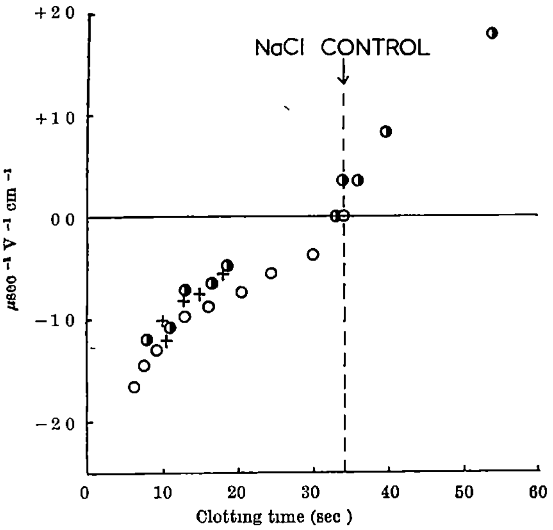

Fig 1 Curves to show relationships between clotting time and electrophoretic mobility, positive or negative, of various lecithin mixtures Mobilities were carried out in 0 045 *M tris* in 0 1 *M* sodium chloride at *p*H 7 2 Clotting times represent means of three measurements

○—○, 0 16 μmole lecithin plus varying amounts of dicetyl phosphoric acid, ◐—◐, 0 16 μmole lecithin plus 0 045 μ*M* dicetyl phosphoric acid to which is added varying concentrations of C_{22}-pyridinium bromide, ×—×, 0 16 μmole lecithin plus varying concentrations of phosphatidyl ethanolamine

first at 3,000 r p m for 10 min and then at 20,000 r p m for 15 min To 0 1 ml of such plasma were added 0 1 ml of the mixed phospholipid emulsion, 0 1 ml of 1 100,000 'Stypven' brand (Burroughs Wellcome and Co) Russel viper venom in water and finally 0 1 ml 0 025 *M* calcium chloride All reactants were maintained at 37° C and the clotting reactions were followed at this same temperature

Fig 1 shows the relationship between the electrophoretic mobilities of the various lecithin/dicetyl phosphoric acid mixtures added to plasma and the observed clotting times It can be seen that as the negative charge on the suspension increases, its ability to accelerate the clotting induced in plasma by Russel viper venon also increases

The suspension promoting the most rapid clotting has a mobility of 1 6 sec $^{-1}$ volt^{-1} cm $^{-1}$ This agrees remarkably closely with the value for the particles present in the brain extract described by Bell and Alton[6] (Table 1) Lecithin/phosphatidyl ethanolamine mixtures are also negatively charged (due to the partial ionization of the amine group at *p*H 7 2) and the mobility/clotting time relationships coincide with those of lecithin/dicetyl phosphoric acid mixtures (Table 1 and Fig 1)

That the surface charge is, in all probability, intimately concerned in the reaction is emphasized by the observation that progressive neutralization of the negative charge of the suspension by the addition of C_{22}-pyridinium bromide to the lipid mixture results in a proportionately decreased effectiveness as evidenced by the prolongation of clotting time (Fig 1) Addition of excess C_{22}-pyridinium bromide yields a positively charged lecithin/dicetyl phosphoric acid suspension causing an extended clotting time This latter observation, too, holds important implications for the possible role of orientated surfaces in the whole process of blood clotting

Table 1 COMPARISON OF VARIOUS PHOSPHOLIPID MIXTURES AND THEIR COAGULANT ACTION

Phospholipid mixture	Mobility (μsec $^{-1}$ volt^{-1} cm $^{-1}$)	'Stypven time' (sec) av of 3 readings
Tris/saline control	0	24
0 163 μmole lecithin	Iso-electric	24
0 115 μmole lecithin + 0 035 μmole dicetyl phosphoric acid	−1 6	7 0
0 034 μmole lecithin + 0 120 μmole phosphatidyl ethanolamine	−1 04	8 0
Brain phospholipids, Bell and Alton (ref 6)	−1 69	7 9
Platelets	−1 2	8 7
0 163 μmole lecithin + 0 05 μmole dicetylphosphoric acid + 0 08 μmole C_{22}-pyridinium bromide	+1 8	34 0

The effects on clotting time of other amphipathic molecules having alternative anions, for example, fatty acids, are at present under investigation However, in view of the slightly different affinities of calcium ions for phosphate as compared with carboxyl anions, it is quite probable that the optimum mobilities of suspensions activated by fatty acid, in *tris*-buffered saline, may be different from those reported in Table 1

Final appraisal of the relationships between the surface charge of orientated phospholipids and their possible catalytic role in enhancing blood clotting must await measurement of clotting time in the presence of a constant surface area of test lipid

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HISTOCHEMISTRY

Histochemical Examinations of Chymotrypsin-like Esterases

IN 1953 Gomori[1] discovered a tissue enzyme which hydrolysed chloracetoxy naphthoic acid anilide Mast cells were found to have a large amount of this enzyme activity Based on the demonstration that the time required to achieve a given colour intensity in an enzymatic histochemical system is inversely proportional to enzyme activity[2], it was estimated that mast cells had 30 times the activity of liver and five times the activity of neutrophiles Later, Benditt and Arase[3] were able to show that the enzyme in mast cells strikingly resembled bovine α-chymotrypsin The present work has carried the resemblance further and examined the distribution of the enzyme in the rat, utilizing a newly synthesized series of esters of naphthoic acid anilide ('Napthol *AS*')

The presence of a phenyl group in the acidic portion of an ester is an established character of specific chymotrypsin substrates[4]. Esters of 'Napthol *AS*' were therefore synthesized beginning with the benzoxy ester and sequentially adding a methylene group between the carboxyl carbon and the phenyl ring, giving the phenylacetoxy, phenyl proprionoxy and phenylbutyroxy esters

The use of diazo coupling with the free naphthol released by hydrolysis to measure enzyme activity in the test-tube was unsatisfactory because of (1) the relatively slow coupling which precluded tion, based on the efflux of sodium, increases the sodium uptake to 33 pmoles/cm.^{2}/stimulus. By direct recording, each 4-sec. stimulus was found to elicit, on the average, 4–5 repetitively produced spikes. Thus, the sodium uptake per spike is 7–8 pmoles/cm.^{2}. Using the highest value for the spike in uterine smooth muscle of 73 mV.[5] it can be readily shown that movement of this amount of sodium is capable of discharging a membrane capacity of 9–11 μF./cm.^{2}, a value which closely resembles that of other vertebrate excitable membranes. On the other hand, if the membrane capacity of the rabbit myometrial cell is assumed to be the same as that of frog sartorius muscle fibre or that of sheep Purkinje fibre[6], the observed and estimated amount of sodium moved could cause a change of membrane potential of about 70 mV. Thus, even without correcting for diffusion delay, which is appreciable in this tissue, the results strongly suggest that the ionic hypothesis of excitation can be applied to the rabbit myometrium.

The half-time of extracellular elution of sodium-22 is 10·3 ± 0·71 min. (mean ± *S.E.* of 22 preparations). From this and the thickness of the preparations the diffusion constant of sodium was calculated to be $0{\cdot}202 \times 10^{-6}$ to $1{\cdot}16 \times 10^{-6}$ cm.^{2}/sec. At the maximum, this is one-fifth the value in frog sartorius muscle[7]. The slow extracellular diffusion together with a higher intracellular sodium concentration[8] could conceivably allow local accumulations of sodium even when the entire preparation is immersed in sodium-deficient media. Probably such an explanation can account for the observation that action potentials in smooth muscles seem to be independent of the external sodium concentration.

This work was supported in part by a grant (*A*–1713) from the National Institutes of Health, U.S. Department of Health, Education and Welfare. Support for one of us (D. Z.) was derived from funds allotted by the U.S. Public Health Service and the National Science Foundation.

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Effect of Some Smooth Muscle Stimulants on the Motor Response of the Isolated Guinea Pig Vas Deferens to Hypogastric Nerve Stimulation

RECENTLY potentiation of the response of the cat nictitating membrane to sympathetic nerve stimulation has been shown to occur with serotonin[1] and substance *P* (ref. 2). Burn and Rand[3] have found that circulating noradrenaline increases the responses of organs to sympathetic stimulation.

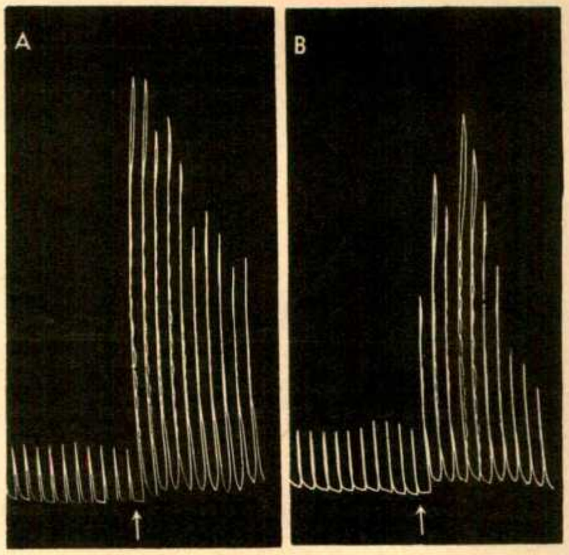

Fig. 1. Contractions of the isolated guinea pig vas deferens in response to hypogastric nerve stimulation (10 V., 2 msec., 5 pulses/sec.) for 5 sec. at 2-min. intervals; bath volume 50 ml. *A*, At arrow, addition of 0·01 μgm. acetylcholine; *B*, at arrow, addition of 5 μgm. adrenaline

Some results are presented here showing that other smooth-muscle stimulants can modify the motor response to nerve stimulation of sympathetically innervated smooth muscular organs. The vas deferens was chosen because this organ is stimulated by most of the smooth muscular stimulants occurring in the body.

The vas deferens with the hypogastric nerve was prepared according to the method described by Huković[4]. The organ was placed in a 50-ml. bath containing Tyrode solution at 37° C. and aerated with oxygen. The contractions were recorded with a frontal writing lever on a smoked drum. The hypogastric nerve was placed on stimulating electrodes and immersed in liquid paraffin. The nerve was stimulated every 1 or 2 min. for 5–10 sec. with a stimulation of supramaximal strength (8–15 V.) and a duration of 2 msec., the frequency being in the range 5–20 per sec. The responses remained for at least 5–6 hr.

Contractions of the isolated guinea pig vas deferens could be elicited by addition to the bath fluid of adrenaline, noradrenaline, acetylcholine, histamine and serotonin although the concentrations required were rather high. Concentrations of at least 5–10 μgm./ml. of adrenaline and noradrenaline had to be used to obtain a moderate contraction. The effective dose of acetylcholine was lower (1–10 μgm./ml.) and those of histamine and serotonin were higher (10–80 μgm./ml.). In general these substances could be ranked in the following order: acetylcholine > adrenaline > noradrenaline > histamine > serotonin.

It was observed, however, that doses of smooth muscular stimulants, which were too small to induce contractions of the isolated vas deferens, caused a conspicuous potentiation of the motor response of the organ to nerve stimulation. Doses ten to a thousand times smaller than those required for eliciting a moderate contraction of the organ increased the response to nerve stimulation two or three times or even more without any gross changes in the basal tone of the ductus. The ability for potentiation followed the same order as that for stimulation. The

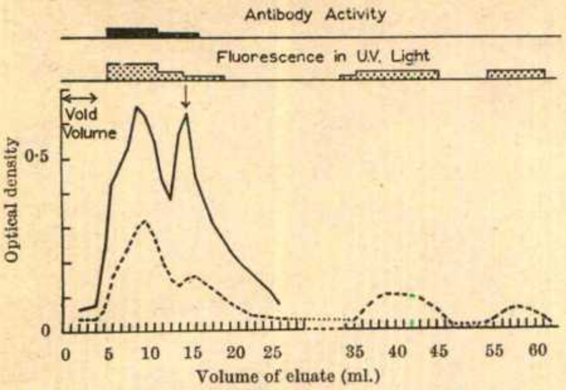

Fig. 1. Elution diagram for an 8-ml. volume of γ-globulin fraction of rabbit anti-human γ-globulin labelled with fluorescein isothiocyanate. The column of 'Sephadex *G*-25', 11 cm. × 0·6 cm. diameter, at room temperature, was eluted with phosphate saline buffer *p*H 7·4. Millilitre eluates were collected. Maximal fluorescence and antibody activity corresponded to eluates of maximal protein and dye absorption. Absorption at 280 mμ, ——; absorption at 490 mμ (dye), - - -

Examination in agar-gel diffusion plates (Fig. 2) shows sharp limitation of antibody activity to the volume of eluate corresponding to the volume of original protein solution and to that giving maximal absorption at 280 mμ (that is, little or no dilution of antibody activity). This was confirmed by the finding that negligible alteration in antibody titre occurred in the eluates. Protein recoveries of up to 95 per cent were obtained in volumes of eluate equivalent to the original volume of the conjugate.

Apart from the use described here, cross-linked dextran gels can also be profitably used for the concentration of fluorescent antibody activity where this is weak, using the method suggested by Flodin, Gelotte and Porath[8] for concentrating solutes of high molecular weight.

Purification of dye-protein conjugates by this method appears to be quicker, simpler and more efficient than other methods[9] at present in use.

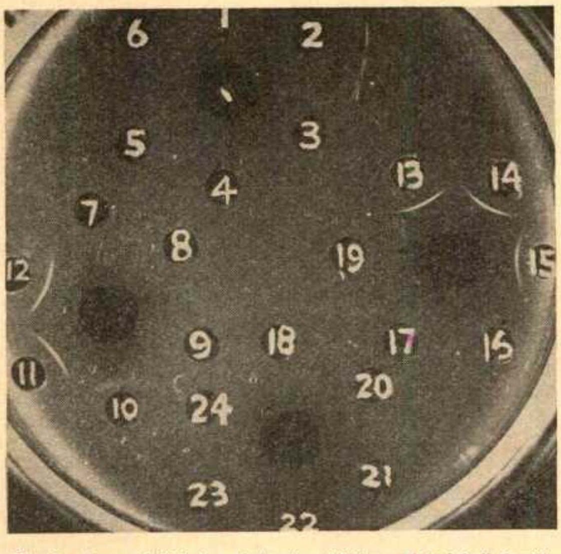

Fig. 2. Agar gel diffusion plate of anti-β-lipoprotein labelled with fluorescein isothiocyanate against β-lipoprotein. After eluting from the column of 'Sephadex *G*–25', samples were taken from each of the ml. eluates and put up against β-lipoprotein. Holes 1–9, representing the void volume of the column, show no precipitation, whereas holes 10–16, representing the volume of protein solution originally put on column, show precipitation. Holes 17–24 show no precipitation

Our thanks are due to Pharmacia Ltd., Uppsala, for free samples of 'Sephadex', and to Dr. C. Ricketts, Medical Research Council Unit, Accident Hospital, Birmingham, for his advice.

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PHYSIOLOGY

Sodium Influx and Excitation in Uterine Smooth Muscle

The applicability of the ionic hypothesis of excitation, more specifically the transient increase of sodium permeability during an action potential[1], has not been extended to smooth muscles in general. This is due largely to serious technical difficulties in impaling the small cells of smooth muscles, as shown by the comparatively low and widely variable values of membrane potentials for smooth muscles. Direct tests of the effect of sodium deprivation on the action potential are therefore rather difficult to make. Additional confusion has been caused by claims that action potentials persist in media containing very little sodium[2] or no sodium at all[3].

An alternative approach to test the applicability of the ionic hypothesis to smooth muscle is to determine the sodium influx associated with each impulse to see whether the charge carried by the sodium ions moving across the excitable membrane can account quantitatively for the displacement of the membrane potential occurring during an action potential. This approach has been used in paired comparisons on isolated myometrial strips from œstrogen-dominated uteri of rabbits. The strips were incubated in a sodium-22 containing Krebs's bicarbonate solution. One of the pair was left unstimulated, and at the incubating temperature of 24° C. spontaneous activity was infrequent. The other of the pair was stimulated in end-to-end fashion by 4-sec. long, 60-c./s. a.c. stimuli, once every minute for about 2 hr. When the relative amounts of sodium-22 eluted from the muscles were plotted against time, the resulting curves could be fitted by exponential equations with two terms. From the intercept values and the rate constants of both terms, the total intracellular sodium-22 at the beginning of the elution was calculated according to the method discussed by Huxley[4].

In paired comparisons of muscle strips allowed to recover for 3 hr. before being loaded with sodium-22 the extra uptake of sodium due to applied stimulation was 16·3 pmoles/cm.^{2}/4 sec. stimulus (mean of 6 pairs, with total stimuli of 120, *S.E.* ± 3·06). Since the stimuli were applied once every minute, allowance must be made for the sodium that entered the cell but was then extruded between stimuli. This correc-

98 per cent of the salts and only 10–15 per cent of the activity are lost during dialysis But as only small quantities of lysozyme are involved in the preparation it was necessary to eliminate all the salts without any further loss of activity This is obtained by filtration on 'Sephadex *G*–50' column 50 × 1 2 cm. (buffer, pyridine 0 3 *M*, HAc 0 1 *M*; *p*H 5 5) (Fig. 2).

Chromatographically pure lysozyme from human milk After re-chromatography on carboxymethylcellulose, the lysozyme from human milk is obtained with a constant specific activity Furthermore, no increase of the purity was observed after filtration on 'Sephadex *G*–50' The fact that the lysozyme obtained in this way is chromatographically pure has been again verified by submitting the enzyme to a ion-exchange (partition) chromatography on 'Amberlite *CG*–50' The lytic enzyme can be chromatographed on a 20 × 0 9 cm. column buffered with a 0 2 *M* phosphate buffer of *p*H 7 0, without any change of the *p*H, the molarity or the temperature of the buffer during the chromatography (elution after 50 ml)

Some properties of the lysozyme The lysozyme from human milk, as all previously examined lysozymes[1], is very stable at acid *p*H it resists to 100° C for 3 min at *p*H 4 5 without any significant loss of activity, it is, however, unstable at alkaline *p*H at *p*H 7 5 a loss of 30 per cent of the activity occurs after 1 min at 100° C If the specific activity is by definition 1 for hen's egg white lysozyme, it is about 2 for human milk lysozyme The molecular weight is approximately 15,000 (experiments made with calibrated 'Cellophane' membranes) The first results concerning the quantitative amino-acid composition indicate that the new lysozyme is not identical with hen's egg white lysozyme

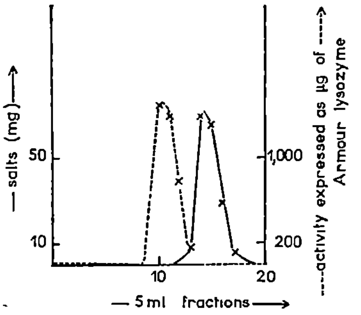

Fig 2

In any event the new lytic enzyme is a lysozyme as it possesses the principal properties of this group it is a basic protein, with a molecular weight near 15,000, stable at acid *p*H unstable at alkaline *p*H, active against suspensions of *M lysodeikticus*, and liberating by its action on an appropriate substrate compounds which can be detected by reagents for reducing sugars and for *N*-acetylhexosamines

We are indebted to Mrs Bertrand (Ecole de Puériculture, Paris) for human milk

This work was supported by grant *FG–Fr*–106 from the U S Department of Agriculture

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Purification and Concentration of Dye-Protein Conjugates by Gel Filtration

It was shown by Porath and Flodin[1] that filtration through cross-linked dextran gels served as a simple method for the separation of materials of varying molecular size This procedure has now been found to be of particular value for the purification of antibody-fluorescent dye conjugates for use in the Coon's[2] technique, since unconjugated dye and dye impurities are removed so that non-specific staining of the tissues has thereby been greatly reduced or abolished

Conjugates of rhodamine[3] and fluorescein isothiocyanate[4] with (*a*) the γ-globulin fraction separated by cold ethanol[5] of rabbit antisera directed against human γ-globulin and β-lipoprotein, (*b*) with whole goat anti-rabbit antiserum and with whole rabbit anti-hen antiserum, were treated as follows Columns of 'Sephadex *G*–25' (Pharmacia, Ltd , Uppsala) were prepared by shaking 10 gm of 'Medium Sephadex *G*–25' (100–250 mesh) with 200 ml of sodium chloride phosphate buffer at *p*H 7 4 for 1 hr allowing the gel to sediment and decanting the supernatant (including any fine particles) This procedure was repeated twice The swollen gel was then suspended in a fresh volume of 200 ml of buffer and slurried into a burette, the lower end of which was plugged with glass wool Columns varying in length from 10 to 30 cm × 0 6 cm diameter were constructed, care being taken to obtain even packing of the gel The 'void volume' of the column was determined with indian ink

2–5 ml aliquots of the protein solutions labelled with dye were applied to the top of the column and allowed to filter through at room temperature When the conjugate had soaked into the gel, it was eluted with phosphate-saline buffer at *p*H 7 4 Measured volumes of the eluate were collected, the optical density measured at 280 mμ for protein, at 490 mμ (absorption maximum for fluorescein) and 560 mμ (absorption maximum for rhodamine) Aliquots from each eluate were also examined by the Ouchterlony[6] technique and its modification by Gell[7] against the appropriate antigens for identification and titration of antibody The eluted conjugate was compared with the original (untreated) material by paper electrophoresis It was also tested by application to sections cut at – 25° C of mouse- and rat-liver taken from animals injected intravenously with the appropriate antigens and killed immediately, and to human pathological material

It can be seen from the elution diagram (Fig 1) that the peak due to protein corresponds to that due to dye in the volume of eluate immediately following the void volume of the column It was repeatedly observed that a second protein peak (marked by an arrow in Fig 1) occurred, accompanied by only a slight peak in dye absorption, suggesting partial fractionation of protein which, under the conditions employed for conjugation in these experiments, was less completely labelled with fluorochrome Further elution of the column gave fractions giving the characteristic absorption of dye alone, unaccompanied by protein

This sequence could also be observed visually since, during elution, a band of coloured conjugates could be seen to separate from coloured bands of free dye. In the case of rhodamine isothiocyanate, four or five bands differing in colour were seen, suggesting the presence of several impurities in the batch utilized

It is interesting that each of the major glycolipids occurring in plants (the sulpholipid and two β-D-galactosyl diglycerides[8,9]) possesses a linkage which may be cleaved by β-galactosidase.

We are indebted to Prof. R G. Wolfe, of the University of Oregon, for the β-galactosidase preparation, and to Prof F J Reithel, of the same University, for his interest in this work

This work was supported by a grant from the U S National Science Foundation

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Interaction between Lysozyme and a Phospholipid of Neoplastic Tissue (Oncolipin)

LYSOZYME is a basic protein, tending to form complexes with fatty acids, aliphatic long-chain alcohols[1], alkyl sulphates[1,2], colic acids[3], and cephalins[4] Interaction promoted by lysozyme towards Rous sarcoma phosphatides is of particular interest[5]

We have examined the possibility of lysozyme producing insoluble complexes with a phospholipid (oncolipin) isolated in malignant tissues by Aoyama[6]

Tests on interaction have been performed on phosphate 0 067 buffers, using Sorensen's technique

Oncolipin was isolated from Yoshida's ascite sarcoma in rats This sarcomatous phospholipid is suspended in saline solution and the above suspension is used for preparing the 0 01 per cent solution of the substance contained in phosphate buffers, at *p*H ranging from 5 9 to 8 2

Table 1 TURBIDITY RELATED TO HYDROGEN-ION CONCENTRATION, IN SÖRENSEN'S PHOSPHATE BUFFERS

Sample (per cent)	Hydrogen-ion concentration (*p*H) 5 9	6 6	7 1	7 6	8 0	8 2
Lysozyme 0 05	0 004	0 004	0 004	0 004	0 004	0 004
Oncolipin 0 01	0 022	0 020	0 024	0 027	0 029	0 027
Oncolipin 0 01 + Lysozyme 0 001	0 036	0 034	0 032	0 038	0 041	0 038
Oncolipin 0 01 + Lysozyme 0 005	0 051	0 053	0 061	0 065	0 068	0 068

The 0 01 per cent solution of oncolipin in phosphate buffers is slightly turbid By addition of lysozyme, the turbidity becomes more evident, and proportionally to the added quantity of the enzyme

It is concluded that lysozyme interacts with oncolipin, at saline hydrogen-ion concentrations, by promoting formation of insoluble complexes This behaviour of lysozyme is like that of another basic protein, namely salmine, being also an insoluble product interacting with oncolipin, at *p*H 6 8–7 0 (ref 6)

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Lysozyme from Human Milk

CONTINUING our research on lysozymes from different origins[1], we were interested in the purification of a human lysozyme Some preliminary experiments have indicated that human placenta, saliva and spleen contain lysozymes, approximately 4, 10 and 40 mgm /kgm respectively (quantities expressed in mgm of hen's egg white lysozyme). In contrast with cow's milk, we have found that human milk contains a lysozyme (40 mgm /l expressed in mgm of hen's egg white lysozyme)

We now report the isolation of this new lysozyme which we obtained in a chromatographically pure state.

The purification includes three steps

(1) Adsorption of the lysozyme on 'Amberlite *CG*–50' buffered with a 0 2 *M* phosphate buffer of *p*H 6 5 50 ml of 'Amberlite' are added to 1 litre of milk and the mixture is stirred for 4 hr The resin is then washed with water and the lysozyme is eluted with a 0 8 *M* phosphate buffer of *p*H 6 5. After dialysis against water at 2° C and lyophilization, a 'primary material' is obtained the lysozyme has undergone a 250-fold purification

(2) Chromatography of the primary material on carboxymethylcellulose The conditions are indicated in Fig 1 200 mgm of the primary material are chromatographed on a 40 × 1 8 cm column at 20° Lysozyme activity was determined by its bacteriolytic action on *Micrococcus lysodeikticus*[2] After re-chromatography of the lysozyme-containing fractions in the same conditions, a symmetrical peak, active against *M lysodeikticus*, is obtained where the specific activity is constant In this way, the primary material was again purified 7–8 times

(3) Desalting of human lysozyme on 'Sephadex *G*–50' The lysozyme-containing fractions after chromatography on carboxymethylcellulose are pooled, dialysed against water at 2° C and lyophilized

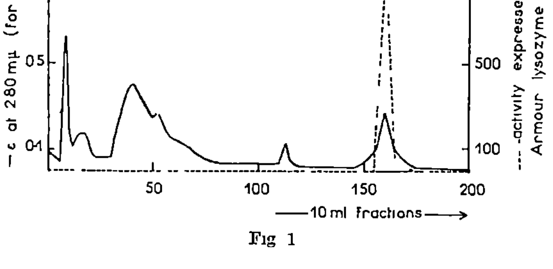

Fig 1

unpublished results) A serious criticism of all this work is that the identification of cystine, or its oxidation product cysteic acid, depends on non-specific colour reactions, and its presence is inferred rather than proved

It is now shown that to isolate the 2 4-dinitrophenyl derivative of cysteic acid from silk hydrolysates in quantities sufficient for positive identification by analysis is possible The material isolated can be determined quantitatively, for example, colorimetrically using a Spekker photoelectric absorptiometer, and the minimum cystine content of silk can thus be found Preliminary work indicates that for *Bombyx mori* this value is about 0 13 per cent The analytical aspects of this work will be discussed elsewhere, but in agreement with other workers[1,2] it seems very probable that this figure is near the true value The isolation of the cystine derivative was performed as follows

Silk fibroin (*Bombyx mori*) was degummed in solution of *p*H 1 5 at 95° C (ref 5), the usual alkaline method being avoided to prevent destruction of cystine The washed and dried fibroin (10 gm) was refluxed for 8 hr with hydrochloric acid (150 ml) and the solution evaporated to dryness *in vacuo* over solid sodium hydroxide The residue was dissolved in water (200 ml) neutralized with sodium bicarbonate (20 gm) and further sodium bicarbonate (40 gm) was added together with 2 4-dinitrofluorobenzene (25 ml) in ethanol (300 ml) The whole was shaken for 12 hr in an open flask and filtered The residue was washed with water (100 ml) and the washings, together with more water (300 ml), were added to the alcoholic solution The solution of the dinitrophenyl derivatives was extracted with ether (ethereal solution rejected), acidified with concentrated hydrochloric acid and extracted twice more with ether The ethereal solution of the dinitrophenyl derivatives was evaporated to dryness, 4 per cent performic acid (50 ml) added, the solution allowed to stand overnight and again evaporated to dryness The residue was shaken for 1 hr with water (50 ml), filtered and the solid extracted twice more with two further volumes (50 ml each) of water The combined aqueous extracts were extracted twice with ether (ethereal extracts rejected) and evaporated to 5 ml

Electrophoresis of this solution was carried out using a Shandon continuous electrophoresis apparatus (No 2520) with Whatman 3 *MM* paper using 33 per cent acetic acid as solvent It was more satisfactory to dispense with the nylon envelopes enclosing the electrodes Using 300 V which gave a current of 16 m amp the yellow solution of the dinitrophenyl derivatives separated into two sharp bands Band I moved vertically down the paper with a slight deviation towards the cathode, the solution from this band being rejected A second band (II) of equal intensity moved strongly towards the anode The solution from band II was collected and evaporated to dryness The orange-brown very deliquescent solid was dissolved in acetone, filtered and carbon tetrachloride added to the solution The whole was evaporated to dryness and kept over phosphorus pentoxide This material and the authentic dinitrophenyl derivative of cysteic acid migrated identically when subjected to electrophoresis Yield, 35 4 mgm

Found (Weiler and Strauss) C, 32 8, H, 3 0, N, 11 8, S, 9 0 $\mathrm{C_9H_9O_9N_3S}$ requires C, 32 3, H, 2 7, N, 12 5, S, 9 5 Synthetic $\mathrm{HO_3SCH_2CH(COOH)\text{-}NHC_6H_3(NO_2)_2}$ (found) C 32 0, H, 3 0, N, 11 9, S, 9 3

We wish to thank the Wool Textile Research Council for financial assistance towards this investigation

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Hydrolysis of α-Sulphoquinovosides by β-Galactosidase

Every photosynthetic tissue so far examined has been found to contain a high concentration of a sulpholipid[1] the structure of which has been elucidated as 6-sulpho-α-D-quinovopyranosyl-(1→1′)-2′,3′-diacyl-D-glycerol[2,3] A high concentration of the deacylated form of the sulpholipid, *G*-quinSO$_3$H, occurs in *Chlorella* and *Scenedesmus*[4] This unique glycoside is cleaved to yield glycerol and 6-sulpho-D-quinovose by a highly purified preparation of β-galactosidase from *Escherichia coli ML* 308 (ref 5)

Enzymatic reactions were carried out at *p*H 7 0 25° C with tracer amounts of *G*-quinSO$_3$H labelled with sulphur-35 with or without synthetic carrier[6] The hydrolysis followed typical first-order reaction kinetics when the radioactive substrate (approximately 10^{-8} *M*) was used without carrier Hydrolysis of *G*-quinSO$_3$H by β-galactosidase was inhibited by the addition of a normal substrate, β-D-galactopyranosyl glycerol (kindly supplied by Prof H E Carter), to the reaction medium, suggesting that the reactions of the enzyme with both types of substrate take place at the same site on the enzyme

2-Hydroxyethyl or 3-hydroxypropyl sulphoquinovoside exhibited susceptibility to hydrolysis by β-galactosidase The methyl, ethyl and *n*-propyl glycosides were, however, completely resistant The β-glycopyranoside and the furanoside isomers as well as α-D-glucopyranosyl-(1→1′)-glycerol were not affected by the enzyme Sequoyitol and (+)-pinitol (kindly supplied by Dr Laurens Anderson) did not affect the hydrolysis of the β-galactoside and *G*-quinSO$_3$H, even though they are related to each other

These observations indicated that the hydrolyses of the 6-sulphoquinovosides are abnormal but highly specific β-Galactosidase has been considered strictly specific in its substrate requirement Only glycosides with the β-D-galactopyranoside structure have been considered susceptible to its hydrolytic action[7] Since the enzyme attacks certain sulphoquinovosides, as well as β-galactosides, the active site[7] should bind both types of substrate Although it is difficult to establish a mechanism at this stage, it is interesting that the distances between the oxygen atoms on carbons 1 and 4 are equal in both substrates and that the 2′- or 3′-hydroxyl group of the glycerol and one of the oxygen atoms of the sulphonic acid of *G*-quinSO$_3$H may occupy relative positions identical with those of the hydroxyl groups of carbons 2 and 3, respectively, of the β-D-galactopyranoside Since a hydroxyl group of the aglycone was essential for the susceptibility to β-galactosidase action, it must play a part in determining the specificity of the enzyme gelatin.) In an earlier communication[3] dealing mainly with inhibition of gelation of gelatin by alkaline copper (II), it was reported that a cobalt (II) complex prepared at *p*H 11.5 also prevented gelation. The correct reading is that a *p*H 11.5 nickel (II) complex, λ_{max} 435 mμ, prevented gelation, and that on acidification gelation occurred. The cobalt complex reported earlier was formed at higher alkalinity and was actually the cobalt (III) complex. At that time the cobalt (II) complex was not observed because the very slow adjustment of *p*H permitted oxidation to complex *B* without forming a noticeable amount of complex *A*.

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Formation of 2-Heptanone from Caprylic Acid by Spores of Various Filamentous Fungi

IT was shown recently that washed, non-germinating spores of *Penicillium roqueforti* are capable of producing 2-heptanone from caprylic acid[1]. In examining the mechanism of this reaction[2], other fungal spores were examined for their ability to produce a ketone from the fatty acid. It is significant that spores from almost half the fungi selected converted caprylic acid to heptanone, thus indicating the more general occurrence of a reaction thought to be limited to only a few moulds.

The fungi were grown on tomato juice–agar slants in Roux bottles, and after incubation at 25° C. for 3–10 days (depending on the growth-rate of a particular fungus) the spores were collected by adding water to the bottle and scraping the surface of the mycelium with a flattened metal rod. This mixture was then filtered through several layers of cheesecloth to remove bits of agar and hyphal cells. The spores were removed from suspension by centrifugation and then 'washed' several times in distilled water by centrifugation. The spore concentration was adjusted to 10^9/ml. by using a conventional hæmacytometer. Ketone-forming activity remained constant over a period of several months when the spores were stored in water at 4° C.

To avoid using large amounts of spores, which required more time in culturing and collection, the reaction was conducted in Warburg vessels. The reaction mixture consisted of 1.0 ml. of spore suspension, 1.0 ml. of 0.5 *M* phosphate buffer at *p*H 6.0 or 7.0 and 0.2 ml. of 0.1 *M* sodium caprylate; 0.2 ml. of 20 per cent potassium hydroxide was placed in the centre well to absorb carbon dioxide. Thus, the oxygen consumption of the spores could be followed manometrically and the general direction and rate of the reaction could be observed. Heptanone production was proportional to the consumption of oxygen; if no uptake of oxygen was observed, no heptanone was produced. After 3–5 hr. the Warburg vessels were removed from the bath and the contents of the vessels were steam-distilled directly into 1.0 ml. of a 0.1 per cent solution of 2,4-dinitrophenylhydrazine. The samples, collected in the hydrazine solution, were stored at 4° C. over-night. The following day the hydrazone crystals were washed, recrystallized from hot 95 per cent ethanol and the melting points determined. The hydrazones were also chromatographed at room temperature in a closed glass cylinder with absolute methanol/water (9 : 1 by volume) as the developing solvent. The compounds were spotted on a sheet of Whatman No. 1 filter paper and after 6 hr. development the R_F of the unknown was compared with that of the known hydrazone of 2-heptanone.

It is noteworthy that spores from the majority of the *Aspergillus* sp. (9 out of 11) and *Penicillium* sp. (9 out of 12) and some related moulds including *Paecilomyces varioti* and *Scaputariopsis brevicqulis* rapidly converted caprylate to 2-heptanone. However, none of the spores of Mucorales (*Mucor hiemalis*, *Mucor mucedo*, *Phycomyces blakesleeanus*, *Rhizopus stolonifer*, *Thamnidium elegans*, and others) was capable of the reaction.

Spores of *Penicillium roqueforti* and *Aspergillus ochraceus* were found to produce identically the same amount of 2-heptanone. Some of the fungal spores produced more ketone at *p*H 6.0 and others more at *p*H 7.0. Although the reaction with some of the spores may have occurred at a lower or higher *p*H, our only interest in these particular investigations was to determine the range of the reaction among the fungi selected. The pathway for the conversion of fatty acids to ketones by spores of filamentous fungi is being investigated and the results of these experiments will be published shortly.

We are indebted to Prof. W. F. Whittingham and Prof. K. B. Raper for furnishing the cultures for these experiments. This work was supported in part by grant *E*-1201 from the U.S. National Institutes of Health.

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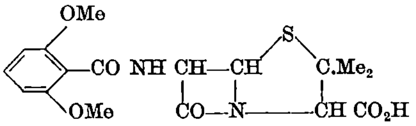
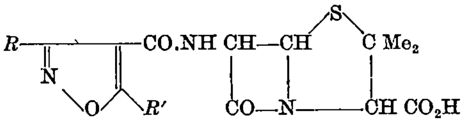
Isolation of a Cystine Derivative from Silk

CONSIDERABLE doubt exists as to whether silk fibroin (*Bombyx mori*) contains a small amount of cystine or whether this amino-acid is entirely absent. Apart from any biological significance, even a very small quantity of cystine, depending on its mode of incorporation, could have a considerable influence on the molecular weight of fibroin.

Schroeder and Kay[1], using ion-exchange chromatography, reported the presence of 0.17 per cent cystine in silk, and later Zuber, Ziegler and Zahn[2] estimated the cystine by an electrophoretic method after oxidation of the fibroin as 0.12 per cent. In recent reviews on silk[3,4], however, no reference is made to its cystine content and it has been asserted that sulphur-containing amino-acids are entirely absent and the claim that silk fibroin contains 0.12 per cent cystine cannot be reconciled with the analytical results so far available[4]. In these laboratories it has been found possible apparently to detect cystine in silk hydrolysates by both paper and ion-exchange chromatography and the Shinohara method (Earland, C., Stell, J. G. P., Lodge, R. S., and Raven, D. J., 1·3 and 35° C all have the same half-life of 160 min. as determined by a previously reported method[4] whereas the half-life of methicillin under the same conditions is between 2 and 3 minutes.



(I)



(II)

A solution of 5-methyl-3-phenylisoxazole-4-carbonyl chloride in acetone was added to a stirred solution of 6-aminopenicillanic acid and sodium bicarbonate in aqueous acetone. After 1 hr acetone was removed, the mixture was acidified, and 5-methyl-3-phenyl-4-isoxazolylpenicillin was extracted into methyl isobutyl ketone. The clarified extracts were then treated with sodium 2-ethylhexoate in isopropanol, whereupon the hydrated sodium salt of the penicillin crystallized in high yield, m.p. 188° (decomp.), $[\alpha]_D^{20}$ + 201° (*c* = 1 in water) (Found: C, 52·0; H, 4·9; N, 9·0; S, 7·1; Na, 5·2. $C_{19}H_{18}N_3O_5SNa, H_2O$ requires: C, 51·7; H, 4·5; N, 9·5; S, 7·3; Na, 5·2 per cent). A similar reaction with 3-methyl-5-phenyl-isoxazole-4-carbonyl chloride gave the anhydrous sodium salt of 3-methyl-5-phenyl-4-isoxazolylpenicillin, m.p. 239° (decomp.), $[\alpha]_D^{20}$ + 226° (*c* = 1 in water) (Found: C, 53·7; H, 4·7; N, 9·8; S, 7·6; Na, 5·0. $C_{19}H_{18}N_3O_5SNa$ requires: C, 53·9; H, 4·3; N, 9·9; S, 7·6; Na, 5·4 per cent.)

Details of the preparation of many analogous 3,5-disubstituted 4-isoxazolylpenicillins will be reported elsewhere. Several of the products are at present undergoing clinical evaluation.

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Cobalt Complexes of Proteins and Polypeptides

WE wish to present evidence for the formation of two types of cobalt complexes of proteins and polypeptides in which cobalt is probably bound to the peptide groups. A variety of proteins (for example, gelatin, pepsin, edestin, lysozyme, chymotrypsinogen), as well as tetraglycine and poly-DL-alanine, give the complexes, but most of the work has been carried out with commercial gelatin.

Complex *A* is obtained at high alkalinity as a clear violet solution, λ_{max} 560 mμ. On acidification of the violet complex immediately after formation, the pink colour of cobalt (II) ion is restored. A typical preparation is made as follows. To a mixture of 10 ml. of 10 per cent aqueous gelatin, 1 ml. of 0·35 *M* cobalt (II) sulphate and 4 ml. water, there is added quickly 5 ml. of 4 *M* potassium hydroxide (final hydroxide concentration 1 *M*). The optical density is about 0·3 (0·1-cm cell) at 560 mμ. Complexes have also been prepared starting with 2 per cent gelatin solutions, the final alkali concentration also being 1 *M*. The more dilute gelatin was used when spectra of complexes of higher cobalt to peptide ratios were measured. The highest molar ratio of cobalt to peptide groups that was found to give the clear violet solution was about 1 : 10. At slightly higher ratios the solutions showed a Tyndall effect probably caused by colloidal cobaltous hydroxide, but were transparent by transmitted light. At still higher cobalt to peptide ratios, the rose-coloured form of cobalt (II) hydroxide was precipitated.

The lowest hydroxide concentration at which a fluid complex of gelatin can be formed is about 0·3 *M*. Below 0·3 *M* alkali a jelly of gelatin combined with the blue form of cobalt (II) hydroxide is produced.

The red-brown complex *B* is formed on ageing of complex *A* in air, the transformation beginning in a few minutes in the case of gelatin, in a few seconds with some other proteins. In an open test-tube the change to red-brown is observed to proceed slowly from the top downwards. A similar change is produced immediately by addition of hydrogen peroxide or hypobromite ion. When a gelatin–cobalt solution was mixed with alkali in vacuum, the violet colour persisted for 22 hr (although some brown precipitate formed toward the end), at which time air was admitted, resulting in conversion to complex *B* in a few minutes. The absorption maximum of complex *B* is 460–470 mμ. Acidification of complex *B* does not restore the cobalt (II) colour, but gives a light brown solution of λ_{max} 480 mμ.

Apparently *A* is a cobalt (II) complex, while *B* is a cobalt (III) complex. Ligation of cobalt to the peptide groups, with ionization of the peptide hydrogen, is suggested by the high alkalinity required and by the lack of reactivity of poly-L-proline, poly-L-hydroxyproline or poly-sarcosine. The complexes of gelatin with high cobalt contents do not gel at 0°, while a control lacking cobalt, but at the same alkali concentration, does gel. The solutions gel after acidification. By analogy with the biuret-type copper and nickel complexes, which are known to be peptide complexes[1] and also do not gel, we may regard the cobalt complexes as peptide complexes. Tetraglycine binds one cobalt to two tetraglycines, assuming the cobalt to be octahedral it is possible that the ligating groups are the six peptide groups and not the terminal groups. Complex *B* is probably related to the wine-red complexes of cobalt (III) with glycylglycine and glycylglycylglycine which Manyak, Murphy and Martell proposed as having cobalt–peptide (as well as cobalt–amino and cobalt–carboxyl) bonds[2]. The spectra of complexes *A* and *B* prepared from gelatins in which all the amino-groups had been acetylated or 70 per cent of the guanidino groups removed by alkaline hypobromite were the same as those of the complexes of the parent gelatin. (To prevent oxidation of complex *A* a few μgm. of bisulphite were required to destroy traces of positive bromine not removed by dialysis of the hypobromite-treated

Consistently low calcium values were obtained by the method of direct dilution with water as might be expected due to the presence of interfering phosphorus and protein. The other three methods of preparing solutions for measurement by the atomic absorption technique gave results which were in close agreement and which were in good correlation with calcium values obtained by oxalate–permanganate titration.

Experiments on the recovery of calcium added to saliva in known amounts indicated that, with the exception of direct dilution with water, atomic absorption analysis gave acceptable results. Saliva samples diluted with ethylenediamine tetraacetate acid gave an average recovery of 98.6 per cent and deproteinized saliva containing strontium suppressor gave an average recovery of 99.0 per cent.

From the results in Table 1 it may be deduced that the secretion of the submaxillary salivary glands (6.8 mgm. per cent calcium) contains about 50 per cent more calcium than that of the parotid gland (4.1 mgm. per cent calcium) under conditions of exogenous stimulation. The higher frequency of calculus surrounding lower anterior teeth and their relative immunity to dental caries may be attributable to their proximity to the orifices of the ducts of the submaxillary glands and the higher calcium content of this secretion.

Using Schneyer's data[5] for the proportion of individual gland pair secretions of total stimulated saliva (submaxillary 63 per cent, parotid 34 per cent) and neglecting the contribution of sublingual and other minor glands, the calcium content of whole saliva was calculated as 5.9 mgm. per cent

$$\left(\frac{6.1 \times 63 + 4.1 \times 34}{97}\right)$$

Values for calcium content of whole saliva shown in Tables 1 and 2 are in good agreement with this theoretical amount.

The 'debris' and particulate matter which is inevitably present in whole saliva interferes with some of the volumetric and colorimetric methods for determining salivary calcium content. Many investigators have therefore carried out preliminary centrifugation of the whole saliva. Provided that there is no clogging of the capillary tube of the atomizer, the atomic absorption spectrophotometric method can be applied directly to whole saliva diluted in ethylenediamine tetraacetic acid without prior centrifugation. Table 2 shows the results of duplicate calcium analyses on five samples of whole saliva both before treatment and on the supernatant and precipitate following centrifugation. My results indicate an approximately 22 per cent lower calcium value for centrifuged saliva.

Table 2. CALCIUM CONTENT OF WHOLE SALIVA* (MGM./100 ML.) ESTIMATED BY DIRECT DILUTION WITH ETHYLENEDIAMINE TETRAACETIC ACID

Saliva	Untreated saliva	Centrifuged† Supernatant	Centrifuged† Precipitate
E. N.	6.52	5.08	1.58
	6.52	5.08	1.68
E. N.	6.00	4.73	2.06
	5.75	4.73	—
E. N.	6.35	4.25	2.78
	6.52	4.08	2.78
H. S.	6.00	5.28	1.25
	6.15	5.08	1.38
B. G.	5.42	4.40	1.25
	5.42	4.40	1.15

* Stimulated by paraffin.

† Centrifuged at 4,000–4,500 r.p.m. for 10 min.

With stimulation by citric acid, very little variation was found in the calcium content of parotid and submaxillary saliva collected as consecutive aliquots during 1.5-min. intervals for 15 min. Over the period studied the glands showed no evidence of depletion of the calcium content of the secretion. There appeared to be no appreciable difference in the calcium content of right and left parotid gland saliva collected simultaneously or in the flow-rate.

I am indebted to Dr. J. B. Willis for his suggestions and Dr. K. W. Knox for his interest and encouragement in these experiments. I also wish to thank Mr. B. Gow for his invaluable assistance in assembling the atomic absorption equipment. This work was supported by the National Health and Medical Research Council of Australia.

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New Penicillins Stable towards Both Acid and Penicillinase

FOLLOWING on the isolation in these Laboratories of 6-aminopenicillanic acid[1], we have used it to synthesize many novel penicillins, with particular emphasis on structures which resist inactivation by staphylococcal penicillinase. One class of penicillins possessing this valuable property has been obtained by acylating 6-aminopenicillanic acid with aromatic or heteroaromatic carboxylic acid chlorides substituted in both *ortho* positions. When the ring structure of the side-chain acid is six-membered, almost any pair of *ortho* substituents confer marked stability towards penicillinase, but when it is five-membered one or both of the substituents must be relatively bulky, for example, phenyl or substituted phenyl. Such findings illustrate the primary importance of steric effects in determining stability towards penicillinase. In this respect the chemical constitution of the side-chain is of much less significance, although it has a profound influence on antibacterial activity and on stability towards acids.

The first such penicillin to be synthesized[2], 2,6-dimethoxyphenylpenicillin (I, methicillin), has proved very effective in staphylococcal infections although, since it is unstable to acid and is poorly absorbed when given by mouth, it has to be administered by injection. We have now prepared a series of 3,5-disubstituted 4-isoxazolylpenicillins[3] (II) many of which, especially those in which one of the *ortho* substituents is a phenyl or substituted phenyl group and the other is a methyl or other lower alkyl group, exhibit high antibacterial activity both *in vitro* and *in vivo*. (We thank Dr. G. N. Rolinson, Mr. D. M. Brown, and their colleagues for carrying out these tests.) Furthermore, they are relatively stable at low pH and suitable for oral administration. Thus, 5-methyl-3-phenyl-4-isoxazolylpenicillin (II, $R = \text{Ph}$, $R' = \text{Me}$), 3-methyl-5-phenyl-4-isoxazolylpenicillin (II, $R = \text{Me}$, $R' = \text{Ph}$), and phenoxymethylpenicillin (penicillin V) in 50 per cent aqueous ethanol at pH

Rotational Analysis of the $A^2\Pi$–$X^2\Sigma^+$ System of MgCl

ALTHOUGH the internuclear distances in the diatomic halides of the typical elements of Groups I and III are known, often very precisely, few values are available for the diatomic alkaline earth halides. Following the rotational analysis[1] of the $C^2\Pi$–$X^2\Sigma^+$ system of CaCl, we have now completed an analysis of the 0–0 and part of the 0–1 bands of the $A^2\Pi$–$X^2\Sigma^+$ system of MgCl, from absorption spectrograms taken in a third order of a 6.5-m. grating instrument.

The analysis of the longer-wave-length sub-bands of the 0–0 and 0–1 bands was straightforward, but lines of the shorter wave-length sub-bands are badly blended and overlapped, and the 1–0 bands are too poorly resolved for analysis. However, a satisfactory check was provided by the analysis of the longer wave-length component of the 0–0 band of ^{37}MgCl. The bands observed present no unusual features. As in CaCl, the spin-splitting in the ground-state was too small to be detected, so that the satellite branches were everywhere blended with the main branches. Λ-type doubling in the $^2\Pi$ state is also very small, and the lines in the shorter wave-length sub-bands, presumed to involve $^2\Pi_{\frac{1}{2}}$, are too overlapped to show whether this state is, in fact, inverted, as expected from the likely electron configuration.

Table 1. SUMMARY OF CONSTANTS (^{35}MgCl)

State	T_0	ΔG_{01}	B_0	$10^3\ \alpha$	$10^7\ D$	r_e, Å
$A^2\Pi_{1/2}$	26,535.89		0.25116	(1.8)	2.2_5	2.171_6
$A^2\Pi_{3/2}$	26,481.95		0.24914	(1.8)	2.2_5	2.176_4
$X^2\Sigma^+$	0	462.12	0.24423	1.5_8	2.5_2	2.198_6

A summary of the constants obtained in the present work is given in Table 1. The value for the internuclear distance in the ground-state is r_e = 2.199 Å, not significantly different from the value 2.18 ± 0.02 Å obtained for the gaseous dihalide, $MgCl_2$, by electron diffraction[2].

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BIOCHEMISTRY

Application of Atomic Absorption Spectroscopy to the Determination of Calcium in Saliva

THE importance of salivary calcium concentration of the environment in the solubility of tooth and enamel is readily apparent. As with other biological fluids, numerous methods, ranging from classical oxalate-permanganate titration and emission flame photometry to the more recent complexometric and colorimetric techniques, have been used to estimate the calcium content of saliva. Unfortunately most of these methods have certain disadvantages. The oxalate-permanganate method requires relatively large amounts of saliva, time-consuming centrifugation, washing and titration within a narrow temperature-range. Its accuracy has been questioned because of incomplete precipitation of the oxalate or loss of some calcium oxalate during the washing process[1]. Another devious procedure involves a complexometric analysis of calcium and magnesium in saliva followed by a separate colorimetric determination of magnesium, calcium being obtained as the difference[2]. Several complexon-titration techniques using eriochrome black *T*, murexide or plasmo corinth indicators were tested on saliva. They were found unsatisfactory because the colour shift at the end-point was either too gradual and indistinct or, in the case of murexide, the colour faded too rapidly.

Table 1. CALCIUM CONTENT (MGM./100 ML.) OF SALIVA ESTIMATED BY DIFFERENT METHODS

Saliva	Type	(*a*) Saliva diluted with water	(*b*) Saliva diluted with ethylene-diamine tetra-acetic acid	(*c*) Saliva deprotein-ized strontium suppressor	(*d*) Saliva deprotein-ized lanthanum suppressor	Oxalate permanganate method (Clark-Collip)
H. S.	Whole saliva*	—	5.50	5.48	5.68	—
A. T.		—	7.25	7.40	7.50	7.42
J. F.		—	7.15	7.54	7.50	7.52
E. N. ‡		3.70	5.08	5.15	—	—
E. N.	Parotid †	2.26	3.35	3.29	—	3.42
A. T.		3.39	5.55	5.55	—	—
H. S.		2.9	3.7	3.2	—	3.6
H. S.		2.46	3.76	3.75	—	3.71
B. G.		2.48	4.23	4.13	—	3.99
E. N.	Sub-maxillary‡	4.63	7.39	7.37	—	—
H. S.		3.99	6.40	6.40	—	6.95
H. S.		5.02	6.55	6.85	—	—

* Whole saliva was stimulated by paraffin.
Saliva stimulated by application of citric acid to tongue.
‡ Sample centrifuged to remove particulate matter.

Willis's method[3] for the determination of calcium in blood serum by atomic absorption spectroscopy has been adapted for salivary calcium analysis and found to be accurate and rapid, requiring as little as 0.1 ml. of saliva. The apparatus used in these experiments has been previously described[4]. A Hilger *D* 290 monochromator was found satisfactory in this system.

Certain normal constituents of saliva can interfere in the determination of calcium by the atomic absorption method. Sodium enhances calcium absorption, phosphorus depresses the calcium absorption and the interference of protein appears to be variable, depending on the type of flame used[3]. Saliva can be deproteinized by the standard procedure of adding trichloracetic acid and centrifugation. Large concentrations of [Sr^{++}] or [La^{+++}] can compete with calcium for combination with phosphorus and suppress this interference. Ethylenediamine tetraacetic acid, by complexing calcium, can also overcome phosphorus interference and presumably compete with proteins for calcium.

The results of calcium estimations on saliva are shown in Table 1. The saliva was diluted 1 : 10 by one of the following methods: (*a*) direct dilution with water, (*b*) dilution with water and solution of the disodium salt of ethylenediamine tetraacetic acid, final concentration 10,000 p.p.m. ethylenediamine tetraacetic acid, (*c*) dilution with water, strontium chloride solution and trichloracetic acid: final concentration 2,500 p.p.m. strontium and 4 per cent trichloracetic acid. The sample was centrifuged and measurements made on the supernatant, (*d*) similar to method (*c*) but lanthanum chloride was used as a phosphate 'suppressor', final concentration 10,000 p.p.m. lanthanum.

The saliva thus prepared was measured relative to standard calcium solutions containing respectively (*a*) 60 p.p.m. sodium (approximate level in 1 : 10 diluted saliva), (*b*) 10,000 p.p.m. ethylenediamine tetraacetic acid, (*c*) 5,500 p.p.m. strontium, 60 p.p.m. sodium and 4 per cent trichloracetic acid, (*d*) 10,000 p.p.m. lanthanum, 60 p.p.m. sodium and 4 per cent trichloracetic acid.

Calcutta, for the electrical resistivity measurements This communication is published by the permission of the Director, Central Building Research Institute, Roorkee.

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Sedimentation of β-Lactoglobulin (*A,B*) under Dissociating Conditions in Acid Solution

Evidence that β-lactoglobulin molecules dissociate into halves in the *p*H region 1 6–4 has been presented by Timasheff, Townend and Weinberger[1-3], and they have shown[3] that there is reasonable agreement between the results obtained by the complementary methods of ultracentrifugation and light-scattering The overall picture which emerges is of a dumb-bell-shaped[4] lactoglobulin molecule, of axial ratio 2, dissociating reversibly at 25° below *p*H 4 into two spherical sub-units of equal size According to light-scattering data, and on the assumption of a molecule of weight 36,000, the dissociation constant at *p*H 1 6 is 2 5 × 10^{-4} mol /l. and at *p*H 3 5 is 4 3 × 10^{-6} mol /l [3]

We have now attempted to carry a comparison of the two methods a stage further, and to calculate the implications for sedimentation of accepting these values from light-scattering of the dissociation constants

The main effect of dissociation on sedimentation is seen in a departure of the sedimentation velocity–concentration curve from the normal form Weak dissociation leads to a high maximum, whereas strong dissociation leads to a lower maximum, or if sufficiently strong, to no maximum and only a change of slope[5] In Fig 1 theoretical curves are shown for sedimentation at *p*H 1 6 and 3 5 The sedimentation velocity–concentration curves marked *a* and *b* have been constructed on the basis of the light-scattering dissociation constants, together with the following minimal assumptions (1) The limiting sedimentation coefficient of the sub-unit (monomer) is 1 89 svedbergs. (2) The whole molecule (dimer) moves relatively faster than the sub-unit by the factor $2^{2/3}$ because of its weight, and 1/1 044 because of its shape[6], that is, $s_2 = 1\ 52\ s_1$ (3) The sedimentation coefficient for each species considered alone obeys the relation $s = s_0\ (1/1 + gc)$, where g is a constant (4) At *p*H 1 6, the maximum value of the sedimentation coefficient, measured using the boundary median, is 2 34 svedbergs This fixes the value of g as 0 1 (gm /100 ml $)^{-1}$

Whether the curves are based on a weight average[7], or the position of the median of the boundary[5,8], is not very material for this simple monomer–dimer system, as was shown earlier[5]

The experimental points in Fig 1 are taken from the work of Townend, Weinberger and Timasheff[3], and include one check point not hitherto published (Timasheff and Townend, personal communication) It is seen that they lie fairly near the theoretical curves *a* and *b* when considered as a whole, but that there is an obvious discrepancy at low concentrations at *p*H 3 5 It is difficult to see how a dissociation constant as great as 4 3 × 10^{-6} mol /l can be reconciled with the sedimentation results at the two lower concentrations there The effect on the position of the theoretical curve of reducing the value of the dissociation constant ten-fold is shown in Fig 1 (curve *c*) It will be interesting to see whether future work on purified *A* and *B* lactoglobulin eliminates this conflict between the interpretation of light-scattering and ultracentrifuge data.

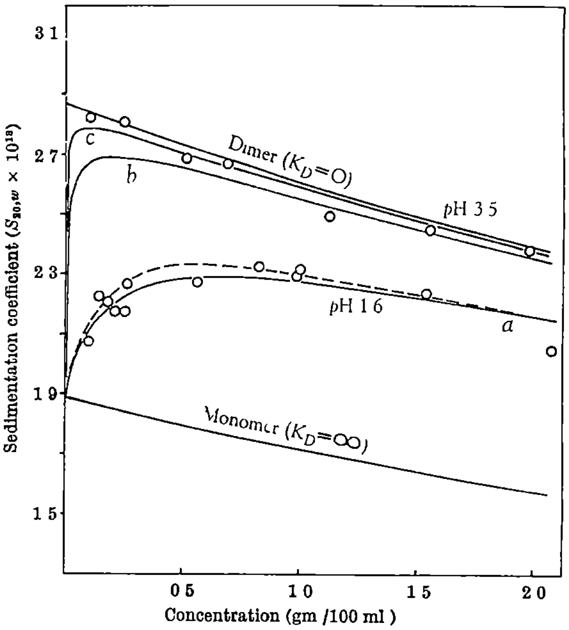

Fig 1. Sedimentation of lactoglobulin (*A,B*) under dissociating conditions *a*, K_D = 2 5 × 10^{-4} mol /l , *b* K_D = 4 3 × 10^{-6} mol /l , *c*, K_D = 4 3 × 10^{-7} mol /l ——, Weight average (theory), ---, median of boundary (theory), ○, experiment (Townend, Weinberger and Timasheff, ref 3)

The value of g is in itself of some interest, since it is about twice that to be expected for a non-reacting protein near its isoelectric point This may be a reflexion of the fact that the lactoglobulin molecules are sedimenting in a highly charged condition, and that electrostatic repulsion is reducing the number of near neighbours, and hence their contribution to the net velocity of sedimentation[9]

This work was supported in part by a grant from the Agricultural Research Service, U S Department of Agriculture

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exact structure of the platinum–chloro-alumina complex is unknown, but attempts to fit six co-ordinate platinum into assumed models of the alumina surface should be fruitful.

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Puzzolanic Activity and Semiconductivity of Fired Kaolin and Kaolinitic Clay

It is known that the reaction of a puzzolan with lime in the presence of water is a chemisorption process[1], and that the puzzolanic activity of a material is enhanced when its crystal structure breaks down, that is, when the material is in a highly disordered state. The enhancement of puzzolanic activity is attributed to the increased reactivity of the materials in the disordered state[2] or its lattice being in strained condition. Similarly, the observed high puzzolanic activity of volcanic ash, a natural puzzolan in the glassy phase, is considered to be the result of the highly strained condition of its lattice[3].

On the other hand, a chemisorption process depends on the electrical characteristics of the reactants[4] and the breakdown of structure of a crystal, say, through the action of heat, may produce crystals which may be semiconductors. Clay minerals, for example, on firing are changed into an intimate mixture of oxides, some of which are semiconductors. It is also possible that the defects produced in the crystal when heated under appropriate conditions may induce semiconducting properties[5]. It is therefore suggested that the effect of disorders or defects present in a natural puzzolan or introduced into a material to improve its puzzolanic activity by heating (as in burnt clays) is to render the material a semiconductor, then the reactivity with lime of a puzzolan may be a semiconductor surface reaction.

Results of work on the puzzolanic activity of kaolin (unfired, fired and fired with zinc oxide) and of a fired kaolinitic clay (fired with or without admixture of zinc oxide) have been published recently[6,7]. Electrical resistivities of some of these materials, kaolin (unfired, fired at 800° C. and fired with 0.01 per cent zinc oxide) and a kaolinitic clay (fired alone and with 0.01 per cent zinc oxide added to it), have been measured to see if these materials were semiconductors. Results are given in Fig. 1, which shows that (*a*) these materials are semiconductors (kaolin undergoes crystallographic changes during firing, hence for kaolin the values represent the resistivities of the products formed during heating) and (*b*) resistivities of these materials increase slightly when heated with zinc oxide.

If the puzzolanic activity of a material depends on its semiconducting properties, then its puzzolanic activity should alter with its electrical resistivity. When kaolin and kaolinitic clay are fired with zinc oxide there is an increase in electrical resistivity (Fig. 1). There is also an increase in puzzolanic activity (in terms of compressive strength and adsorption of lime) of these materials when fired with zinc oxide[6,7]. Similarly, there is an increase in resistivity of kaolin on firing (Fig. 1) and there is also a corresponding increase in both compressive strength and adsorption of lime of kaolin upon firing[6].

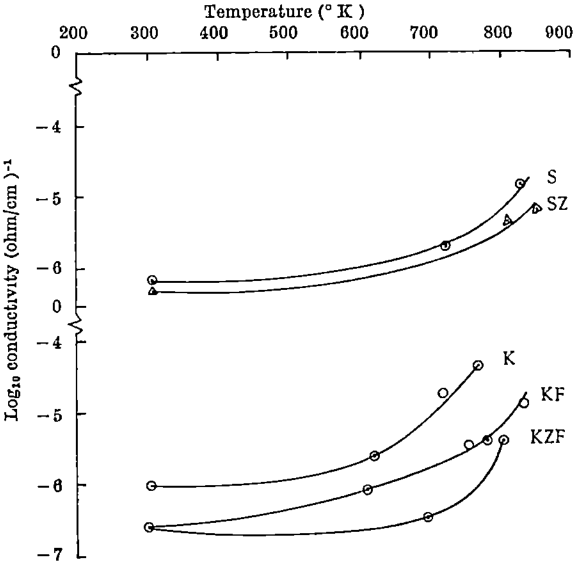

Fig. 1. Variation of electrical conductivity with temperature. K, Kaolin; KF, kaolin fired at 800° C.; KZF, kaolin + 0.01 per cent zinc oxide fired at 800° C.; S, clay fired at 800° C.; SZ, clay fired with 0.01 per cent zinc oxide at 800° C.

If adsorption of lime by a puzzolan in the presence of water is a chemisorption process on the surface of a semiconductor, there should be a change in the resistivity of the puzzolan after it has adsorbed lime in presence of water. Accordingly, resistance measurements of kaolin and kaolin fired at 800° C. were carried out at room temperature. These materials were then allowed to adsorb lime in the presence of water and their resistances (after having been thoroughly washed and dried) were again measured under similar conditions. It was found that there was a difference in the resistivities of these materials before and after they had adsorbed lime[8]. These resistance measurements were, however, not very reliable.

A natural puzzolan with a glassy structure can be a semiconductor. It is the short-range order (bonding predominantly covalent) which determines semiconductivity rather than the long-range order (structure). The general conditions to be fulfilled by a material in order that it may be a semiconductor have been given by Mooser and Pearson[9]. It will be seen that it is quite possible that many natural puzzolans satisfy these conditions.

If reactivity with lime of a puzzolan is a semiconductor surface reaction, the formation of a compound on the surface of a puzzolan will involve electron transfer and will be guided by the chemical potentials in the adsorbate and in the puzzolan[4] (that is, Fermi-level). Obviously only those semiconducting materials which have suitable Fermi-levels can behave as puzzolans.

It is therefore suggested that (i) for a material to have puzzolanic properties it must be a semiconductor; glassy-phase puzzolans are probably glassy-phase semiconductors, and (ii) reactivity with lime of a puzzolan then may be considered to be a semiconductor surface reaction.

I thank Shri A. K. Dutta, research officer of the Indian Association for the Cultivation of Science, much higher pressures and lower temperatures used in this work Indeed, recent work by Rudolph and Melton[15] and by Field[16] has shown that higher molecular weight ions become more important in the mass spectra of acetylene and of ethylene, respectively, when high pressures (up to 350μ in ethylene) are used

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CHEMISTRY

Valence State of Platinum–Alumina Catalysts

Platinum-on-alumina reforming catalysts are widely used in the petroleum industry Recent work in this laboratory has shown that catalysts containing soluble platinum more readily convert paraffins to aromatics[1] Soluble platinum is that which will dissolve in either dilute hydrofluoric acid or acetylacetone. It is believed to exist as a compound involving the alumina support and the chloride present in the catalyst, and is in the plus-four valence state after solution in dilute hydrofluoric acid

To establish the valence state of soluble platinum in the catalyst, ultra-violet spectrophotometric measurements have been made on catalysts specially prepared on alumina aerogels[2] In addition, the reflectance of finely ground samples of previously pelleted platinum-on-alumina catalysts has been measured

Before spectral analyses of the transparent aerogels could be made, the platinum–chloro-alumina complex was converted to the platinum–iodo-alumina complex The exchange is necessary because the platinum–chloro-alumina complex absorbs in a region of the ultra-violet spectrum (260 mμ) where scattering by the alumina aerogel is too high to allow observation of the peak Absorption of the platinum-iodo-alumina complex in the near ultra-violet (360 mμ), however, is free of light-scattering The platinum-iodo-alumina complex was made from an all-soluble platinum–chloro-alumina aerogel catalyst by carefully treating an evacuated aerogel with a solution of potassium iodide in absolute methanol and normal pentane The exchange of chloride for iodide is straightforward, with no oxidation of platinum in the potassium iodide solution[3] The absorption spectra for aqueous iodoplatinic acid and the platinum–iodo-alumina aerogel are shown in Fig 1. Measurements were made with a Cary model 11-*MS* spectrophotometer A slight shift of the platinum–iodo-alumina peak to a longer wave-length for the aerogel, as compared with aqueous H_2PtI_6, might be due to additional complexing with the alumina Because divalent platinous iodide does not absorb in this region[3], these spectra indicate platinum, as complex on the catalyst, is present in the plus-four valence state Iodide exchange of a platinum–chloro-alumina aerogel that was insoluble in hydrofluoric acid showed no ultra-violet absorption, as indicated by the dashed line

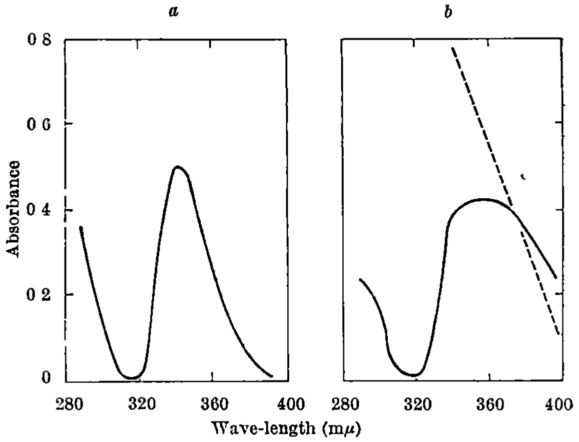

Fig 1 Ultra-violet spectra *a*, Aqueous $PtI_6^{=}$, *b*, catalyst treated with potassium iodide

Reflectance spectra of platinum catalysts are shown in Fig 2 The results were obtained with a Beckman model *DU* reflectance attachment on samples ground finer than 100 mesh Magnesium carbonate was the reference standard An absorption spectrum of aqueous chloroplatinic acid is included for reference The soluble platinum catalyst shows a small peak slightly shifted from the peak for aqueous $PtCl_6^{=}$. A sample of alumina alone and the platinum catalyst insoluble in hydrofluoric acid show no peaks

Platinum in the soluble form is thus in the octahedral plus-four valence state in the catalyst The

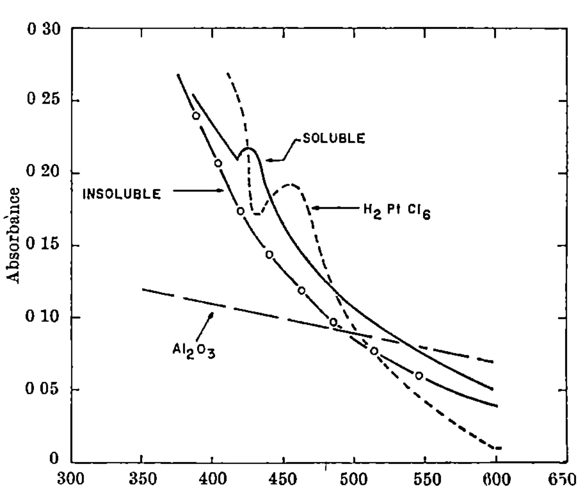

Fig 2 Reflectance spectra

warmer part was sublimed on to the cooler part. Such crystals were small in size (less than 1μ) and showed atypical shapes (Fig. 4).

As for the sublimation of the frozen droplets made up of tap water as material, it was once observed, when the sublimation occurred from inside the margin of the droplet, that the sublimed area expanded in all directions taking constantly a hexagonal shape (Fig. 5).

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RADIATION CHEMISTRY

Production of High Molecular Weight Products from the Irradiation of Methane with 4-MeV. Electrons

THE effects of ionizing radiations on gaseous methane have been examined previously and the yields of hydrogen and of higher hydrocarbons up to pentane determined to within what appear to be small limits of error[1,2]. Hydrogen is the major product, while the amounts of gaseous hydrocarbons are much less and decrease rapidly with increase in molecular weight. However, there is a 10–30 per cent excess of elemental hydrogen in the products[1-3]. In fact, a large excess of hydrogen seems to be a common feature of the radiation chemistry of gaseous hydrocarbons, though not of liquids[4-6]. It has been known for some time that massive radiation doses lead to the production of liquid products from low-molecular-weight saturated hydrocarbons[7-9], while more recently a more detailed study of liquid product formation from methane and other gaseous hydrocarbons has been reported[10,11]. The nature of the liquid products and their significance in the overall radiolytic process have not been examined.

We have irradiated purified (99·98 per cent) gaseous methane in 'Pyrex' vessels with electrons from a linear accelerator. The dose-rate was about 10^{18} eV. cm.$^{-3}$ min.$^{-1}$ in methane at 1 atm., 15° C. The dosimetry was based on the yield of ethane, $G = 2{\cdot}00$, from methane after short irradiations ($<$ 1 per cent decomposition). Gaseous products were analysed by gas chromatography, liquid products by gas chromatography and mass spectrometry. The liquid was collected by centrifugation. Irradiations giving up to 30 per cent methane decomposition were carried out.

The production of gaseous products was very similar to that found by Lampe[1] except that we were unable to detect unsaturated products irrespective of the dose given. No hydrocarbons above pentanes could be detected in the gas.

Of the liquid product collected, only a small fraction (about 10 per cent) was detectable by gas chromatography at 180° C., and consisted of C_6–C_{12} hydrocarbons, the proportion of C_6 and C_7 being less than that of C_8 and C_9. A further analysis on a much less sensitive temperature programmed gas chromatography apparatus showed that the major portion of the liquid consisted of C_{14} to C_{20} hydrocarbons. A mass spectrometric analysis gave the same overall picture and also showed not only the presence of hydrocarbons with up to 27 carbon atoms per molecule, but also the absence of significant amounts of unsaturated material. Furthermore, even higher hydrocarbons were undoubtedly produced which were not collected by centrifuging the reaction vessel. Irradiation of a methane–argon mixture containing 0·65 electron fraction argon gave essentially the same yield of liquid products as with pure methane. In this case the yield was based on the total energy absorbed in the mixture, using electron fractions to convert from pure methane to the methane–argon mixture. Examination of the gas chromatograms showed, however, that the C_6–C_{12} fraction of the product from the methane–argon mixture was only about half that of the corresponding fraction obtained from pure methane. This shift to higher molecular weight products in argon–methane mixtures may have been due to a gross energy transfer from the argon to methane, resulting in a larger energy deposition per gram of methane in the mixture than in the pure methane. However, irradiation of pure methane to twice the dose given to 50–50 argon–methane mixtures still showed a much larger fraction of low molecular weight liquid products from the pure methane.

The conclusion is drawn that a process occurs both in pure methane and in methane–argon mixtures which results in the formation of high molecular weight products, and that this process is enhanced in the presence of argon (but not of xenon, as shown by other experiments).

Other work on the same systems in this Laboratory has shown some similarity in the behaviour of methane and methane–argon mixtures when irradiated to low doses. For example, the yields of ethane and propane are practically unchanged at argon electron fractions 0–0·9. However, the hydrogen yield, based as usual on the total energy given to the mixtures, is actually greater in the mixtures than in pure methane. It is noteworthy that the additional hydrogen is not scavengable. Also, the *n*-butane/isobutane ratio is significantly increased. In xenon and helium, however, the behaviour is quite different, for example, the *n*-butane/isobutane ratio in methane–xenon mixtures is only about one-third of that observed in mixtures with argon.

The results outlined briefly here indicate that a process occurs in irradiated methane which is enhanced in mixtures with argon, this process giving rise to both increased yields of high molecular weight products and of molecular hydrogen. Since only about half the hydrogen from pure methane is scavengable and the non-scavengable fraction is increased in argon mixtures, it appears likely that a non-radical process is responsible.

It is suggested that the high molecular weight products and non-scavengable hydrogen are produced by ion-molecule reactions[12,13] of the type:

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$
$$C_2H_5^+ + CH_4 \rightarrow C_3H_7^+ + H_2, \text{ etc.}$$

Melton[14] has recently shown mass spectrometrically that there is a large increase in the CH_3^+/CH_4^+ ratio on addition of argon to methane. The fact that $C_3H_7^+$, or any other higher ion, has not been detected in mass spectrometric studies of methane is not good evidence that this ion is absent or unimportant at the recording of that derivative for the 129-keV. line of iridium 191; the source was about 60 μc., the absorber was a strip of iridium foil 5 mm. wide and 0·05 mm. thick, and the recording took about 1 hr. The entire assembly can fit into the working gap of an electromagnet.

It is hoped to publish a more detailed description of the method, with applications, elsewhere.

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Investigation with the Electron Microscope of the Sublimation of Ice Crystals at Low Temperatures

INVESTIGATIONS of ice crystals by means of the electron microscope have been made by several investigators[1-4], but most of them were based on replication techniques. Recently, Fernández-Morán[5] reported the results obtained by low-temperature microscopy; he succeeded in stabilizing ice crystals deposited on a cold substrate by maintaining them at low temperatures during observation in the electron microscope with a microbeam of very low intensity. It was noted by him that the ice crystals were predominantly cubic and hexagonal in shape at −120° C. and at −100° to 90° C., respectively, and actually showed corresponding sharp-ring diffraction patterns.

I have been investigating the freeze-drying process of micro-organisms by the use of an electron microscope in which a specimen-cooling device and a 16-mm. cinematograph were incorporated[6]. When the freeze-drying process of yeast cells was being investigated, I happened to observe the process of sublimation of ice crystals which were deposited on the film mounted on a specimen grid.

The electron microscope used for this experiment was a model 6*A*-type made by the Japan Electron Co. Yeast cell suspension as an experimental material was sprayed on a carbon–collodion film which had been designed to keep the specimen from contamination during irradiation with the electron beam. The droplets sprayed on the film were rapidly frozen to −196° C. by immersing them into liquid nitrogen. The specimen cartridge was quickly transferred into the specimen chamber of the electron microscope and settled in the specimen holder which was pre-cooled to −140° − −160° C. by contact with a cooling device refrigerated by liquid nitrogen. Electron beam intensity was controlled to be as low as possible so that the beam might not make artefacts. The electron current density used in this experiment was $5 \cdot 6 \times 10^{-4}$ amp./cm.^{2} which was sufficient for taking a cinematograph at a moving speed of 1·5 frames/sec.

Throughout the specimen field, large circular particles with diameters of 20–50μ were scattered. These proved to be droplets of yeast cell suspensions when observed in a subsequent drying process. In the interspaces numerous small particles could be seen. These particles were of cubic, hexagonal, round or other atypical shape with sizes varying from 1 to 5μ; it was quite probable that they were the ice crystals made up of water vapour condensed on the collodion film during transfer from liquid nitrogen to the specimen chamber. Electron diffraction patterns were also obtained corresponding to several crystal types.

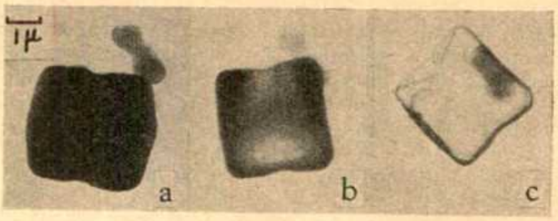


Fig. 1

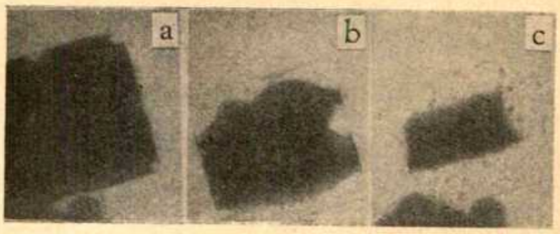


Fig. 2

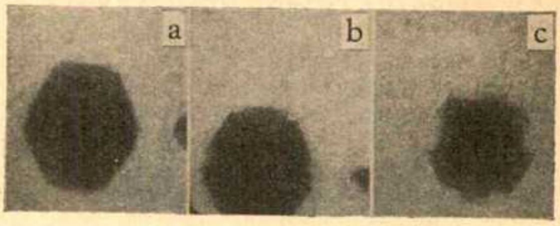
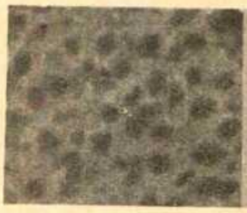


Fig. 3



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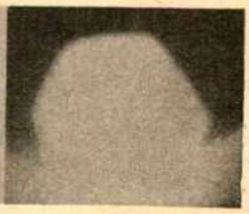

Fig. 5

After the refrigerant reservoir was raised from the specimen holder, the temperature of the specimen increased gradually. When it reached −90° or −80° C., not exactly measured, the ice crystals began to sublime. As sublimation proceeded, crystals decreased in size and sometimes suddenly disappeared from the field, being probably removed from the collodion film to the vacuum pump. In some cases, ice crystals maintained their contour even after sublimation. This fact was considered to be a kind of replication caused by the electron beam in the microscope during sublimation (Fig. 1,*a*, *b*, *c*). In rare cases it happened that the crystals of cubic and hexagonal types sublimed in such a manner that several 'worm-eaten' spots appeared at the corners of their margins (Figs. 2*a*, *b*, *c* and 3*a*, *b*, *c*). In one case where it was supposed that a temperature gradient may have arisen within the specimen, the water vapour which sublimed from ice crystals at the

Table 1. Results of Potassium–Argon Dating

Material	K_2O (per cent)	Volume of argon (mm.³) / Weight of sample (gm.)	Atmospheric contamination (per cent)	δV_r	δt	Age and estimated error (m. yr.)
96392 Hornblende, Kennack Gneiss at Kennack Sands 734 E. 165 N.	0·577 ± 0·0032	0·008658	10·7	4·0	3·33	366 ± 20
96396 Hornblende, Landewednack Hornblende Schist at Pencra Head 810 E. 225 N	0·375 ± 0·0034	0·006108	16·5	2·0	3·37	371 ± 20
96389 Hornblende, Landewednack Hornblende Schist at Pen Olver 713 E. 118 N.	0·425 ± 0·0019	0·00798	12	3·8	3·94	442 ± 24
96395 Hornblende Amphibolite at Predannack Head 663 E. 160 N.	0·211 ± 0·0040	0·003768	27·2	3·0	3·25	357 ± 20
96394 Hornblende Granulite at Pol Cornick 670 E. 153 N.	0·242 ± 0·0050	0·004650	3·2	7·4	4·33	492 ± 26
Biotite from Eddystone Rock.	8·03 ± 0·0901	0·1156	4·8	0·3	3·40	375 ± 17

$\lambda\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$

$\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$

δV_r = Percentage standard deviation in volume of radiogenic argon due to uncertainty in isotope ratios of argon sample.

δt = Error in age in millions of years that would be caused by a 1 per cent error in either proportion of potassium oxide or volume of radiogenic argon.

Grid references to localities refer to the National Grid.

Biotite separated from the biotite gneiss of the Eddystone Rock (obtained through the courtesy of Dr. P. A. Sabine, Geological Survey) gave a Caledonian age (375 million years) by the total volume method. This rock has in the past been generally correlated with the metamorphics of the Lizard[4]. From the experience in the Lizard it cannot be assumed that this Caledonian age is the age of crystallization of the gneiss, but it can be said that the Eddystone Gneiss forms part of the metamorphic terrain affected by the Caledonian orogenic events.

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PHYSICS

Method of Measuring the Mössbauer Effect

The Mössbauer effect[1] is of great value because of the extremely high energy resolution it provides, but it is often very difficult to measure, because it may be very small (for example, about 0·07 × 10^{-2} per cent for zinc-67 in zinc metal[2]). Common values are about 1 per cent of the total absorption, and even these may be attained only at very low temperatures. A differential absorption of this order is very difficult to measure with precision, particularly at low temperatures, because the stability of the equipment must be exceptionally high. For these reasons, most of the detailed applications have involved the use of the 14·4-keV. line of iron-57, which gives about 14 per cent differential absorption at room temperature. The parent nuclide is cobalt-57, which is difficult to prepare; moreover, the internal magnetic fields in iron cause a hyperfine splitting, and the low energy (14·4 keV.) makes the radiation difficult to use. Further, very strong sources are needed, because the distance from source to counter is large and because the source is used for only a small proportion of the total time. Finally, the velocity of the source relative to the absorber varies (owing to the curvature of the path) and is known only to 10 per cent or so if a system similar to Mössbauer's original one is used.

Fig. 1 shows schematically a system designed to overcome these disadvantages. The phosphor *P* (of a scintillation counter) is placed inside an open-ended drum *D*, which bears the absorber (one, not two). The source *S* is mounted on a vibrating strip *V*. The axis of rotation of *D* lies below the line joining *S* to *P*, so the absorber has a known effective velocity relative to *S*. The drum (diameter 3 in.) is driven via a continuously variable gear; the velocity is swept evenly continuously through zero. The thick copper strip *V* is clamped at *C* and performs bending vibrations, thus causing the source to vibrate along the line *S–P* (a motor-driven cam provides the motion). The strip dips into liquid nitrogen. The lower edge of the drum also dips into liquid nitrogen. The alternating signal from the ratemeter is fed to a phase-sensitive detector, the output of which is the first derivative of the Mössbauer line. Fig. 2 shows a

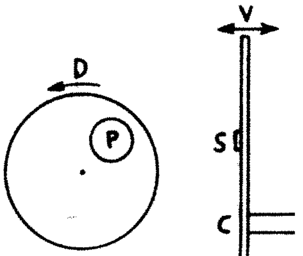

Fig. 1

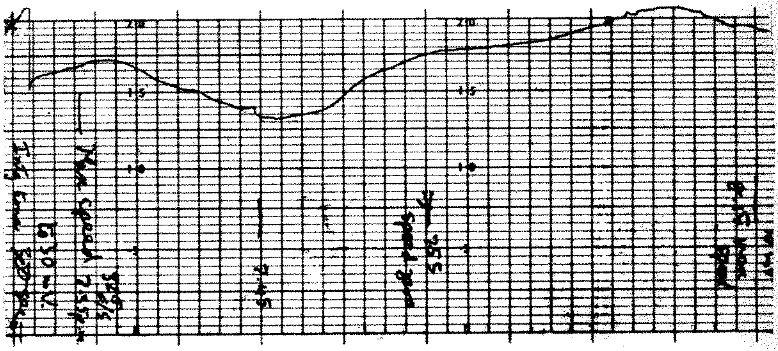

Fig. 2

Table 1. RESULTS OF AGE DETERMINATIONS ON GRANITE FROM MAHÉ ISLAND, SEYCHELLES ARCHIPELAGO

Material	Reference	Method	Volume of argon (mm.^{3}) / Weight of sample (gm.)	K_2O (per cent)	Atmospheric contamination (per cent)	Apparent age and estimated error (m. yr.)
Grey granite, **total** rock	*K*/27	Total volume	0·0967	4·48	5·0	536±23*
	L/6		0·0965	,,	5·1	534±23*
	K/39		0·0854	,,	1·7	495±22*
	L/15		0·0885	,,	3·9	501±22*
	L/16		0·0941	,,	3·5	531±23*
						av. = 519±9*
Pink granite, total rock	*L*/9	Total volume	0·0898	4·63	3·1	497±22*
	K/41		0·0918	,,	2·9	507±22*
	L/23		0·0973	,,	3·8	530±23*
						av. = 511±10
Biotite from pink granite	*L*/29	Isotope dilution	0·1399	5·26	2·3	654±17

$\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$.
$\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$.
* Errors given are those arising from experimental procedure.
True errors which would take into account argon loss are much greater—see text.

suggesting an affinity between Africa and the Seychelles. This would be consistent with the view that the islands represent a continental fragment left during the widening of the Indian Ocean.

Samples of hornblende from Mahé and other islands of the Archipelago have been received from Dr. B. H. Baker of the Ministry of Commerce, Industry and Communications, Mines and Geological Department, Nairobi. Work has begun on these, and upon basaltic material from typical oceanic islands in the Atlantic.

We are especially grateful to the Governor of the Seychelles for providing the samples on which the measurements were made. Also we wish to thank Dr. D. H. Matthews of the Department of Geodesy and Geophysics, Cambridge, for his help and interest in this work, and Dr. S. O. Agrell of the Department of Mineralogy and Petrology, Cambridge, for preparing thin sections of the rocks. We are indebted to the Department of Scientific and Industrial Research for financing the project, which is part of a programme of investigation into the geochronology of oceanic rocks.

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Age Determinations of Rocks in the Lizard (Cornwall) Area

A PREVIOUS attempt to date the major geological events in the Lizard area[1] using the potassium–argon total volume method on biotites and muscovites gave a Caledonian age of 391 million years for the youngest intrusives, the Kennack Gneiss, but gave an anomalously younger age of 356 million years for the regionally metamorphosed Old Lizard Head Series. A series of independent measurements by Dodson[2] using the isotope dilution method gave a similar pattern with the younger age (350 million years) from muscovite from the Old Lizard Head Series and the older age (368 million years) from the Kennack Gneiss and also from biotite from the Landewednack Hornblende Schist, co-metamorphic with the Old Lizard Series. The differences between our results and those of Dodson are of the same magnitude evident in comparative measurements previously described[3].

Potassium–argon measurements have now been made on five hornblendes collected from the Kennack Gneiss (96392), the regionally metamorphosed Landewednack Hornblende Schist (96389, 96396) and the dynamothermal aureole of the peridotite (96394, 96395). The argon measurements were made by the method of isotope dilution. Potassium oxide was determined by six or eight measurements on different samples using the flame photometer. The standard deviations of these measurements are given in Table 1. Because of the low potassium content of the hornblendes the main source of error in the age values lies in the potassium oxide determinations.

The hornblende 96392 is from the same specimen of Kennack Gneiss that gave a 397-million year age from biotite. The two specimens of Landewednack Hornblende Schist are interbedded and co-metamorphic with the Old Lizard Head Series which gave an average age of 356 million years for muscovite. One hornblende (96396) gives an age agreeing with that of the Kennack Gneiss but the other (96389) gives an appreciably older age.

The two hornblendes from the metamorphic aureole of the peridotite have very different ages. No. 96395 is from the green-brown hornblende amphibolite assemblage at about 800 yards from the nearest peridotite outcrop. This gives an age (357 million years) compatible with the Caledonian ages previously described. No. 96394 is a brown hornblende from a band of hornblende–hypersthene–augite granulite immediately in contact with the peridotite. The brown hornblende gives a much greater age of 492 million years, more in harmony with the geological evidence that the regional metamorphism and peridotite intrusion are much earlier than the intrusion of the basaltic dykes and Kennack Gneiss.

The interpretation placed on these results is that the Caledonian age of 360–390 million years is a true one for the intrusion of Kennack Gneiss. This was also a period of partial or complete loss of argon for pre-existing metamorphic assemblages. The specimen of rather massive Landewednack Hornblende Schist and particularly the hornblende granulite interbanded with the peridotite at the peridotite margin may have been partly insulated from the Caledonian effects and reflect partial argon loss from Pre-Cambrian or Cambrian minerals. Hercynian thrusting may also have caused loss of argon, particularly from micas in the flat-foliated, less competent Old Lizard Head Series.

The present results are significant as further evidence that hornblendes of low potassium content can give meaningful ages in determinations by the potassium–argon method.

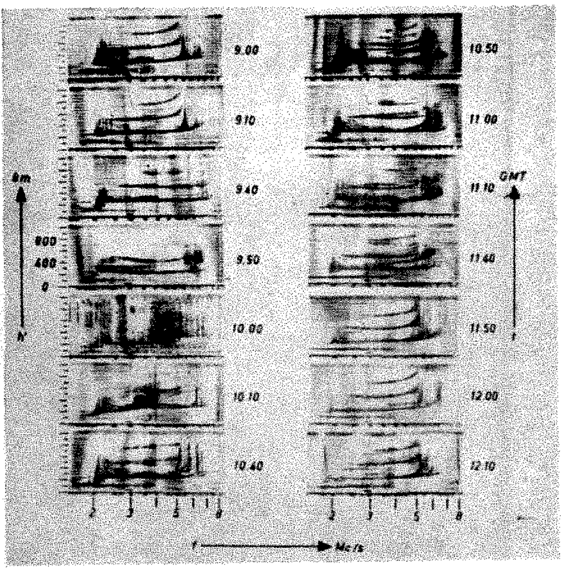

Fig. 3. Ionograms obtained at Sodankylä on October 31, 1961, showing a disturbance caused by the round-the-world ionospheric waves

On October 31, at 8.29.27 G.M.T., another atomic explosion occurred in Novaya Zemlya at a distance of 1,190 km. The deflexion of the microbarograph due to this explosion was about a third of the deflexions due to the round-the-world waves of the big explosion. Further, the ionospheric disturbance due to the latter ones was more than one hour too late to be originated from the smaller explosion. It can, therefore, be said that the smaller explosion had no detectable effect on the ionosphere.

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Potassium-Argon Age Determinations on Granite from the Island of Mahé in the Seychelles Archipelago

The Seychelles Archipelago is situated in the Indian Ocean some 1,100 miles to the east of Mombasa. It is made up of nineteen islands and a number of islets of which all but two are granitic in composition. The only two coral islands in the group are Bird and Denis, which lie at a distance of about sixty miles north of Victoria, Mahé. Mahé itself is the largest of the islands, being seventeen miles long and up to seven miles wide. Rugged peaks of granite rise to a height of almost 3,000 ft. above sea-level and show marked erosion features. Praslin, Silhouette and Digue are the other main granitic islands of the group.

Throughout the islands, the most common rock type is pink or grey amphibolite granite. Syenite occurs on Longue Island and in the southern part of Silhouette. In various localities the plutonics are intruded by dykes of varied composition.

Sedimentary rocks are absent, though the occurrence of andalusite-hornfels on the islands of Silhouette, Cerf and Stag[1,2] may indicate that clay slates once existed.

The granitic islands lie in the centre of an extensive submarine platform that is surrounded by depths of more than 1,000 fathoms and completely separated both from the Carlsberg Ridge and from Madagascar. By any criteria other than petrographic, the islands are of oceanic type.

The age of the group is not known, but the degree of erosion suggests prolonged action by the agents of weathering. Coral fragments are to be found cemented on to the granite at heights varying between 10 and 30 ft. above low-tide level. In addition to this evidence of recent elevation, Gardiner[3] considers that a more ancient uplift of some 200 ft. occurred.

Age determinations have been carried out on samples of pink and grey granite from the island of Mahé. Owing to the small amount of biotite present in the particular samples used in this work and its partially decayed state, preliminary measurements were made on samples of the whole rock. Most of the potassium in the material is situated in the feldspars, consequently owing to loss of argon by diffusion, a total rock determination would yield an age less than the true value. However, these measurements yielded an average minimum age for the rock of 515 million years. The close agreement of the total rock ages of the pink and grey granites (Table 1) does suggest that they were formed simultaneously.

A few grams of altered biotite were separated from the pink granite in an attempt to determine its absolute age. This result is shown in Table 1 and substantiates the preliminary results.

The total-volume method was used in age determinations on the whole rock and the technique of isotope dilution used in the measurement of the biotite sample.

Owing to the coarse and variable nature of the granite it is possible that the samples used in the potassium oxide analyses of the total rock might not have been identical to those used in the argon measurements. In view of this, the combined errors arising from uncertainties in content of potassium oxide and volume of argon measurement are estimated at ± 5 per cent. Experimental errors are, however, insignificant compared with those caused by loss of argon from the feldspars. Experience has shown that 30 per cent of the argon can easily have been lost by diffusion. Though the biotite is partially altered, losses should not be of this magnitude. In Table 1 the apparent ages together with their estimated experimental errors are given. Errors arising from loss of argon cannot be accurately evaluated and have not been included.

From these measurements it is concluded that Mahé Island and most probably all the granitic islands of the Seychelles Archipelago came into existence during the very late Pre-Cambrian. Most oceanic islands are considered to be younger than Mid-Cretaceous, consequently the Seychelles are out of place chronologically and petrologically.

It is interesting to note that a large number of minerals from the continent of Africa have ages around 630 million years[4]. If the age yielded by the biotite sample is a true one, it could be evidence

LETTERS TO THE EDITORS

GEOPHYSICS

Round-the-World Sound Waves produced by the Nuclear Explosion on October 30, 1961, and their Effect on the Ionosphere at Sodankylä

The heavy nuclear explosion on October 30, 1961, at 8.33.33 G.M.T. at a distance of 1,160 km. in Novaya Zemlya (presumably at tropospheric heights) was recorded at Sodankylä by means of a seismograph, a microbarograph, a magnetograph, and a vertical incidence ionosonde. The deflexion of the microbarograph took place at 9.42 G.M.T. with an amplitude of about ± 1 mb. The ionosonde showed a sudden, heavy disturbance in the *E* and *F* regions at 9 G.M.T., that is, 42 min. before the deflexion of the microbarograph (Fig. 1).

On October 31, the microbarograph again showed two very distinct and strong deflexions, namely, at 18.32 and 21.28 G.M.T. These deflexions are interpreted as being caused by round-the-world waves due to the same nuclear explosion, one being propagated in the backward, the other in the forward, direction (Fig. 2). The mean velocity deduced from these round-the-world waves is 311 m./sec. and is in good agreement with calculations based on mean atmospheric conditions below the stratopause. The wave-lengths in the recorded wave-trains lie between 198 and 58 km., and the waves are supposed to have been guided in the spherical shell between the ground and the stratopause.

Because there were no pronounced magnetic effects during the ionospheric disturbance, it seemed to be clear that the latter was not caused by excessive ionization but by a shock-wave passing over Sodankylä at ionospheric heights. This shock-wave would lose its excessive velocity and be propagated later as a sound wave in the temperature minimum between the stratopause and the lower thermosphere. If the waves are long, of the same order of magnitude as those measured on the ground, and if the formula which is giving the absorption loss[1] and which is normally applied to shorter wave-lengths will hold approximately for these wave-lengths, they can be propagated around the world without excessive attenuation. On the other hand, the intensity of the returning waves in the vicinity of the explosion site is greatly increased by focusing. Therefore, it seems reasonable to assume that these waves are strong enough to cause a detectable ionospheric effect when passing over Sodankylä.

The velocity of sound waves at ionospheric heights was estimated using rocket data[2]. The mean value obtained for the velocity was 420 m./sec., which is equivalent to round-the-world travelling time of 27 hr. On the other hand, according to the estimation mentioned above, the attenuation of such a sound wave travelling 40,000 km. and having a wave-length of 200 km. does not exceed 20 db. below heights of 150 km.

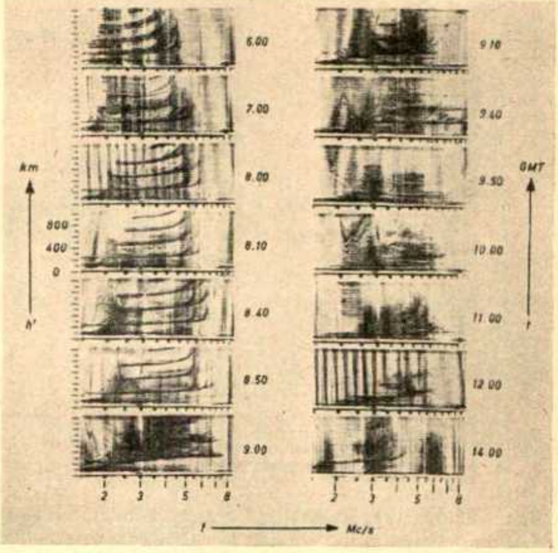


Fig. 1. Ionograms obtained at Sodankylä on October 30, 1961, showing an ionospheric disturbance caused by the nuclear explosion in Novaya Zemlya

The expected effects on the ionosphere are, indeed, to be seen on the ionograms (Fig. 3) at 9.50–11.40 G.M.T. on October 31. Although the ionograms are not completely continuous with time, we suppose that both the forward and backward propagated waves exist. This is because of the special shape of some of the oblique reflexions due to the slow sweeping speed of 4 min. over the frequency-range shown. We think that the end of the north-eastward travelling wave-train is to be seen on the ionogram at 10.50 G.M.T., whereas the end of the south-westward travelling one is to be seen on the ionogram at 11.40 G.M.T.

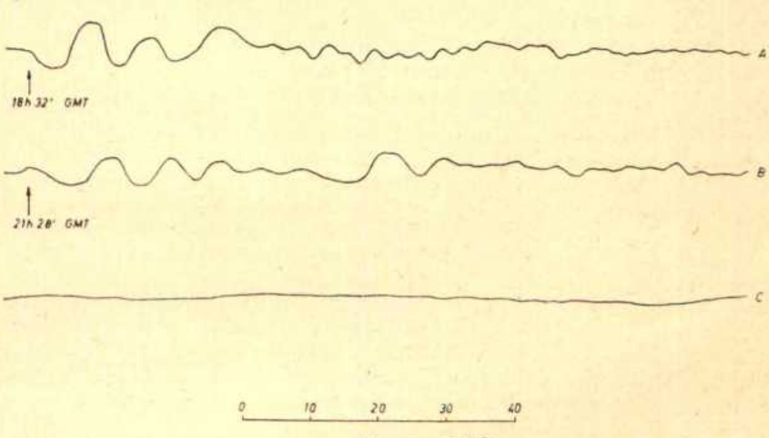
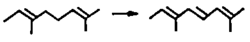
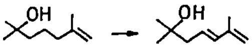
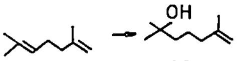
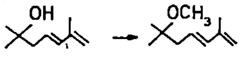
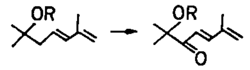


Fig. 2. Recordings of the microbarograph at Sodankylä on October 31, 1961. *A* and *B* are the deflexions of the backward and forward waves, respectively. *C* is a trace obtained under normal conditions. Pressure increases upwards

Table 3 THE FIVE TYPES OF STEP-REACTION INVOLVED IN THE BIOSYNTHESIS OF CAROTENOIDS IN PURPLE BACTERIA

	Chemical nature	Structure of molecule at site of attack	Occurrence
1	Dehydrogenation		I→II, II→III, III→IV, IV→V
2.	Dehydrogenation		IX→XI, X→XII, XV→XVI
3	Hydration		V→IX, XI(*a*)→X(*a*), IV→XV
4	Methylation		IX→XI(*a*), XII→VIII XVI→XIII
5	Introduction of a keto group		*XIII→XIV, XVI→XVII

* R = H or CH_3

dehydrogenations across a C—C bond, like those required by structures X and XI, are exceedingly common enzymatic reactions

The scheme outlined in Fig 1 supposes a total of 15 step-reactions subsequent to phytoene These 15 reactions can be grouped into five different types, depicted diagrammatically in Table 3 Each type of reaction is defined by two parameters (*a*) the chemical nature of the change that occurs, and (*b*) the structure of the molecule immediately adjacent to the site of attack In principle, a total of five enzymes might therefore be sufficient to catalyse all the reactions shown in Fig 1 The biological evidence, suggests, however, that the actual number is somewhat greater In the genus *Rhodospirillum*, *Rhodospirillum molischianum* produces only lycopene and rhodopin, *Rhodospirillum photometricum* produces lycopene, rhodopin, rhodovibrin and anhydro-rhodovibrin, and *Rhodospirillum rubrum*, all the carotenoids of the *A* group These differences can be accounted for enzymatically only by the supposition that specific enzymes catalyse the two similar successive dehydrogenations of type 2 involved in this sequence Both these enzymes are lacking in *Rhodospirillum molischianum*, one is present in *Rhodospirillum photometricum* and both are present in *Rhodospirillum rubrum*

The divergence of biosynthetic pathways subsequent to the formation of neurosporene, which leads in most purple bacteria to the exclusive formation of either group *A* or group *B* pigments, implies that the dehydrogenation of neurosporene to lycopene is enzymatically distinct from the preceding dehydrogenations which result in the formation of neurosporene from phytoene, even though it also belongs to type 1 On the other hand, the accumulation patterns observed in mutants rather strongly suggest that the three dehydrogenations which result in the conversion of phytoene to neurosporene may all be catalysed by a single enzyme Both in purple bacteria and in other organisms synthesizing carotenoids, mutants which accumulate phytoene alone have been frequently isolated Such mutants must have lost the enzyme required to convert phytoene to phytofluene But mutants which can be interpreted as having lost the enzyme that converts phytofluene to ζ-carotene, or ζ-carotene to neurosporene, have never been found When a mutational block leads to the accumulation of phytofluene and ζ-carotene, these substances are invariably accompanied by much larger amounts of more oxidized carotenoids (for example, neurosporene and chloroxanthin in the green mutant of *Rhodopseudomonas spheroides*) These otherwise puzzling facts become readily intelligible on the hypothesis that all the dehydrogenations between phytoene and neurosporene are catalysed by the same enzyme, loss of this enzyme necessarily resulting in the accumulation of phytoene alone

A full account of the chemical investigations by one of us (S L J) on the structures of the group *A* carotenoids and a further discussion of the present topic will be published elsewhere

We wish to acknowledge the kindness of Prof B C L Weedon in communicating to us the chemical structures of certain of the carotenoids discussed, prior to their publication

Note added in proof The biosynthetic scheme shown in Fig 1 predicts the existence of an intermediate between rhodopin (IX) and anhydro-rhodovibrin (XI), since this conversion requires both a dehydrogenation of type 2 and a methylation The expected intermediate, 3,4-dehydro-rhodopin, has recently been discovered by one of us (S L J) in *Rhodopseudomonas palustris* The structure of this new group *A* carotenoid has been unequivocally established by the determination of its nuclear magnetic resonance spectrum (Jackman, L M, and Jensen, S L, *Acta Chem Scand*, in the press) The position of the chromophore in 3,4-dehydrorhodopin provides further support for the assignment of structures X and XI to rhodovibrin and anhydrorhodovibrin, respectively

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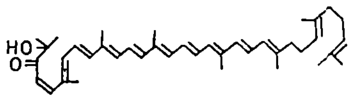
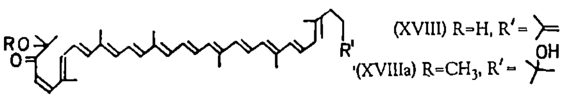


(XVII)



(XVIII) R=H, R'=

(XVIIIa) R=CH_3, R'=

When the wild-type of *Rhodopseudomonas spheroides* is cultivated under semi-aerobic conditions, its carotenoid composition changes markedly from that characteristic of anaerobic (photosynthetic) cultures[7] Pigments *Y* and OH-*Y* virtually disappear, the major carotenoids are spheroidenone and OH-*R*, accompanied by smaller amounts of a very highly unsaturated carotenoid which has been termed *P*512 (ref 1) The adsorptive properties reported for this carotenoid[1,9] suggest that it contains at least one hydroxyl group The positions of the absorption maxima in visible light fit with the assumption of 12 carbon-carbon double bonds and a keto group in conjugation The biosynthesis of *P*512 can be interpreted as resulting from the dehydrogenation of OH-*R* or spheroidenone by a reaction of the type characteristic of the phytoene-lycopene sequence, suggesting structure XVIII or XVIII*a* for this carotenoid.

We plan to investigate further the chemical structures of OH-*Y*, OH-*R* and *P*512

Conclusions

The complete scheme which we propose for the biosynthesis of the carotenoids of the purple bacteria is shown in Fig 1 It should be noted that in this scheme structures X and XI have been employed for rhodovibrin and anhydro-rhodovibrin, respectively, even though the chemical data now available do not exclude structures X*a* and XI*a* The preference for X and XI rests on the fact that these structures make it possible to formulate the conversion of lycopene to anhydro-rhodovibrin, and of anhydro-rhodovibrin to spirilloxanthin, as reactions strictly analogous to the conversion of neurosporene to pigment *Y* Furthermore, α,ω-dehydrogenations of the type which must occur in the biosynthesis of spirilloxanthin assuming structures X*a* and XI*a* are not known to occur as enzymatic reactions, whereas

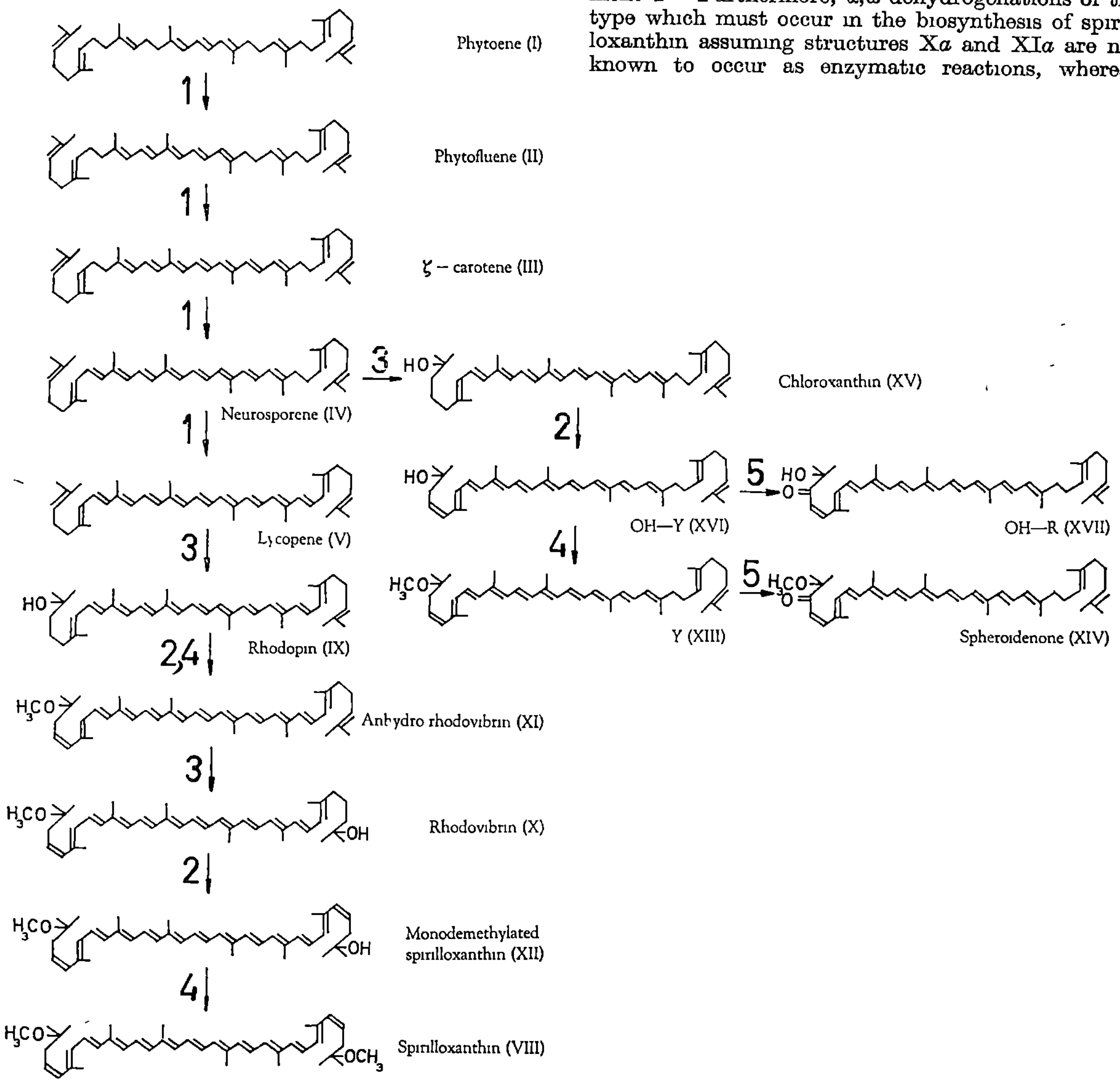
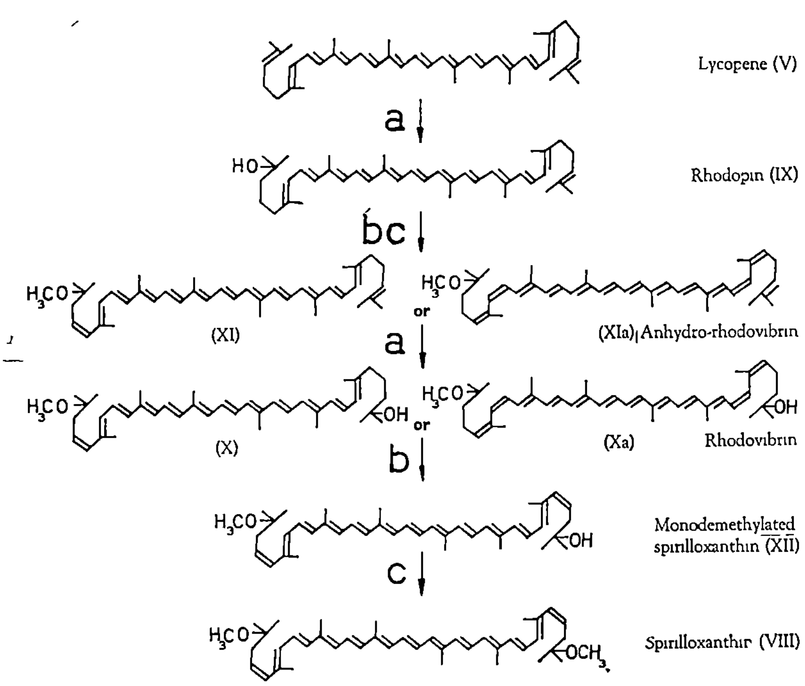


Fig 1 Structural scheme for the pathway of carotenoid biosynthesis in purple bacteria



(VIII)[26–28], and to the demonstration that the monohydroxylycopene formed in *Rhodospirillum rubrum* is identical with rhodopin, not with lycoxanthin (VI). Structure IX has been assigned to rhodopin[29,30]. Furthermore, the structures of the pigments known as OH-*P*481, *P*481 and OH-spirilloxanthin have been largely determined. OH-*P*481 is identical with rhodovibrin, and has either structure X or structure X*a* (ref. 31). *P*481 (anhydro-rhodovibrin) has either structure XI or structure XI*a* (ref. 32). In the case of these two pigments, the uncertainty concerns the exact position of the chromophore in the molecule. OH-spirilloxanthin is a monodemethylated spirilloxanthin (XII) (ref. 28).

In the light of these chemical facts, a simple and consistent interpretation of the biosynthetic transformations between lycopene and spirilloxanthin can now be offered[32]. These transformations involve three different types of chemical reactions, each of which takes place twice in the overall sequence:

(*a*) The hydroxylation step: addition of water to an isopropylidene double bond, with formation of a tertiary alcohol.

(*b*) The dehydrogenation step: introduction of an additional double bond in conjugation with the already existing chromophore, either at the same side as the hydroxyl group (assuming formulæ X and XI for rhodovibrin and anhydro-rhodovibrin) or by α,ω-dehydrogenation (assuming formulæ X*a* and XI*a* for the two compounds in question). For reasons of biochemical analogy which will be shortly discussed, we prefer the first formulation and thus favour formulæ X and XI.

(*c*) The methylation step: methylation of a tertiary hydroxyl group.

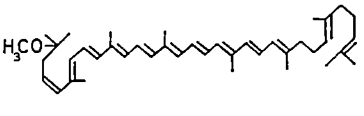
The initial reaction which leads to the formation of the group *A* pigments is a dehydrogenation: the conversion of neurosporene to lycopene. This reaction is not a dehydrogenation of type *b*, but is analogous rather to the successive dehydrogenations which result in the conversion of phytoene to neurosporene.

Biosynthesis of the Group *B* Carotenoids

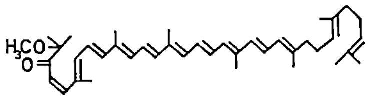
Goodwin, Land and Sissins[8], who first investigated the chemical structure of pigment *Y* and spheroidenone (pigment *R*), showed that they were monomethoxy compounds, and that spheroidenone had a conjugated keto group. The actual structures which they proposed[7,33] were, however, incorrect. Definitive structures for pigment *Y* (XIII) and spheroidenone (XIV) have recently been established by Barber, Jackman, Weedon and Yokoyama[34]. Pigment *Y* can be visualized as consisting of half a spirilloxanthin molecule (VIII) coupled with the more saturated half of the neurosporene molecule (IV). This, in turn, suggests that a sequence of reactions similar to those operative in spirilloxanthin synthesis must occur in the synthesis of pigment *Y* from neurosporene. Structurally speaking, this would require the formation of compounds XV and XVI as intermediates.

Structure XV has recently been suggested for chloroxanthin by Barber, Jackman, Weedon and Yokoyama[34]. Chloroxanthin, first characterized by Nakayama[9], is a monohydroxy derivative of neurosporene that accumulates together with neurosporene in the green mutant of *Rhodopseudomonas spheroides*, which is totally blocked in the synthesis of pigment *Y* and spheroidenone.

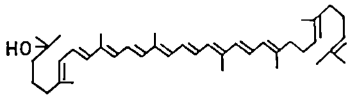
In the wild-type of *Rhodopseudomonas spheroides* grown anaerobically in the light, pigment *Y* and spheroidenone are always accompanied by smaller amounts of related monohydroxy compounds. The properties so far reported for OH–*Y* (refs. 7 and 9) are in accord with those which can be predicted for structure XVI. We therefore suggest that OH–*Y* is non-methylated and is the immediate biosynthetic precursor of *Y*. A similar chemical relationship can be postulated between speroidenone and OH–*R*, in which case the latter should possess structure XVII. The limited chemical data available[7,9] are in accord with such a supposition.



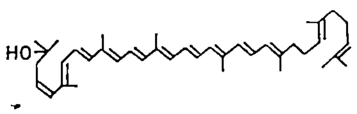
Y (XIII)



Spheroidenone (XIV)



(XV)



(XVI)

is phytoene[7], the specific site of the enzymatic defect is thus reasonably interpreted as lying at the step between phytoene and phytofluene

The biosynthetic origin of the group *B* pigments has been investigated in *Rhodopseudomonas spheroides*, from which a series of mutants with specific blocks in carotenoid synthesis has been isolated The patterns of accumulation in these mutants (Table 2) are most readily interpreted in terms of the following sequence

$$\text{phytoene} \rightarrow \text{phytofluene} \rightarrow \zeta\text{-carotene} \rightarrow \left\{ \begin{array}{l} \text{neurosporene} \\ \text{chloroxanthin} \end{array} \right\} \rightarrow Y \rightarrow \text{spheroidenone}$$

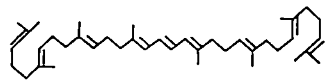
The biochemical data accordingly suggest that the pigments of groups *A* and *B* are synthesized from a common series of more saturated carotenoids originating with phytoene, the specific pathways diverging from one another subsequent to the formation of neurosporene In support of the hypothesis of a common origin for the pigments of both groups, it should be added that traces of neurosporene, ζ-carotene and phytofluene can be found in exponentially growing cells of the wild types of both *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides*[4,7]

The biological experiments which led to these interpretations of carotenoid synthesis in purple bacteria were carried out several years ago At that time, there were many uncertainties and some misconceptions concerning the chemical structures of the compounds involved Consequently, it was not then possible to formulate in chemical terms the postulated reaction sequences, or to establish the chemical nature of the individual step-reactions. These uncertainties and misconceptions concerning carotenoid structure have been largely eliminated by recent chemical work, which reveals that the earlier biological data in fact conform with a rather simple and chemically intelligible biosynthetic scheme We shall consider in turn the nature of the common primary step-reactions and of the specific step-reactions which lead to the group *A* and group *B* pigments

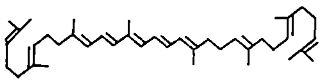
The Common Primary Step-Reactions

The structure of phytoene (I) was well established by the work of Rabourn and Quackenbush[12] The same workers afterwards assigned structures to phytofluene (II) and ζ-carotene (III)[13,14], although the chemical evidence for these structures has not yet been published Zechmeister[15] also recently suggested structure II for phytofluene Structures I, II and III for these carotenoids have been confirmed in an elegant fashion by Davis, Jackman, Siddons and Weedon[16] Neurosporene was long considered to be identical with 5,6,5′,6′-tetrahydrolycopene, synthesized by Eugster, Linner, Trivedi and Karrer[17], and thus to be a structural isomer of ζ-carotene (III) The improbability of an isomeric relationship between ζ-carotene and neurosporene in the light of the chemical evidence available had been pointed out by several authors[18,19], but definite proof that neurosporene is a dihydrolycopene with structure IV has only recently been furnished by Davis, Jackman, Siddons and Weedon[16]

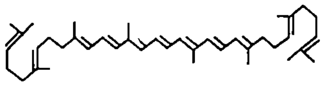
Phytoene is characterized by the possession of a centrally located chromophore with 3 conjugated double bonds, and of 6 isolated double bonds, the molecule thus possesses complete bilateral symmetry



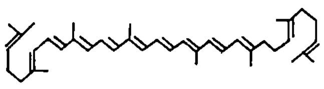
Phytoene (I)



Phytofluene (II)



ζ – carotene (III)



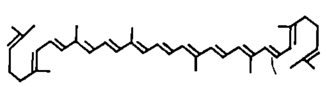
Neurosporene (IV)

The successive formation of phytofluene, ζ-carotene and neurosporene from phytoene evidently involves a series of stepwise dehydrogenations at each step, a new double bond is introduced alternatively to the left and to the right side of the phytoene chromophore Each double bond so introduced brings into conjugation one of the previously isolated double bonds, and the length of the chromophore is accordingly increased by two conjugated double bonds at each step The biosynthesis of neurosporene from phytoene therefore proceeds by three successive dehydrogenations, identical in mechanism and differing from one another only in the specific points on the polyene chain at which they take place

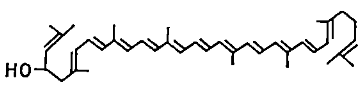
A reaction sequence of this type was in essence first proposed by Zechmeister[20], on the basis of chemical considerations, to account for the biological origin of the coloured carotenoids This hypothesis was further elaborated by Porter and Lincoln[21], who used it to explain the genetic and biochemical data concerning carotenoid synthesis in tomato fruits The so-called 'Porter–Lincoln sequence', which is in fact the only coherent general hypothesis ever advanced to explain the biological formation of coloured carotenoids, was long treated with scepticism by most workers in the field (see, for example, refs 22 and 23) Such scepticism can no longer be justified

Biosynthesis of the Group *A* Carotenoids

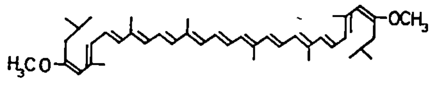
At the time when the biosynthetic pathway from lycopene to spirilloxanthin was determined in *Rhodospirillum rubrum*, the structures of three members of the sequence were assumed to be established lycopene (V)[3], lycoxanthin (VI)[8,24] and spirilloxanthin (VII)[25]



(V)



(VI)



(VII)

However, recent chemical investigations have led to a revision of the structure of spirilloxanthin induced by electrical stimulation of reticular formation, involve (at least as a component of a more complex mechanism) the breakdown of high-energy phosphorus compounds Triggered by the impulses generated by reticular neurones it sets in motion the processes leading to lactic acid formation Our results confirmed the finding of previous workers[4] that the brain lactic acid does not rapidly disappear from the tissue when once it has been formed

From these considerations it follows that, unless it is believed that changes produced by electrical stimulation of brain stem reticular formation in our experiments are not of a physiological nature, it must be inferred that physiological processes resulting from an increased functional activity of reticular neurones (such as arousal or increased attention) are associated with increased glycolytic activity and the liberation of the lactic acid in the brain

We thank Dr R M C Dawson, Institute of the Department of Animal Physiology, Cambridge, for kind advice regarding the method of lactic acid estimation

This work was supported in part by a grant from the Yugoslav Foundation for Scientific Research, contract No 490/1

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BIOSYNTHESIS OF CAROTENOIDS IN PURPLE BACTERIA: A RE-EVALUATION BASED ON CONSIDERATIONS OF CHEMICAL STRUCTURE

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THE photosynthetic apparatus of purple bacteria contains a distinctive array of carotenoid pigments they are aliphatic, and mostly bear methoxyl groups Five main pigments, together with their monohydroxy derivatives, have been found in the species so far examined lycopene, *P*481 (anhydrorhodovibrin), spirilloxanthin, pigment *Y* and spheroidenone (formerly known as pigment *R*) On the basis of their biological distribution, these ten pigments can be subdivided into two main groups (Table 1) Some species of purple bacteria contain exclusively pigments of the lycopene, *P*481 and spirilloxanthin types (group *A* pigments), others, exclusively those of the *Y* and spheroidenone types (group *B* pigments) These regularities in distribution in themselves strongly suggest that the pigments of groups *A* and *B* represent the products of two divergent biosynthetic pathways Stoichiometric interconversions of pigments belonging to each group have been demonstrated to take place in certain circumstances within the bacterial cell, and this and the nature of the observed conversions establish unequivocally two sets of biosynthetic interrelationships[4-6]

$$\text{lycopene} \rightarrow P481 \rightarrow \text{spirilloxanthin, and}$$
$$Y \rightarrow \text{spheroidenone}$$

Table 1 NATURAL DISTRIBUTION OF THE MAJOR CAROTENOIDS IN PURPLE BACTERIA

	Group *A*			Group *B*		
	Lycopene	*P*481	Spirilloxanthin	*Y*	Spheroidenone	
Rhodospirillum molischianum	+	–	–	–	–	refs 1, 2
Rhodospirillum photometricum	+	+	–	–	–	
Rhodospirillum rubrum	+	+	+	–	–	
Rhodopseudomonas palustris	+	+	+	–	–	
Chromatium	+	+	+	–	–	ref 3
Rhodopseudomonas spheroides	–	–	–	+	+	refs 1, 2
Rhodopseudomonas capsulatus	(+)	–	–	+	+	
Rhodopseudomonas gelatinosa	–	–	(+?)	+	+	

In *Rhodospirillum rubrum*, synthesis of all group *A* pigments can be suppressed by bacterial growth in the presence of an appropriate concentration of diphenylamine In these circumstances, there is a massive intracellular accumulation of carotenoids more saturated than lycopene (phytoene, phytofluene, ζ-carotene, neurosporene) After removal of the diphenylamine, these reduced carotenoids can be converted within the cell to the typical group *A* pigments The analysis of the diphenylamine effect in *Rhodospirillum rubrum* has permitted the formulation of the following biosynthetic sequence for the group *A* pigments[4]

$$\begin{array}{c}\text{phyto-}\\\text{fluene}\end{array} \rightarrow \begin{array}{c}\zeta\text{-caro-}\\\text{tene}\end{array} \rightarrow \begin{array}{c}\text{neuro-}\\\text{sporene}\end{array} \rightarrow \left.\begin{array}{l}\text{lycopene}\\\text{OH-lycopene}\end{array}\right\} \rightarrow$$
$$P481 \rightarrow \text{OH-}P481 \rightarrow \left\}\begin{array}{l}\text{spirilloxanthin}\\\text{OH-spirilloxanthin}\end{array}\right.$$

So far, only one mutant of *Rhodospirillum rubrum* with a specific block in carotenoid synthesis has been found The only carotenoid produced by this mutant

Table 2 CAROTENOID PIGMENTS IN THE WILD-TYPE AND IN 3 MUTANT CLASSES OF *Rhodopseudomonas spheroides* GROWN UNDER ANAEROBIC CONDITIONS IN THE LIGHT

	Phytoene	Phytofluene	ζ-Carotene	Neurosporene, chloroxanthin	*Y*, OH–*Y*	Spheroidenone, OH–*R*	
Wild type	–	tr	tr	tr	+	+	(refs 8, 9, 11)
Brown mutant	–	tr	tr	+	+	tr	(ref 9)
Green mutant	–	tr	tr	+	–	–	(ref 10)
Blue-green mutant	+	—	—	—	—	–	(ref 11)

Starting from these investigations and the well-established role of the reticular formation in the regulation of the states of wakefulness and sleep, the present investigation was carried out to find out if similar biochemical changes take place in the brain following electrical stimulation of the brain-stem reticular formation and if they could be correlated with concomitant changes in electrical activity

Young rats of 50–90 gm anæsthetized with 'Nembutal' (50 mgm /kgm body-weight) were used in the experiments Recording electrodes were placed on the frontal and parietal cortex A fine stimulating electrode (0 01 in diameter) was inserted stereotaxically in the brain-stem reticular formation according to co-ordinates based on measurements previously made on several animals of similar age and size The operative procedure was completed in 10 min after initiation of anæsthesia in all experiments Stimulating and recording was programmed in such a way as to permit immersion of the animals into liquid air exactly at the end of the twentieth minute after 'Nembutal' injection Thus, the period of anæsthesia was maintained constant in all the experiments Electrical stimuli (0 3 m amp , 300 cycles/sec and 0 25 m sec pulse duration), delivered by a Grass stimulator model 4*S*, were given for two periods of 10 sec , with an interval of 5 sec between the two stimulations The electrocorticogram was recorded throughout the experiment using a Reega Alvar apparatus This provided an index of the blood and oxygen supply to the brain during the experiment Details of the stimulation and recording technique are published in ref 3

Rats were divided into eight groups (each consisting of at least seven animals) Rats from the first (control) group received only an injection of 'Nembutal', those from the second (control) group were placed in the stereotaxic instrument, incision was made and holes drilled in the skull, the third (control) group consisted of rats, with cortical and reticular electrodes inserted, which were not subjected to stimulation Animals belonging to the fifth, sixth, seventh and eighth group were stimulated as described and dropped in the liquid air 30 sec , 1 min , 3 and 5 min respectively, after cessation of electrical stimulation In order to secure rapid fixation of biochemical changes and prevent post-mortem glycolysis, animals were killed by immersion in the liquid air Reasons for freezing the whole animal have been described (ref 4). To facilitate dissection, the frozen animals were immediately transferred into a deep-freezer at – 20° C Rapidly dissected with cooled instruments, brains (sectioned just in front of the cerebellum) were dropped in the liquid air and powdered in a previously cooled crusher Lactic acid was estimated according to the method of Friedemann *et al.* with modifications introduced by other authors[5]

The results are illustrated in Fig 1 In the first (control) group of animals, killed after being in anæsthesia for 20 min , the lactic acid content ranged from 6 5 to 10 5 mgm per cent with a mean of 8 9 (broken line in Fig. 1) These figures are in agreement with values obtained by other authors under similar conditions[4] Estimations of the lactic acid content of the brain of rats comprising the second and third (control) group of animals gave somewhat higher values than those of the first group (12 0 and 12 5 mgm. per cent respectively—full line in Fig 1) This showed that fixing the animal into the stereotaxic apparatus and the operative procedure used, already raised to some extent the lactic acid content of the brain It is conceivable, therefore, that this level should be taken as a basis for comparing values obtained after electrical stimulation As seen in Fig 1, electrical stimulation of brain stem reticular formation was consistently found to be followed by a rapid and significant increase of the lactic acid content of the brain ($P > 0\ 001$ with Fisher's t test) No such high values were obtained following focal stimulation of cerebral cortex under identical conditions or even after strong hippocampal after-discharge produced by insertion of electrode into this structure Experiments in which the period between the cessation of electrical stimulation and immersion of animal into the liquid air was varied from 30 sec to 5 min (Fig 1) showed that this increase was of short duration, followed by a return of the lactic acid within the limits of control variations 5 min after discontinuing the stimulation

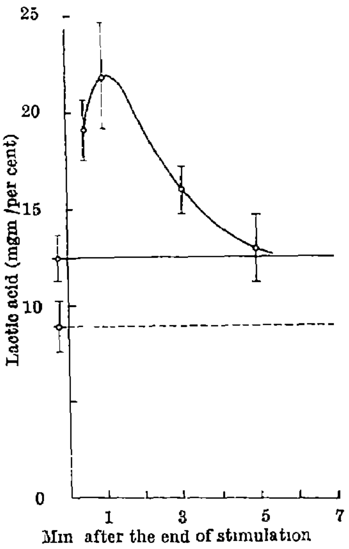

Fig 1

It should be pointed out that raising the lactic acid outlasted the transient desynchronization of the electrocorticogram When increase of lactic acid was at a peak (that is, between 30 sec and 1 min after discontinuing the stimulation) the pre-stimulation type of electrical activity was usually recorded

Formation of the lactic acid in the brain under normal physiological conditions has been demonstrated by Gibbs *et al* Experiments of Richter and Dawson showing that the lactic acid content of the brain is lowered in normal sleep and increased in emotional excitement provided further evidence that the rate of lactic acid formation varies with the functional activity of the brain[4] In another series of experiments these authors reported that electrical stimulation of the brain produces a rapid fall in the phosphocreatine content and transient rise in the hexose phosphate fraction followed by their return to normal level 15–45 sec after electrical stimulation, indicating the dynamic balance between the breakdown and re-synthesis of high-energy phosphate bonds[7]

Results reported in the present article also suggest that biochemical events, underlying the 'dynamogenesis' or increased functional activity of the brain

Table 3*

Nature	Action	Influence of ether	Adsorption on barium sulphate	Thermo- stable	Thermo- labile	Neutralization by toluidine blue and protamine sulphate	Generation during fibrino- or proteo-lysis
I Fibrin	Immediate	–	–	4°, 1 (month)	56°, 10 (min)	–	–
Heparin	Immediate	–	+	4°, 1 (month) 100°, 10 (min)		+	–
II Lipoprotein	Immediate	+	–	4°, 1 (month) 56°, 10 (min)	60°, 10 (min)	+	–
III Lipoprotein	Progressive	+	–	4°, 1 (month) 56°, 10 (min)	60°, 10 (min)	–	–
IV	Immediate	–	–	60°, 10 (min)	70°, 10 (min)	–	–
V Euglobulin	Immediate	–	–	56°, 10 (min)	100°, 10 (min)	–	–
VI Fibrinopeptide	Immediate	–	–		60°, 30 (min)	Partially	+
Brain antithrombin (lipid?)	Immediate	+		4°, 1 (month) 60°, 20 (min) 100°, 10 (min)		–	–
Normal cerebrospinal fluid antithrombin (lipoprotein?)	Progressive	+	–	45°, 60 (min)	75°, 5 (min) 100°, 1 (min)	Partially (?)	–
Pathological fluid antithrombin	Progressive		+		75°, 5 (min) (?)		?

* Refs 11–15

but do not destroy the antithrombin activity of normal cerebrospinal fluid This activity is not adsorbed by barium sulphate or destroyed by heating (45°, 1 hr), but disappears after addition of ether or heating at 75° for 10 min and at 100° for 1 min

The considerable antithrombin activity of the pathological cerebrospinal fluid is due either to the increase of physiological antithrombin or to the appearance of an abnormal antithrombin(s ?) The last possibility is even more probable because the pathological antithrombin is adsorbed on barium sulphate which does not adsorb the physiological antithrombin of cerebrospinal fluid The pathological antithrombin activity seems to be higher in the acute phase of inflammatory (meningitis) than in chronic neurological diseases (especially degenerative diseases, for example, progressive palsy) and to decrease with improvement of clinical and laboratory signs The relationship between the antithrombin of pathological cerebrospinal fluid and antithrombin I and VI, trypsin (present in inflammatory cerebrospinal fluid)[10] or other proteo-fibrino(geno)lytic agents can be established only after further investigations Contrary to the behaviour of blood antithrombins, the pathological antithrombin of cerebrospinal fluid is adsorbed on barium sulphate and has a progressive action (Table 3)

The question as to where the antithrombin of normal and pathological cerebrospinal fluid is formed and how it enters the fluid cannot be answered Also the significance of, and part played by, the normal and pathological antithrombin of cerebrospinal fluid remain unknown The investigations on the hæmostatic (especially antithrombin) activity of normal and pathological fluid can possibly help the diagnosis, differentiation and prognosis of certain neurological diseases.

The investigations were supported by the Rockefeller Foundation and by the Polish Institute of Sciences in the United States

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EFFECTS OF ELECTRICAL STIMULATION OF RETICULAR FORMATION ON THE LACTIC ACID CONTENT OF THE RAT BRAIN

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IT is customary, especially in the literature on electrophysiology, to speak about the 'activating', 'energizing', or 'dynamogenic' action of the brain-stem reticular formation on the electrical activity of the cerebral cortex Since the pioneer work of Moruzzi and Magoun[1], a great deal of information has been accumulated concerning the electrical manifestations of this activating process and its neuronal mechanism However, little is known about the metabolical correlates of this activation, that is, about the biochemical events triggered by impulses from reticular neurones It is generally assumed that any form of stimulation or departure from the resting state might start glycolytic processes, with the liberation of inorganic phosphorus and lactic acid It has been reported that the metabolical activity of the brain is increased in trauma, shock, convulsions and emotional excitement and reduced in anæsthesia and sleep[2] blood[9]. Gohr and Scholl[1] found in cerebrospinal fluid an inhibitor of thrombin generation which has no effect on the already generated thrombin. They denied any identity between this inhibitor and heparin, while Kafka[2-4] denied the existence of an antithrombin in cerebrospinal fluid at all.

In spite of the last negative statements we decided to re-investigate the problem of the antithrombin activity of normal and pathological cerebrospinal fluid.

(1) The cerebrospinal fluid was collected by lumbar puncture and centrifuged for 10 min. at 3,000 r.p.m. in order to sediment and eliminate the morphological elements. The determinations were performed after storing cerebrospinal fluid for 6 hr. at 4° C. (2) The primary solution of thrombin ('Thrombofort'—Richter plus distilled water) was diluted with veronal–acetate buffer in equal volumes in order to obtain a secondary solution. Only a part of the primary solution was used to prepare the secondary one, which allowed us to standardize the activity of the first thrombin solution. 0.2 ml. of the secondary solution must clot 0.2 ml. of oxalated deprothrombinized plasma within 30 ± 2 sec. (3) Deprothrombinized plasma was prepared from a pooled oxalated plasma from at least five blood-donors by shaking for 10 min. with barium sulphate (1 : 10) and subsequent centrifugation. This plasma, free from factors II, VII, IX and X, and containing factors V, VIII and XI (plasma thromboplastin antecedent), did not clot for at least 15 min. after the addition of equal volumes of thromboplastin and *M*/40 solution of calcium chloride.

To determine thrombin inactivation 0.4 ml. of the primary thrombin solution was mixed with 0.4 ml. of cerebrospinal fluid. Immediately, 0.2 ml. of this mixture was added to 0.2 ml. of oxalated deprothrombinized plasma. The coagulation time of the mixture was determined. The determination was carried out again after a 10- and 20-min. incubation of thrombin solution with cerebrospinal fluid. In a control reaction 0.2 ml. of veronal–acetate buffer (*p*H 7.35) was used instead of cerebrospinal fluid. All determinations were performed at room temperature.

The first group of experiments concerned twenty specimens of fluid showing no pathological changes in the routine laboratory tests (protein, globulin, sugar and chloride levels, pleocytosis, Nonne-Apelt, Weichbrodt and Pandy reactions). Table 1 shows that after 10- and 20-min. incubation the thrombin activity of the experimental mixture decreases slowly and progressively in the presence of normal cerebrospinal fluid. A certain tendency to a greater prolongation of the coagulation time in the presence of a higher concentration of proteins appeared, but was neither stable and repeatable nor statistically significant. The comparative investigations showed that the antithrombin of normal cerebrospinal fluid is considerably weaker than the antithrombin of blood and of pathological cerebrospinal fluid. Toluidine blue (0.05 ml. of a 2.5 mgm./ml. concentration) and protamine sulphate (0.05 ml. of a 1 mgm./ml. concentration) reduced, but did not abolish, the antithrombin activity of cerebrospinal fluid as compared with control reactions with distilled water (0.05 ml.). The antithrombin activity of normal cerebrospinal fluid did not change after adsorption on barium sulphate, but disappeared after addition of ether. Heating for 1 hr. at 45° C. did not influence essentially the antithrombin activity of normal cerebrospinal fluid. After 10 min. at 75° and 1 min. at 100° the antithrombin activity of normal cerebrospinal fluid disappeared.

Table 1

	No. of cases	Time for incubation (min.) 0		10		20	
Normal cerebrospinal fluid	20	29.75	(30)*	33.55	(30)	37.37	(31)
Normal serum	10	27.4	(30)	95.5	(30)	205.6	(31)
Toluidine blue	10	27.6	(29.6)	30.4	(34.4)	33.6	(39.4)
Protamine sulphate	10	27.1	(29.7)	30.6	(34.4)	33.9	(38.5)
Barium sulphate	10	29.45	(29.1)	32.85	(33.3)	36.1	(38.2)
	5†	*29.6*	*(29.5)*	*37.6*	*(70.2)*	*42.4*	*(120.6)*
Ether	5	29.2	(29.8)	28.5	(34.3)	30.8	(38.5)
75°, 5 min.	5	29.4	(29.2)	31.2	(33.4)	32	(38)

The mean values of the residual uninactivated thrombin time are given in seconds.
* Mean values of control determinations (in brackets).
† Normal cerebrospinal fluid in ordinary type, pathological fluid in italics.

Table 2

	Diagnosis	Incubation time (min.) 0	10	20	Proteins mgm. per cent	Globulins mgm. per cent	Pleocytosis	Nonne-Apelt, Weichbrodt and Pandy tests
1	Tuberculous meningitis	30	170	308	210	0.06	312	+
2	,, ,,	27	43	60	45	0.04	37	+
3	,, ,,	28	36	48	30	0.03	42	+
4	,, ,,	29.5	66	143	30	0.03	31	+
5	,, ,,	30	56	126	30	0.03	24	+
6	Pneumococcal meningitis	29	34	41	85	—	40	+
7	,, ,,	31*	165	623	480	—	1,690	+
		29	52	99	300	—	479	+
		29	44	78	—	—	240	+
		30.5	43	56	160	—	115	+
8	Meningitis	26	32.5	40	60	—	8/3	+
9	Encephalitis and meningitis	32	38	45	105	0.1	98	+
10	,, ,,	29	33	38	55	—	13/3	+
11	Hydrocephalus	26	42	64	240	—	25	+
		25.5†	29	41	320	—	'Many'	+
12	Progressive palsy	30	37	45	76	0.2	243	+

The values of the residual uninactivated thrombin time are given in seconds.
* Determinations of October 14, 17, 19, and 24, 1960.
† Ventricular cerebrospinal fluid.

The second group of experiments concerned twelve specimens of fluid with abnormal results in the laboratory tests (Table 2). The inactivation of thrombin in pathological fluid is greater than in normal fluid and sometimes even greater than in serum. The results show no correlation between the protein-level or pleocytosis and antithrombin activity of the pathological cerebrospinal fluid. In one case of pneumococcal meningitis the antithrombin activity decreased in parallel to the improvement of clinical signs and laboratory tests. In a patient with hydrocephaly the cerebrospinal fluid obtained by ventricular puncture showed a lower antithrombin activity than the fluid obtained by lumbar puncture. The pathological antithrombin activity disappears after adsorption on barium sulphate and after 10 min. heating at 75° (Table 1).

Our results provide evidence for the existence of an antithrombin activity in normal and pathological cerebrospinal fluid. We have been able to determine some of their physico-chemical and biological properties which allowed us to differentiate between the normal and pathological antithrombins of cerebrospinal fluid and the blood antithrombins (Table 3).

The antithrombin of normal cerebrospinal fluid has a progressive action considerably weaker than that of the blood antithrombin or of pathological fluid. Toluidine blue and protamine sulphate reduce

Table 1 VIRUS AND INFECTIVE RIBONUCLEIC ACID CONTENT OF ULTRACENTRIFUGE FRACTIONS OF FOOT-AND-MOUTH DISEASE VIRUS BEFORE AND AFTER ACIDIFICATION

	Virus titre in mice (log ID_{50}/ml)*	Ribonucleic acid titre in mice (log ID_{50}/ml)†	Complement-fixing activity‡
Initial virus diluted 1 to 10 in 0 1 per cent sodium dodecyl sulphate	7 2	3 2	94 2
Top 5 ml	5 0	1 6	17 8
Bottom 5 ml	7 6	3 4	72 4
Acidified virus (*p*H 5 6)	2 6	3 4	117 7
Top 5 ml	—	3 5	56 7
Bottom 5 ml	—	3 2	61 0

* Intraperitoneal inoculation of 0 03 ml

† Intracerebral inoculation of 0 03 ml

‡ Millilitres of 1/30 complement fixed by total 1/10 initial virus or fraction

Table 2. INFECTIVITY OF ACIDIFIED FOOT-AND-MOUTH DISEASE VIRUS BY VARIOUS ROUTES OF INOCULATION

	Titre in mice (log ID_{50}/ml)		Ribonucleic acid titre in mice (log ID_{50}/ml)
	Intra-peritoneal	Intra-cerebral	Intra-cerebral
Initial virus diluted 1 to 10 in 0 1 per cent sodium dodecyl sulphate	7 3	7 8	3 2
Same + 100 μgm ribonuclease per ml	7 3	7 6	—
Acidified virus (*p*H 5 0)	2 0	3 7	3 4
Same + 100 μgm ribonuclease per ml	1 8	1 7	—

by centrifuging the control and acidified virus suspensions at 30,000 r p m for 1 hr in the *L*40 head of the Spinco ultracentrifuge The upper and lower 5-ml fractions from each tube were then separated and titrated for virus content and also extracted with phenol and the infective ribonucleic acid estimated The results in Table 1 show that more than 90 per cent of the infective ribonucleic acid which could be extracted from the control virus was present, as virus, in the lower 5-ml fraction In contrast, equal amounts of infective ribonucleic acid could be extracted from the upper and lower 5-ml fractions of the acidified virus, indicating that the entity from which the infective ribonucleic acid was extracted had a smaller sedimentation constant than the initial virus Complement-fixation tests on the fractions showed that this activity also was distributed equally between the two fractions of the acidified virus, confirming the previous finding of Randrup[7] that the virus had disrupted to yield smaller complement-fixing particles Unacidified virus preparations contained only 20 per cent of the initial complement-fixing activity in the top 5 ml when centrifuged under the same conditions

Although these results showed that acidified virus suspensions contained a fragment which was smaller than the initial virus but which still yielded infective ribonucleic acid on extraction with phenol, they could not be taken as conclusive evidence that the infective ribonucleic acid itself had been released from the virus particle Some evidence has been obtained, however, which makes this seem likely By lowering the *p*H of the virus suspension in 0 1 per cent sodium dodecyl sulphate to 5 0, a product was obtained which was of low infectivity when tested intraperitoneally in mice (Table 2) This suspension was, however, about fifty times more infective when inoculated intracerebrally, but all this extra infectivity could be destroyed by adding 100 μgm ribonuclease per ml Moreover, the infectivity of the acidified virus tested intracerebrally was as high as that of the ribonucleic acid which could be obtained from it by phenol extraction It appears likely, therefore, that the infective material which was left after acidification was mainly the viral ribonucleic acid itself

Some of these experiments have been repeated with a foot-and-mouth disease virus of type *C* (strain 997) grown in tissue culture In these experiments also it was possible to extract infective ribonucleic acid from acidified virus of low virus infectivity These results thus confirm the earlier work of Mussgay[10] and also show that the dissociation of foot-and-mouth disease virus into its nucleic acid and protein components is not confined to one type of the virus

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ANTITHROMBIN ACTIVITY OF NORMAL AND PATHOLOGICAL CEREBROSPINAL FLUID

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THE coagulant activity of cerebrospinal fluid has been ascribed either to the presence of a thromboplastin[1-4] or of a factor-VIII-like[5] activity Some authors[2-4,6] admitted that cerebrospinal fluid may contain small amounts of prothrombin Normal cerebrospinal fluid contains neither fibrinogen nor fibrin, they appear in pathological cerebrospinal fluid when its protein-level increases beyond 100 mgm per cent[7]

Other investigators demonstrated the anticoagulant properties of cerebrospinal fluid In idiopathic hæmorrhage the red clot does not form from the extravasated blood in the presence of cerebrospinal fluid[8] The fibrinogen of tuberculous cerebrospinal fluid clots more slowly than the fibrinogen of the

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Table 3 ESTIMATED QUANTITIES OF MIXED SALIVA AND OF CONTAINED NITROGEN ENTERING THE OVINE RUMEN

State of animal	Mixed saliva output				Saliva nitrogen entering rumen			
	Flow-rate ml /hr	Volume l /day	No of observations	No of sheep	Amount mgm /hr	Amount gm /day	No of observations	No of sheep
Anæsthetized								
Mean	151 5	3 64	7	3	31 4	0 75	54	3
Range	(84 0–207)	(2 02–4 97)			(19 1–47 2)	(0 46–1 13)		
Conscious—not eating								
Mean	258 6	6 21	14	6	19 5	0 47	15	6
Range	(31 2–1,020 0)	(0 75–24 48)			(2 4–76 7)	(0 06–1 84)		
Mean—all data	227 7	5 35			28 8	0 69		

Estimates of the volume of mixed saliva put out per day by anæsthetized and conscious sheep, and of the daily salivary nitrogen input to the reticulo-rumen of these animals are shown in Table 3 The estimate for total daily saliva output by conscious sheep agrees substantially with Somers's[7] estimate of 5 1 (range 3–6 1) Whereas Somers[7] has indicated a mean daily saliva nitrogen input to the reticulo-rumen of 0 7–0 9 gm for conscious sheep, I have found a lower value (mean 0 47 gm /day, range, 0 06–1 84 gm day), but I have obtained close agreement with Somers's estimate[7] for conscious sheep with his value of 0 75 gm /day for anæsthetized animals

The negative relationship between rate of saliva flow and saliva nitrogen previously discussed is apparent in these mean values for anæsthetized and conscious sheep This relationship suggests that despite considerable increase in the secretion of mixed saliva, such as during feeding, the total amount of saliva nitrogen entering the rumen with the ingested feed is not greatly increased

Thanks are due to Dr D F Stewart, associate chief of the Division of Animal Health, Commonwealth Scientific and Industrial Research Organization, McMaster Laboratory, Sydney, for making available animal house facilities Particular thanks are due to Mr H Lahoud, laboratory attendant, and Mr S A S Douglas of the School of Wool Technology for chemical analyses of some of these samples

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DISSOCIATION OF FOOT-AND-MOUTH DISEASE VIRUS INTO ITS NUCLEIC ACID AND PROTEIN COMPONENTS

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IT is well established that preparations of many different animal viruses contain smaller particles in association with the infective virus particle Included in this group are the viruses of influenza[1], rabies[2], poliomyelitis[3], Rift Valley fever[4]. African horse sickness[5] and foot-and-mouth disease[6] In the latter case it has also been shown that the larger 25-mμ infective particle can be dissociated into the 7-mμ particle by lowering the *p*H to about 6 (ref 7) or by heating at 56° C (refs 8 and 9) Recent work by Mussgay[10] showed that lowering the *p*H resulted in the release of the infective ribonucleic acid core of the virus, and Bachrach[11] has since demonstrated that a similar release of the infective ribonucleic acid occurs when the virus is heated at 56° C The latter author, however, was unable to confirm Mussgay's findings with acidified virus[12] The present experiments were carried out in an attempt to resolve the differences between the results of these workers, as their findings have an important bearing on the structure of the infective particle

The infective ribonucleic acid which can be extracted with phenol from foot-and-mouth disease virus is extremely sensitive to environmental ribonuclease[12,13] In experiments designed to show the presence of the infective ribonucleic acid, therefore, it is necessary for the enzyme to be removed from the viral suspensions or for its action to be completely inhibited In most of the experiments described here, vesicular fluid obtained from guinea pigs infected with strain 1 (immunological type *O*) was used as the source of virus This was partly freed from ribonuclease by three successive precipitations with half-saturated ammonium sulphate The precipitated virus was then re-suspended at 1/10 of its initial concentration in 0 1 per cent sodium dodecyl sulphate, which was a concentration sufficient to protect infective viral ribonucleic acid from the action of the ribonuclease remaining in the virus suspension Lowering the *p*H of this suspension to 5 6 led to a considerable reduction in virus titre (measured by intraperitoneal inoculation of 7-day-old mice) As much infective ribonucleic acid could be extracted with phenol from this acidified virus, however, as from the initial virus (Table 1) Infective ribonucleic acid was estimated by intracerebral inoculation of mice, as it is non-infective when administered intraperitoneally

Evidence that acidification had caused breakdown of the virus into smaller particles was obtained

Table 1 RATE OF EXTRUSION OF MIXED SALIVA FROM ŒSOPHAGEALLY FISTULATED SHEEP CONSUMING ROUGHAGE DIETS

Sheep and sampling date	Remarks	Duration of sampling (min)	Weight of feed consumed (gm dry matter)	Percentage recovery of extruded feed (dry matter basis)	Volume of saliva collected (ml)	Rate of extrusion of saliva (ml /min)
	Diet 1					
1 Oct 14, 1955	Animal hungry and in good condition Ate consistently	40	67 9	75	443	11 1
1 Oct 17, 1955	Animal hungry and in good condition Ate consistently	20	42 0	58	76	3 8
1 Oct 19, 1955	Animal distressed due to fistula leakage Hungry, losing weight Ate consistently	38	58 3	95	291	7 7
1 Oct 28, 1955	Animal distressed due to fistula leakage Hungry, losing weight Ate consistently	29	43 2	97	186	6 4
2 April 29, 1959	Animal hungry, ate consistently	25	178 3	71	163	6 5
3 April 29, 1959	Animal hungry, ate consistently	20	165 5	63	240	12 0
	Diet 2					
4 May 2, 1959	Animal hungry, ate consistently	50	38 6	53	515	10 3
5 May 1, 1959	Animal hungry, ate consistently	35	55 8	95	398	11 4
2 May 13, 1959	Animal hungry, ate consistently	70	141 4	39	526	7 5

Note On no occasion reported here were fistulæ blocked either by 'packing' of feed, or by the polythene collection bags pressing against them

docile than others Before sampling, the mouth and the œsophagus were washed out with distilled water At sampling, most, if not all, of the saliva passed via fistula from the conscious sheep and was collected The fluid in these cases was almost invariably ejected as a cohesive volume rather than as a steady trickle On the other hand, the experimental conditions of collection of saliva as it dripped from the gagged mouth of anæsthetized animals were almost constant. It is reasonable to conclude that collection of saliva from these sheep was complete The mean concentration of sodium and potassium in the mixed saliva of one conscious, fistulated sheep (seven half-hourly sub-samples) were 138 5 m equiv /l and 35 7 m equiv /l respectively The electrolyte composition of saliva from anæsthetized sheep was not examined

For all values reported, other than ash and *p*H, there is a wider variation for conscious than for anæsthetized animals Table 2 shows the mean values and their range These findings agree substantially with other estimates appearing in the literature

The large differences in rate of flow, total nitrogen and solids content between anæsthetized and conscious sheep are presumably due to the greater barrage of saliva eliciting stimuli received by the conscious sheep Since the ash contents were similar, it is suggested that most of the difference in total solids content obtained for these two treatments is due to a difference in organic matter content—presumably mucoproteins This is supported by the markedly higher (mean difference 13 2 mgm per cent) nitrogen content of saliva from anæsthetized than from conscious animals

A significant negative relationship obtained between flow-rate of mixed saliva and total nitrogen content for each of two anæsthetized sheep ($r = -0\ 72$, $P < 0\ 001$, 26 degrees of freedom, $r = -0\ 67$, $P < 0\ 001$, 20 degrees of freedom) There were insufficient observations per animal to permit any conclusions about the existence of a similar relationship for saliva from conscious animals

These results relate to the diversion of mixed saliva from anæsthetized sheep for time periods of up to 6 hr after consumption of a diet containing 11 per cent crude protein They support Phillipson and Mangan's[4] suggestion that the nitrogen content of saliva could be diluted by increasing rates of fluid secretion

On the other hand, Somers[5] found a positive relationship between rate of secretion and nitrogen content of mixed saliva ($r = 0\ 9212$, $P < 0\ 001$) His values are independent of time, mine depend on time Therefore the two analyses are not comparable In a later paper Somers[6], when examining a time-dependent relationship between total saliva nitrogen and parotid saliva volume secreted over a 24-hr period for conscious sheep following feeding, showed an apparent positive relationship between these values Diets of different nitrogen contents were used by Somers and by me This, together with the different saliva fluids examined, could explain the two sets of findings

Table 2 SHOWING COMPARISON BETWEEN CERTAIN SALIVA PROPERTIES FOR ANÆSTHETIZED AND CONSCIOUS SHEEP
(Mean values)

Condition of animal	No of sheep used	Flow-rate (ml /min)	Nitrogen (mgm /100 ml)	Solids (mgm /100 ml)	Ash (mgm /100 ml)	*p*H
Anæsthetized, non-fistulated	3					
Mean		2 52	20 73	1,253 9	662 6	8 52
Range		(0 30–8 30)	(18 01–28 12)	(560–1,518)	(240–1,164)	(8 24–8 65)
No of values		(7	(54)	(33)	(33)	(43)
Conscious, fistulated	6					
Mean		4 31	7 52	969 8	609 8	8 24
Range		(0 52–17 0)	(1 75–14 64)	(146 0–1,265 0)	(64 0–840 0)	(8 06–8 47)
No of values		(14)	(15)	(11)	(11)	(3)
Mean—all values		3 71	17 86	1,208 0	649 6	8 50

follows then that there would be two synapse-like sites in this linking mechanism: one is in the vicinity of the plasma membrane or between the plasma membrane and the endoplasmic reticulum, and the other between the reticulum and the contractile system. The 'glycerol effect' would be chemically (for this effect is highly specific to glycerol) or osmotically related to the former.

Details of this work are to be published elsewhere, with further experimental evidence.

We have found, in addition, a conspicuous effect. If, in these experiments, the normal Ringer's fluid is restored, the development of tension in the fibre by electrical stimulation is reduced and then abolished within a few minutes, in spite of the production of the vigorous action potential. We have no explanation at present of this effect.

We thank Prof. T. Nagai for his constant advice and Prof. E. Miyazaki for his encouragement. We are also grateful to Dr. T. Matsushima, Miss K. Abe and Mr. T. Yamamoto for assistance.

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MIXED SALIVA FROM SHEEP

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IT is well known that the parotid glands of adult ruminants produce saliva continuously and their output volumetrically dominates the relatively smaller contribution made by the other salivary glands. Most reports agree that the submaxillary and sublingual glands chiefly function during the consumption of feed. The buccal glands have received little investigation. The saliva arising from the four types of glands is chemically different. There are few reports in the literature on the properties and flow-rates of mixed ovine saliva, especially on the quantity of endogenous nitrogen re-cycled to the rumen.

In many nutritional investigations on sheep there is need for information on the volume of saliva passing to the reticulo-rumen. Denton[1], using parotid fistulated sheep, has noted values for the flow of parotid saliva of animals consuming roughage and succulent rations. The observations of Bath *et al.*[2], conducted with one œsophageal fistulated sheep, have indicated the volume of mixed saliva put out while this animal consumed lucerne chaff—but the interval of time was not recorded.

The two experiments reported here were made during the course of a series of basic investigations on sheep fitted with chronic œsophageal fistulæ[3]. The fistulæ differed in area (2·0–8·0 cm.^{2}) and were all well healed.

Experiment 1 is concerned with minimal estimates of the volume of mixed saliva passing to the reticulo-rumen of sheep while consuming two types of roughage rations. Experiment 2 compares the composition and flow-rates of mixed saliva of anæsthetized sheep and conscious sheep while not consuming feed.

Table 1 shows the flow-rates of mixed saliva achieved on nine occasions in experiment 1 by five œsophageal fistulated sheep when consuming either a 55 per cent wheat grain, 25 per cent wheaten chaff and 20 per cent lucerne chaff ration (diet 1), or good quality lucerne chaff (diet 2) of known moisture contents. Information on duration of sampling, amount of ration consumed and the percentage recovery, via fistula, of extruded feed are also given.

Fistula extrusa was collected in polythene bags fitted around the animals' neck. Saliva flow-rates were estimated in the following manner. The 'free' saliva fluid was carefully separated from the particulate matter of the extrusa by compression and the fluid volume measured. The 'wet' particulate matter was weighed, oven-dried at 100° C. overnight and the moisture loss noted. After correction for the initial feed moisture content, the fluid volume difference between the 'wet' and 'dry' feed extrusa was added to the 'free' (saliva) fluid volume.

It is considered that this summated value represents the volume of saliva extruded from the fistulæ over the sampling period.

All attempts to occlude the œsophagus below the fistula, so as to ensure complete recovery of swallowed matter via fistulæ, resulted in distress to the animal. In experiment 1 an unknown amount of saliva by-passed the fistula and with the food entered the reticulo-rumen. Therefore these results do not represent estimates of the maximum volumes of saliva secreted by these sheep while consuming feed. However, they do reflect rates of mixed saliva secretion that can be achieved, and exceeded, by sheep while eating these roughage rations (mean 8·5 ml./min., range, 3·8–12·0 ml./min.). In contrast, the mean saliva flow-rate for conscious œsophageal fistulated sheep (not eating, experiment 2) was only half as great (4·3 ml./min., range, 0·52–17·0 ml./min.).

For experiment 2, six œsophageal fistulated and three non-fistulated animals were used. Twenty-one observations were made on the diversion and collection of mixed saliva from either conscious fistulated sheep (without concomitant consumption of feed), or orally from anæsthetized non-fistulated sheep ('Kemithal', lateral recumbency). For conscious sheep the collection periods ranged from 6 to 335 min. (mean 71·7 min.), and for the anæsthetized sheep from 268 to 370 min. (mean 300·2 min.).

The experimental conditions for the fistulated sheep varied widely between occasions. Some animals were fasted for 12 hr. before sampling, others were fed immediately before sampling. In some cases food was within the animals' sight—but in no case was the animal consuming its ration when saliva samples were collected. Some animals were more

(1) The time of re-appearance of the twitch and the gradation of the twitches are intimately related to the duration for which the muscle is immersed in Ringer's fluid containing glycerol, and are not affected by the intervals (1–9 min.) at which a stimulation to evoke twitch is given. (2) This phenomenon is recognizable over the wide range of glycerol concentrations (120–840 m*M*), though it becomes obscure at both extremes of concentration—at the higher concentrations, due to the reduced contraction of the re-appeared twitch; at the lower one, due to the small diminution of the mechanical response. (3) Under various hypertonic conditions, produced by adding sodium chloride (150–250 m*M*) or several non-electrolytes (mannitol, 50–400 m*M*; monoacetin $CH_2OH{\cdot}CHOH{\cdot}CH_2OCOCH_3$, 50–300 m*M*; glucose, 200–400 m*M*; sucrose, 200–300 m*M*, and urea, 300 m*M*) to normal Ringer's fluid, only diminution or abolition of twitch occurs; hence the re-appearance of twitch after diminution is highly specific to glycerol, and the term the 'glycerol effect' is quite justified. (4) The 'glycerol effect' appears also in the case of sartorius muscle (13 m*M* potassium chloride) depolarized with potassium, when stimulated in either a longitudinal or a transverse a.c. (50 c./s.) electric field (8–10 V./cm., 40–94 msec.). Another series of experiments showed that the 'glycerol effect' occurs in whole muscle of the frog rectus abdominis, whereas it does not in the frog heart ventricle. We have found that the 'glycerol effect' is recognizable in Ringer's fluid without calcium or potassium or both.

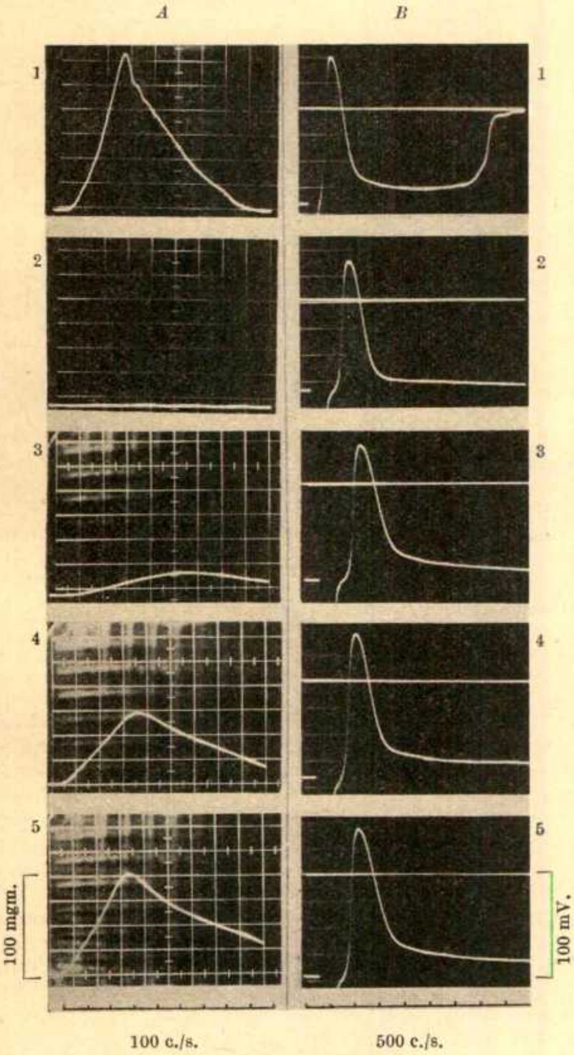

Fig. 1. Mechanical and electrical responses of single muscle fibres of the frog semitendinosus at different times after replacing the Ringer's fluid by the one containing 420 m*M* glycerol

The series of records *A* shows twitch tensions obtained from a single muscle fibre: *A*1, in normal Ringer's fluid; *A*2, *A*3, *A*4 and *A*5, after soaking in glycerol Ringer's fluid for 1 min., 24 min., 44 min. and 60 min., respectively. The series of records *B* shows action potentials obtained from single muscle fibres of a bundle: *B*1, in normal Ringer's fluid (dislodging the micro-electrode caused by the movement of the fibre resulted in the displacement of the tracing to 0 mV. at the right-hand side); *B*2, *B*3, *B*4 and *B*5, after soaking in glycerol Ringer's fluid for 3 min., 26 min., 45 min. and 60 min., respectively

The fibres were stimulated by means of a pair of external electrodes with a square pulse generated from an electronic stimulator at room temperature (17° C.). The tension recorder was a mechano-electronic transducer *RCA* 5734, and glass capillary micro-electrodes filled with 3 *M* potassium chloride (10–30 megohms) were employed to record action potentials. The Ringer's fluid contained 122 m*M* sodium chloride, 2 m*M* potassium biphosphate, 1·3 m*M* calcium chloride and was buffered to *p*H 7·0 with sodium bicarbonate

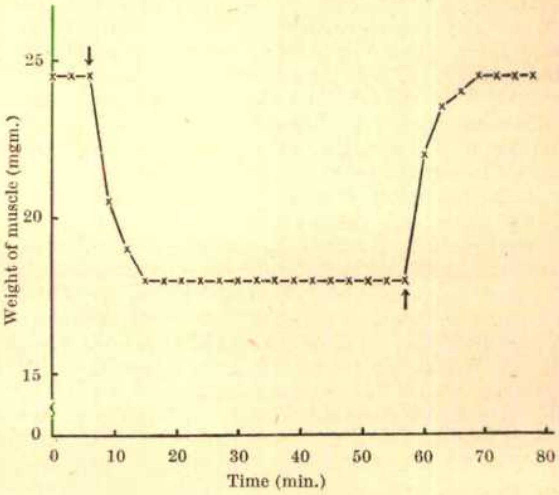

Fig. 2. Osmotic effect of hypertonic Ringer's fluid containing 420 m*M* glycerol on whole muscle of frog sartorius

At ↓, the bathing fluid was exchanged with the glycerol Ringer's fluid, and at ↑, the normal Ringer's fluid was restored. The muscle was weighed on a torsion balance after it was blotted carefully with pieces of filter paper

From these results it is clear that the re-appearance of the mechanical activity takes place as the result of the effect of glycerol on a significant process (or processes) which links the membrane excitation with the contraction and is paralysed under the hypertonic condition. Since glycerol is not considered to diffuse into a muscle fibre, at least within the 10–20 min. in which the 'glycerol effect' begins to appear (no further detectable change in muscle weight occurs at this time (Fig. 2); Bozler, E., personal communication), it is believed that this process exists at the plasma membrane or at its vicinity. It follows also that the contraction both in the transverse a.c. field and in the longitudinal field would not be due to any internal currents flowing within a muscle fibre, but to activation of a process at the membrane or in its vicinity[7,8].

How this 'glycerol effect' occurs is at present difficult to understand. The following explanation is very speculative: we have already pointed out the possibility of the existence of a synapse-like process in the sequence of processes linking excitation with contraction[9]. On the other hand, it is probable, according to Huxley[10], that the *Z*-line or the endoplasmic reticulum is the structure responsible for the electrical spread of excitation in question. It

The presence of pyridoxal and pyridoxal phosphate in the vicinity of isolated Ehrlich ascites tumour cells intensifies their uptake of amino-acids, both under aerobic[9] and anaerobic[10] conditions. These tumour cells quickly take up pyridoxal to an extent calculated to be 1·3–1·4 times that of the extra-cellular level of the vitamin[11]. This is understandable, since Ehrlich ascites tumour cells have been reported to have a greater capacity to take up amino-acids from the blood and concentrate them, than have normal cells[12]. These findings are also compatible with the known primary functions of vitamin B_6 as a coenzyme in amino-acid decarboxylation, transamination and catalytic breakdown of tryptophan. Our observations of the reversing effect by pyridoxine and pyridoxamine on 5-FU and 5-FUDR growth inhibition of *C. albicans* directs attention to the possibility of a new function of vitamin B_6 in nucleic acid metabolism, as well as a possible new mode of action of the 5-fluoropyrimidines. The antagonism between vitamin B_6 and 5-fluoropyrimidines also raises the question as to whether 5-fluoropyrimidine-resistant cancer cells possess a 'built-in' resistance mechanism by virtue of possessing greater levels of B_6 or by their synthesizing greater quantities of the vitamin than do susceptible cancer cells. The effect of vitamin B_6 on 5-fluoro-pyrimidine-intoxicated animals, with and without transplanted tumours, in order to determine the ability of vitamin B_6 to reduce parenteral toxicity of the 5-fluoropyrimidines, is the next area of exploration under consideration. Investigations along some of these lines are under way.

This investigation was supported by a research grant, *G*-7072, of the National Science Foundation, Washington, D.C.

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'GLYCEROL EFFECT' AND THE MECHANISM LINKING EXCITATION OF THE PLASMA MEMBRANE WITH CONTRACTION

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FOR various reasons, it seemed of interest to consider the use of glycerol and/or other non-electrolyte in investigating the mechanism of a 'membrane'. Thus: (1) in the biochemical and biophysical fields[1,2], glycerol has been used for disintegrating the cell membrane, suggesting the possibility that it might have an effect on other membrane systems of the muscle cell, for example, sarcoplasmic reticulum[3]; (2) glycerol is a constituent member of the lipid part of lipoprotein, which seems to form the framework of the membrane system of the cell[4]. We have therefore investigated the relationship between glycerol and the activity of muscle, in an attempt to elucidate the mechanism of the excitation-contraction link of the muscle cell, using the 'glycerol effect' for this purpose.

If a single muscle fibre from the frog's skeletal muscle (m. tibiofibularis and m. semitendinosus of *Rana nigromaculata* or *Rana japonica*) is immersed in a Ringer's fluid which is hypertonic due to the addition of a high concentration of glycerol, say, about 420 m*M*, the mechanical response in twitch declines and then disappears within about 1 min. (Fig. 1*A*2). Nevertheless, such fibres are electrically excitable, and the action and resting potentials are almost unchanged (Fig. 1*B*2), though the rate of rise and fall of the action potential is likely to be slightly reduced. Since the diminution of the mechanical response due to the hypertonic condition and the differential action on the mechanical response and action potential of a muscle fibre have been already reported[5,6], this effect as such is not new. The disappearance of the mechanical response is considered to be due not to the chemical effect of glycerol but to the hypertonicity of the Ringer's fluid used; this view is supported by the fact that the disappearance of the response is accompanied by a considerable loss of weight of muscle (Fig. 2).

About 6–10 min. after immersion in Ringer's fluid containing glycerol, however, a new observation, which we have called the 'glycerol effect', appears. As shown in Fig. 1*A*, in spite of being left in the hypertonic condition, mechanical response in the form of twitch re-appears, the twitch tension rises gradually and reaches 50–70 per cent (maximum value) of the normal within 40–60 min. after re-appearance. It is very interesting that the restoration takes place without any further changes in either action or resting potential (Fig. 1*B*) and even in the weight of muscle (Fig. 2).

These results indicate directly that the disappearance of the ability to develop twitch tension under hypertonic conditions occurs not by alteration of the contractile system due to dehydration, but by paralysis of function of a strategic system linking the excitation of the plasma membrane with the contraction.

A similar phenomenon, namely, the disappearance of the mechanical response followed by its re-appearance is also recognizable in the case of complete tetanus, which is evoked at appropriate intervals to test the contractibility.

Experiments with the isotonic lever on whole muscle of the frog sartorius give the following results:

order of addition of metabolite and antimetabolite was not crucial, both vitamin B_6 and 5-fluoropyrimidines were introduced together with the inoculum. As seen in Tables 1 and 2, inhibition indices for both 5-FU and 5-FUDR for *C. albicans* were small, indicating very high antimetabolite activity of the 5-fluoropyrimidines in the *C. albicans* test system. However, the inhibition indices were not constant for either pyridoxine or pyridoxamine versus 5-FU or 5-FUDR and the antagonism was non-competitive. This would indicate that vitamin B_6 competes with the 5-fluoropyrimidines at more than one site on the cell.

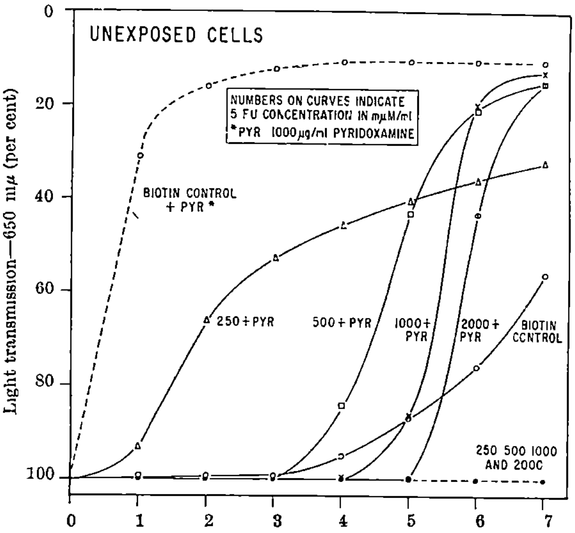

Fig. 4. The reversing action of pyridoxamine (1,000 μgm./ml.) on growth inhibitory effect of 5-FU on previously unexposed cells of *C. albicans* at four levels of 5-FU at 28° C. in basal synthetic culture medium in incubator shaker at 250 cyles/min. Metabolite and antimetabolite introduced together. Biotin control tubes, with and without pyridoxamine, do not contain 5-FU. Note complete inhibition without escape, by 250, 500, 1,000 and 2,000 mμmoles 5-FU/ml., in absence of pyridoxamine. Each point represents an average of triplicate values, arithmetic plot

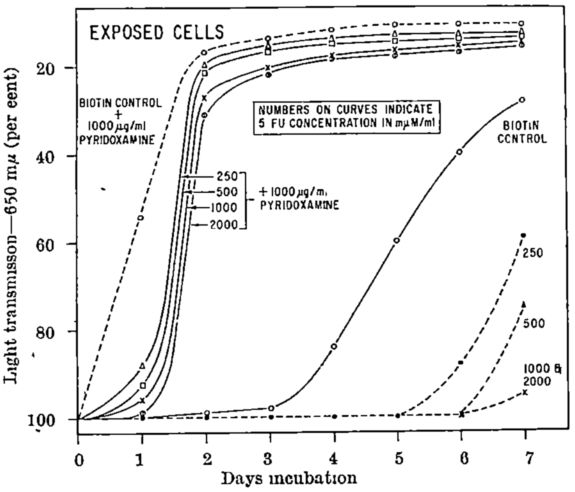

Fig. 5. The reversing action of pyridoxamine (1,000 μgm./ml.) on growth inhibition of 5-FU on cells of *C. albicans* previously in continuous contact for seven days with an 80 per cent inhibitory dose of 5-FU (0·30 μmole/ml.). Test run at four levels of 5-FU at 28° C. in basal synthetic culture medium in incubator shaker at 250 cycles/min. Metabolite and antimetabolite introduced together. Biotin control tubes, with and without pyridoxamine, do not contain 5-FU. Note complete inhibition with escape in tubes containing 250, 500, 1,000 and 2,000 mμmoles 5-FU/ml., in absence of pyridoxamine. Each point represents an average of triplicate values, arithmetic plot

Table 1. AMOUNTS OF 5-FU REQUIRED TO PRODUCE HALF-MAXIMAL INHIBITION OF GROWTH OF *Candida albicans* IN THE PRESENCE OF VARYING AMOUNTS OF PYRIDOXAMINE AND PYRIDOXINE

(Four days incubation at 28° C. in New Brunswick Gyrotory Shaker, 250 cycles/min.)

Metabolite (μmoles/ml.)	5-FU μmoles/ml. (ID_{50})	Inhibition index*
Pyridoxamine		
0	0·27	—
0·005	0·27	—
0·05	0·28	0·28
0·5	0·31	0·08
5·0	0·59	0·06
10·0	0·63	0·04
Pyridoxine		
0	0·27	—
0·005	0·27	—
0·05	0·31	0·8
0·5	0·32	0·1
5·0	0·72	0·09
10·0	0·77	0·05

* Inhibition index = (ID_{50} of 5-FU in presence of metabolite) minus (ID_{50} in absence of metabolite) divided by (concentration of metabolite)

Table 2. AMOUNTS OF 5-FUDR REQUIRED TO PRODUCE HALF-MAXIMAL INHIBITION OF GROWTH OF *Candida albicans* IN THE PRESENCE OF VARYING AMOUNTS OF PYRIDOXAMINE AND PYRIDOXINE

(Four days incubation at 28° C. in New Brunswick Gyrotory Shaker, 250 cycles/min.)

Metabolite (μmoles/ml.)	5-FUDR (μmoles/ml.) (ID_{50})	Inhibition index*
Pyridoxamine		
0	0·021	—
0·05	0·023	0·04
0·5	0·019	0·004
5·0	3·00	0·6
Pyridoxine		
0	0·021	—
0·05	0·021	—
0·5	0·021	—
5·0	0·029	0·0016

* Inhibition index = (ID_{50} of 5-FUDR in presence of metabolite) minus (ID_{50} in absence of metabolite) divided by (concentration of metabolite)

The 5-fluoropyrimidines inhibit mammalian tumours by interfering with synthesis of ribonucleic (RNA) and deoxyribonucleic (DNA) acids[4-6]. They inhibit incorporation of formate, uracil-4-carboxylic acid and pyrimidines into DNA thymine of liver, spleen and the Ehrlich tumour *in vivo*. They also block the incorporation of uracil and uracil-4-carboxylic acid into RNA uracil. The most important site of action is in the inhibition of methylation of uridylic acid to form thymidylic acid[5]. Analogous effects of thymine and uracil metabolism have been shown to occur in microbiological systems, such as *Escherichia coli*[7], *Staphylococcus aureus*[8], and others. It is reasonable, therefore, to postulate that at least one of the inhibitory effects of 5-FU and 5-FUDR on *C. albicans* is due to interference with nucleoprotein synthesis in this organism. Further work, however, will be required to confirm this hypothesis.

In view of the fact that the growth-restricting action of 5-FU and 5-FUDR on *C. albicans* was reversed by vitamin B_6, and that growth of the organism continued to be restricted by increasing concentrations of 5-fluoropyrimidines despite the presence of optimal concentrations of vitamin B_6, we can assume that the 5-fluoropyrimidines interfere in some manner with uptake or utilization of vitamin B_6 by the yeast cell, unless the vitamin was itself involved in nucleoprotein synthesis. We are not aware of any reports that vitamin B_6 is thus involved. We did observe that when exhausted cells of *C. albicans*, both exposed and unexposed to the prior inhibitory effects of 5-FU, were given pyridoxamine, the unduly prolonged lag phase of the organism promptly disappeared (Figs. 4 and 5), indicating a salutary effect on cellular reproduction.

10 mμmoles 5-FUDR/ml or higher with at least 5,000 mμmoles pyridoxamine/ml

Partial purification of the pigment produced by *C albicans* from 5-FU and pyridoxamine was accomplished by de-ionization of filtered supernatant fluid with '*IRC*-50' cationic exchanger, adsorption at pH 3 with 'Darco *G*60' activated charcoal, followed by several washings with distilled water, after which the pigment was eluted from the charcoal with alkaline 50 per cent ethanol, to yield a clear yellow solution The pigmented product behaved as an indicator in this solution

pH	Colour
< 1	No colour
2	Light reddish violet
2 8–3 5	Red
5 5–6 5	Yellowish pink
6 5–11	Yellowish brown
> 12	Yellow

The yellow solution was adjusted with hydrochloric acid to a red colour at pH 3 In ascending paper chromatograms in n-butanol/methanol/benzene/water = 2 1 1 1 (upper layer) for 15 hr at 22° C, the red-coloured product, not fluorescent in ultra-violet light, migrated to R_F 0 43, interspersed, but clearly separable, from five other fluorescent unpigmented spots When tested against *C albicans*, the pigmented, metabolic product was neither growth-inhibitory nor growth-stimulatory in seeded basal synthetic culture medium agar Further purification and characterization of this pigment is proceeding

Order of introduction of metabolite versus antimetabolite in test system Further experiments were conducted to determine whether the order of addition of the metabolite and antimetabolite was crucial for the reversal action of the metabolite Pyridoxine and pyridoxamine were introduced simultaneously, first with 5-FU and then with 5-FUDR, into basal synthetic culture medium, followed immediately by addition of the standard inoculum of vitamin-depleted yeast cells Under these new conditions, pyridoxamine (Fig 3) and pyridoxine were again capable of reversing the toxicity of 5-FU for *C albicans* Substitution of 5-FUDR produced similar results except that pyridoxamine was more effective in reversing 5-FUDR inhibition than was pyridoxine The order of addition of vitamin B_6 and the 5-fluoropyrimidines is therefore not crucial Vitamin B_6 reversed the toxicity of 5-FU for cells in prior contact with 5-FU for 4 days (Fig 2), as well as for cells previously unexposed to 5-FU (Fig 3)

Reversal of 5-FU inhibition by vitamin B_6 using 5-FU exposed and unexposed cells Inocula in the preceding experiments had consisted of vitamin-depleted yeast cells previously unexposed to 5-fluoropyrimidines or to vitamin B_6 We now wished to determine whether yeast cells continually exposed to 60 μgm 5-FU/ml for a 7-day period at 28° C would respond to the reversing action of vitamin B_6 in the same manner as unexposed cells Accordingly, yeast cells that had been incubated 7 days in the shaker in the presence and absence of 5-FU were collected, washed three times with sterile, double-distilled water, re-suspended, and adjusted to 50 per cent light transmission These 7-day-old cells were re-inoculated into fresh tubes of basal synthetic culture medium containing 5-FU, with and without 1,000 μgm pyridoxamine/ml As seen in Figs 4 and 5, a 7-day prior period of incubation, whether in the presence of 60 μgm 5-FU/ml or not, was capable of exhausting the reproductive ability of *C albicans* and prolonging the lag phase of growth to 3 days (biotin control curves) However, when pyridoxamine was added with biotin, the prolonged lag phase was eliminated regardless of whether the cells had been exposed to 5-FU or not (biotin + pyridoxamine curve) This action of pyridoxamine points to it as having an important role in the cellular reproduction of *C albicans*

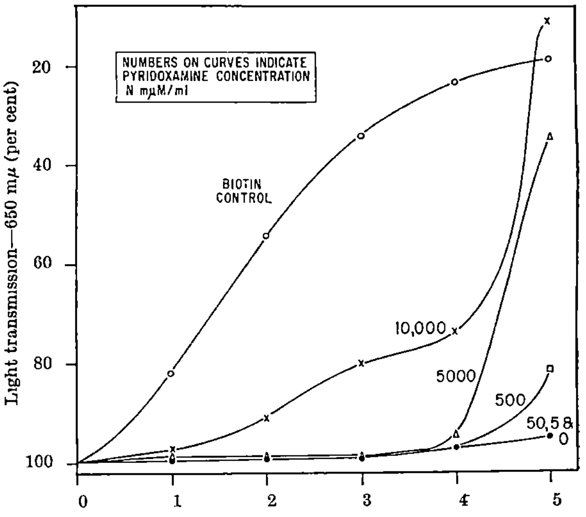

Fig 3 Growth response of *C albicans* and the reversing effect of five levels of pyridoxamine on growth inhibition by 5-FU at 28° C in incubator shaker in basal synthetic culture medium at 250 cycles/min All tubes contain 1,000 mμmoles 5-FU/ml except biotin control Metabolite and antimetabolite introduced together Each point represents an average of triplicate values, arithmetic plot

5-FU exposed yeast cells were completely inhibited by second contact with 5-FU over the range 250–2,000 mμmoles/ml within the 5-day incubation period (Fig 5) However, appreciable escape from inhibition by 5-FU began to occur by the sixth and seventh day, signalling what we interpret as the emergence of 5-FU adapted or resistant cells In the case of unexposed cells, there was also complete inhibition by 5-FU over the same range of concentration, but no escape occurred over a 7-day incubation period (Fig 4) Thus, prior conditioning to 5-FU appears to favour the emergence of 5-FU adapted or resistant cells The future usefulness of 5-FU resistant *C albicans* cells as an indicator system for screening useful compounds for 5-FU-resistant cancer lines, remains to be determined In comparing the speed of reversal by pyridoxamine of growth inhibition of 5-FU exposed yeast cells and unexposed cells, one notes more rapid reversal of growth inhibition of exposed cells than that of unexposed cells (Figs 4 and 5) This may be attributed to (1) the adaptation or increased resistance to 5-FU of the yeast cell exposed twice to 5-FU, (2) to the accumulation of unused growth-stimulatory intermediate metabolites blocked by the prior contact with 5-FU

Type of antagonism between vitamin B_6 and 5-fluoropyrimidines In order to determine whether the antagonism between vitamin B_6 and the 5-fluoropyrimidines was competitive or non-competitive, a determination was made of the amounts of 5-FU and 5-FUDR which were required to cause half-maximal inhibition of growth in the presence of ascending ten-fold concentrations of pyridoxine and pyridoxamine (Tables 1 and 2) Since the water, after which the medium was adjusted to neutrality with 1 *N* sodium hydroxide The basal medium was distributed in 9-ml volumes to optically matched, chemically clean 'Lumetron' glass tubes which were covered with aluminium caps and sterilized by autoclaving at 118° C (12 lb) for 12 min Glass filter-sterilized solutions of varying concentrations of 5-FU, 5-FUDR and vitamin B_6, each contained in a volume of 1 ml , were then added aseptically After being seeded, tubes were incubated at 28° C in a New Brunswick Gyrotory Shaker under constant rotation at 250 cycles per min Daily turbidity measurements were made photometrically with a 401*A* 'Lumetron' colorimeter at 650 mμ The standard inoculum consisted of vitamin-depleted yeast cells prepared by subculturing the organism twice on vitamin-free basal synthetic culture medium agar slopes, with an intervening 7-day incubation period at 37° C By the time of the second sub-culture, growth of the organism had become sparse Vitamin-free washed agar incorporated in the formula was prepared by a previously described method[2] Vitamin-depleted yeast cell suspensions were collected and washed three times in sterile, double-distilled water, adjusted to 50 per cent light transmission at 650 mμ and then inoculated at a standard dose of 0 15 ml suspension per tube

Inhibitory effect of 5-*FU and* 5-*FUDR* Ascending concentrations of 5-fluoropyrimidines caused a progressive restriction of growth of *C albicans* to the point of complete inhibition in the case of 5-FU (Fig 1) and 60–70 per cent inhibition by 5-FUDR This inhibitory effect was observable within the first day of incubation and persisted for the entire 5-day observation period Some escape from the inhibitory effect of 60–90 μgm 5-FU/ml appeared by the fourth day of incubation (Fig 1) However, no escape from inhibition occurred from the effects of 5-FUDR over the same observation period 50 per cent minimal inhibiting dose (ID_{50}) of 5-FU varied from 31 to 37 μgm /ml (0 24–0 28 μmole/ml) from the second to fifth day of incubation, and averaged 34 3 μgm /ml (0 26 μmole/ml) The inhibitory action of 5-FUDR on *C albicans* was approximately ten times that of 5-FU, although, unlike the latter compound, it never produced complete restriction of growth at any concentration The ID_{50} of 5-FUDR varied from 3 9 to 1 86 μgm /ml (0 03–0 014 μmole/ml) from the second to fifth day of incubation, and averaged 2 9 μgm /ml (0 022 μmole/ml)

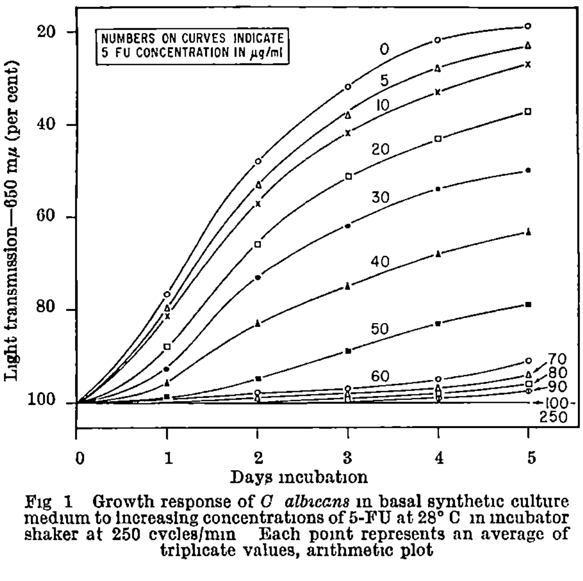

Fig 1 Growth response of *C albicans* in basal synthetic culture medium to increasing concentrations of 5-FU at 28° C in incubator shaker at 250 cycles/min Each point represents an average of triplicate values, arithmetic plot

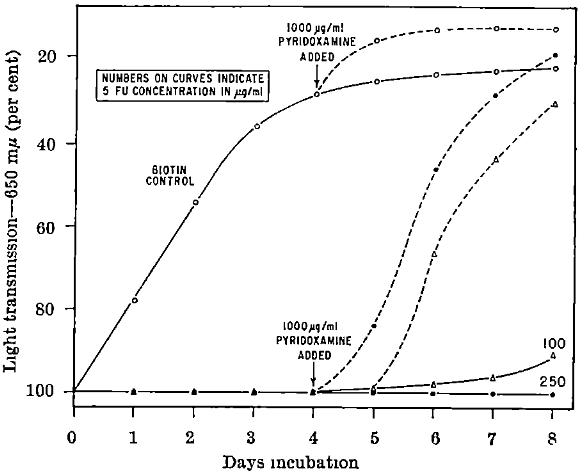

Fig 2 Reversing effect of pyridoxamine (1,000 μgm /ml) on growth-inhibition of cells of *C albicans* previously exposed for four days to high concentrations of 5-FU in basal synthetic culture medium at 28° C in incubator shaker at 250 cycles/min Each point represents an average of triplicate values, arithmetic plot

Reversing effect of pyridoxine and pyridoxamine Stimulated by the newly observed fact that five representative strains of *C albicans* had all shown a partial deficiency for pyridoxamine at an optimal concentration of 1,000 μgm /ml (ref 3), which is beyond the usual concentration for vitamins, we considered the possibility that vitamin B_6 could be involved in cell metabolism of *C albicans* other than as a coenzyme in amino-acid metabolism We therefore applied these vitamins to viable but growth-inhibited yeast suspensions containing ascending concentrations of both 5-FU and 5-FUDR We then observed that the growth-inhibiting effects of the two 5-fluoropyrimidines were reversed by pyridoxine as well as pyridoxamine To our knowledge, this phenomenon has not been reported previously As shown in Fig 2, the addition of 1,000 μgm pyridoxamine/ml (4 1 μmoles/ml) to incubating yeast cells on the fourth day of their continuous exposure to varying concentrations of 5-FU brought about a prompt reversal of growth inhibition The more pronounced reversals occurred at the higher concentrations of 5-FU (50–250 μgm /ml) than lower concentrations Reversal of 5-FU inhibition by pyridoxamine was complete in that final turbidities in pyridoxamine-reversed tubes were approximately equal to, or exceeded, those of biotin control tubes not containing 5-FU (Fig 2)

Extracellular pigment formation A water-soluble, extracellular, pigmented metabolic product of *C albicans*, which is ordinarily not a pigmented yeast, appeared regularly in all seeded tubes of basal synthetic culture medium containing 0 25 μmole 5-FU/ml or higher, whenever the concentration of pyridoxamine exceeded 5 μmoles/ml The coloured product diffused into the supernatant fluid after 24 hr of incubation and progressively increased in concentration with time Pigment was not produced when pyridoxine was substituted for pyridoxamine nor when 5-FU and pyridoxamine were shaken together in distilled water Substitution of 5-FUDR for 5-FU yielded a similar pigment with pyridoxamine, but not with pyridoxine This also occurred after the first day of incubation in tubes containing

The lack of effect of malonate on α-ketoglutarate-stimulated isocitrate oxidation is in favour of the second possibility, since acetoacetate formed by reaction (15) could readily be converted to acetoacetyl-coenzyme A by reaction (13)

Thus, it is possible to explain the stimulations by all the substrates listed in Table 1 in terms of their ability to promote the formation of acetoacetyl-coenzyme A (or other keto-acyl-coenzyme A intermediates of fatty acid synthesis and breakdown) An initial lag was found both in the incorporation of acetate into fatty acid promoted by citrate[1] and in the acetate-promoted oxidation of isocitrate (for example, 0 42 µmole in the first 15 min , 1 25 µmoles in the second and 0 76 µmole in the third) This is to be expected if a catalyst for the oxidation of citrate such as acetoacetyl-coenzyme A has to be formed after the mixing of the components of the reaction mixture

Chappell and Greville[23] have recently reported that the oxidation of isocitrate by rat-liver mitochondria is inhibited by the addition of lewisite or by the removal of phosphate in the presence of dinitrophenol (see also ref 24), procedures known to inhibit the oxidation of α-ketoglutarate They conclude that oxaloacetate formed by the further oxidation of the α-ketoglutarate is necessary for the oxidation of the mitochondrial TPNH This suggestion was supported by their finding that, in the presence of lewisite, catalytic amounts of malate promoted the oxidation of isocitrate

It is possible that the system in rat-liver mitochondria is different from that in rabbit-heart sarcosomes The reaction conditions used in this study were also different from those used by Chappell and Greville In any event, it seems unlikely that oxaloacetate is functioning directly to oxidize mitochondrial TPNH in my experiments for the following reasons (i) acetate decreases the concentration of oxaloacetate, as made evident by the stimulation by acetate of the oxidation of succinate, which is inhibited by oxaloacetate, and by the inhibition by acetate of the oxidation of glutamate which requires oxaloacetate[25], (ii) dinitrophenol which inhibits the oxidation of isocitrate causes increased concentrations of oxaloacetate[25], (iii) glutamate which removes oxaloacetate by transamination also promotes the oxidation of isocitrate, (iv) malonate does not inhibit the α-ketoglutarate-promoted oxidation of isocitrate

I wish to thank Prof E C Slater for his suggestions and criticism and Miss H M Moerdijk for technical assistance

This work was supported in part by research grant *RG*–6569 of the United States Public Health Service

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REVERSAL OF TOXICITY OF 5-FLUOROURACIL AND 5-FLUORODEOXYURIDINE FOR *CANDIDA ALBICANS* BY PYRIDOXINE AND PYRIDOXAMINE

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DURING work on the metabolism of the pathogenic yeast, *Candida albicans*, we observed that the growth of this organism in a synthetic glucose mineral salt medium was markedly inhibited by the anti-cancer fluorinated uracil derivatives, 5-fluorouracil (5-FU, mol wt 130) and 5-fluorodeoxyuridine (5-FUDR, mol wt 246 2) (These compounds were kindly supplied by Hoffmann–La Roche, Inc , Nutley, New Jersey) In following the inhibitory action of these compounds, we noted also that such effects could be promptly reversed by the addition of pyridoxine and pyridoxamine In view of the high toxicity of the 5-fluoropyrimidines and the absence of clinically effective reversing compounds other than thymidine for 5-FUDR (ref 1), application of this observation *in vivo* in human and animal cancer therapy seems to be indicated

The basal synthetic culture medium, in which growth, growth-inhibition and inhibition-reversal occurred, contained the following ingredients glucose, 10 gm , ammonium sulphate, 2 gm , potassium dihydrogen phosphate, 2 gm , crystalline magnesium chloride, 0 2 gm , crystalline calcium chloride, 0·02 gm , crystalline -ferrous chloride, 0 04 gm , crystalline manganous chloride, 0 0015 gm , crystalline sodium molybdate, 0·0015 gm , and biotin, 1,000 mµgm , all dissolved in 900 ml potassium permanganate-oxidized double distilled

Table 1. OXIDATION OF ISOCITRATE BY RABBIT-HEART SARCOSOMES

Substrate or co-factor added	Inhibitor added	$-\Delta$(isocitrate + citrate + aconitate) (µmoles) Exp. 1	Exp. 2	Exps. 3 and 4
None	none	0.75	0.55	0.40
None	dinitrophenol	0.17	0.20	
Acetate	none	2.32	1.57	1.56
Acetate	dinitrophenol	0.68		0.12
Acetate	antimycin	0.18		0.12
Acetate	malonate			0.34
DL-β-Hydroxybutyrate	none	2.83	2.06	1.89
DL-β-Hydroxybutyrate	dinitrophenol	0.69		
DL-β-Hydroxybutyrate	antimycin			0.11
DL-β-Hydroxybutyrate	malonate			0.66
DPN$^+$ (3 mM)	none	1.99		
DPN$^+$ (3 mM)	dinitrophenol	1.40		
DPNase-treated DPN$^+$ (3 mM)	dinitrophenol	0.29		
DPN$^+$ (1 mM)	dinitrophenol	0.80		
α-Ketoglutarate	none		2.00	1.87
α-Ketoglutarate	dinitrophenol			0.82
α-Ketoglutarate	malonate			1.66
Succinate	none			1.69
Succinate	dinitrophenol			0.39
Malate	none			1.36
Malate	dinitrophenol			0.56
Glutamate	none			1.80

Sucrose, 50 mM, potassium chloride, 9.5 mM, ethylenediaminetetraacetate, 1 mM, potassium phosphate buffer (pH 7.5), 50 mM, magnesium chloride, 5 mM, triphosphopyridine nucleotide (TPN$^+$), 0.2 mM, adenosine triphosphate (ATP), 10mM, Ls(+)-isocitrate, 5 mM, coenzyme A, 0.18 mM, glutathione, 1.8 mM, rabbit-heart sarcosomes (isolated in sucrose-ATP, ref. 26), 1.72 mgm. protein in exp. 1, 1.62 mgm. in exp. 2, 1.72 mgm. in exp. 3 and 1.20 mgm. in exp. 4. Other additions, where shown: acetate, 3 mM, DL-β-hydroxybutyrate, 1 mM in exp. 1, 3 mM in exp. 2, 12 mM in exps. 3 and 4, α-ketoglutarate, 30 mM, succinate, 40 mM, L-malate, 30 mM, L-glutamate, 30 mM, 2,4-dinitrophenol, 0.1 mM, antimycin, 1 µgm./ml., malonate, 10–12 mM. 2 per cent ethanol was also present in all flasks of exps. 1 and 2. The reaction was carried out in a shaking manometer flask with 10 per cent potassium hydroxide and a filter paper in the centre well. The reaction was stopped after 40 min. (exp. 1), 45 min. (exp. 2), 60 min. (exps. 3 and 4), at 25° by adding trichloroacetic acid (final conc. 5 per cent). Isocitrate + citrate + aconitate were determined on the neutralized supernatant by measuring the reduction of TPN$^+$ in the presence of isocitrate dehydrogenase and aconitase. The values given for exps. 3 and 4 are the means of two experiments.

promote the rate-limiting reaction (4). It remains to be explained how acetate and the other compounds listed in Table 1 also promote the oxidation of intramitochondrial reduced triphosphopyridine nucleotide (TPNH). It is natural to link this process with the mechanism of fatty acid synthesis, which is known to involve the oxidation of TPNH (refs. 9–11). For example, it seems possible that acetoacetyl-coenzyme A could bring about the transhydrogenase reaction (4) by reactions (6) and (7):

TPNH + acetoacetyl-coenzyme A + H^+ →
TPN$^+$ + β-hydroxybutyryl-coenzyme A (6)

β-Hydroxybutyryl-coenzyme A + DPN$^+$ →
acetoacetyl-coenzyme A + DPNH + H^+ (7)

the sum of which is reaction (4). Experiments to be reported elsewhere have shown the presence of an enzyme catalysing the oxidation of TPNH by acetoacetyl-coenzyme A in digitonin extracts of rabbit-heart sarcosomes. Whether the product of reaction (6) has the L-configuration, which is the substrate for reaction (7) (ref. 12), is of minor importance, since mechanisms for converting the L-form to the D-form are known[13,14].

Acetate can yield acetoacetyl-coenzyme A by reactions (8) + (9) (refs. 15 and 16) or (8) + (10):

Acetate + coenzyme A + ATP →
acetyl-coenzyme A + AMP + pyrophosphate (8)

Acetyl-coenzyme A + malonyl-coenzyme A →
acetoacetyl-coenzyme A + coenzyme A + CO_2 (9)

2 acetyl-coenzyme A ⇌
acetoacetyl-coenzyme A + coenzyme A (10)

The malonyl-coenzyme A could be derived by reaction (2) from the oxalosuccinate formed by oxidation of isocitrate.

β-Hydroxybutyrate can be converted to acetoacetyl-coenzyme A by reactions (11) (ref. 12) and (12):

β-Hydroxybutyrate + coenzyme A + ATP ⇌
β-hydroxybutyryl-coenzyme A + AMP +
pyrophosphate (11)

β-Hydroxybutyryl-coenzyme A + $\frac{1}{2}O_2$ →
acetoacetyl-coenzyme A + H_2O (12)

Table 1 shows that the oxidation of isocitrate with added acetate or β-hydroxybutyrate is inhibited by dinitrophenol as would be expected according to these equations. The slow oxidation in the absence of acetate, which might be due to endogenous fatty acid, is also inhibited by dinitrophenol.

The stimulation of disappearance of isocitrate by succinate or malate in the absence of added acetate could be explained similarly, since these compounds give rise to acetyl-coenzyme A by oxidation of the pyruvate formed by decarboxylation of the oxaloacetate, and to malonyl-coenzyme A by reaction (1). Thus acetoacetyl-coenzyme A can be formed by reaction (9). The contribution of reaction (10) to the formation of acetoacetyl-coenzyme A in the absence of added acetate is likely to be small, because the equilibrium of this reaction is far to the left[15].

The stimulation of isocitrate oxidation by α-ketoglutarate cannot be explained on the same basis since it is not inhibited by malonate. It seems most likely that succinyl-coenzyme A formed by the oxidation of α-ketoglutarate is involved, but how is not certain. It is possible that acetoacetyl-coenzyme A is formed from traces of acetoacetate in the preparation by reaction (13) (refs. 17 and 18):

Succinyl-coenzyme A + acetoacetate ⇌
succinate + acetoacetyl-coenzyme A (13)

although no acetoacetate could be detected in the sarcosomal preparations by Walker's method[19]. The partial sensitivity to dinitrophenol of the stimulation of citrate oxidation by α-ketoglutarate might be due to the appreciable activity of the succinyl-coenzyme A deacylase in the presence of dinitrophenol and absence of fluoride[20]. Glutamate would be expected to act in the same way as α-ketoglutarate, since the oxidation of α-ketoglutarate to succinate is a compulsory step in the oxidation of this substrate by heart sarcosomes[21].

Two explanations for the effect of malonate might be considered: (i) by inhibiting succinic dehydrogenase it prevents the formation of oxaloacetate by the oxidation of isocitrate, with consequent decrease in the amount of acetoacetyl-coenzyme A formed from malonyl-coenzyme A by reaction (9); (ii) malonate causes the loss of acetyl-coenzyme A with formation of free acetoacetate by reaction (15), which is the sum of reactions (14) (ref. 22) and (9):

Malonate + acetoacetyl-coenzyme A ⇌
malonyl-coenzyme A + acetoacetate (14)

Malonyl-coenzyme A + acetyl-coenzyme A →
acetoacetyl-coenzyme A + coenzyme A + CO_2 (9)

Malonate + acetyl-coenzyme A →
acetoacetate + coenzyme A + CO_2 (15)

it would be necessary to determine the exact reasons for the different spectra of the *cis* and *trans* isomers of 4-methylproline, which in turn requires a detailed analysis of these very complex spectra Further research in this direction is in progress

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RELATIONSHIP BETWEEN OXIDATION OF ISOCITRATE AND METABOLISM OF FATTY ACIDS IN RABBIT-HEART SARCOSOMES

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In the presence of adenosine triphosphate (ATP) and [^{14}C]acetate, the oxidation of intermediates of the Krebs cycle by rabbit-heart sarcosomes (mitochondria) is linked with the incorporation of acetate into the mitochondrial long-chain fatty acids[1] It has now been found that two closely related but separate mechanisms are involved, one associated with the oxidation of α-ketoglutarate, succinate or malate and the other with the oxidation of isocitrate The two mechanisms are additive The first is completely sensitive to antimycin and the second is insensitive Since the first step of fatty acid synthesis is the condensation of malonyl-coenzyme A with acetyl-coenzyme A (ref 2), it is thought likely that the Krebs-cycle intermediates provide β-keto carboxylic acids (oxalo-acetate for the first mechanism, oxalosuccinate for the second) for reaction with acetyl-coenzyme A to form malonyl-coenzyme A (cf ref 3)

$$\text{Oxaloacetate} + \text{acetyl-coenzyme A} \rightleftharpoons \text{pyruvate} + \text{malonyl-coenzyme A} \qquad (1)$$

$$\text{Oxalosuccinate} + \text{acetyl-coenzyme A} \rightleftharpoons \alpha\text{-ketoglutarate} + \text{malonyl-coenzyme A} \qquad (2)$$

It is known that isolated heart sarcosomes oxidize added citrate or isocitrate very slowly[4,5] During the investigation of the incorporation of acetate into long-chain fatty acids linked with the oxidation of isocitrate, it was found that the addition of acetate stimulated about six-fold the rate of uptake of oxygen with isocitrate as substrate, and that dinitrophenol abolished this stimulation Measurements of the disappearance of the Krebs-cycle tricarboxylic acids [$-$ Δ(isocitrate + citrate + aconitate)] confirmed that these effects on uptake of oxygen reflected the oxidation of isocitrate

Table 1 shows that a number of other compounds are also able to stimulate the oxidation of isocitrate, namely, β-hydroxybutyrate, α-ketoglutarate, succinate, malate, glutamate or diphosphopyridine nucleotide (DPN) in very high concentrations The effects of α-ketoglutarate and β-hydroxybutyrate, of α-ketoglutarate and acetate, and of diphosphopyridine nucleotide and acetate were not additive Dinitrophenol inhibited the effects of all these compounds except that of diphosphopyridine nucleotide (Table 1 shows that the stimulation by diphosphopyridine nucleotide was the same in the presence and absence of dinitrophenol) Malonate inhibited the stimulation by acetate or β-hydroxybutyrate, but had little effect with α-ketoglutarate (the effect of malonate on the stimulation brought about by the other compounds was not tested)

The measurements shown in Table 1 were carried out under the same conditions as used in work on the incorporation of acetate into long-chain fatty acids[1], that is, in the presence of triphosphopyridine nucleotide (TPN), coenzyme A and adenosine triphosphate, and in the absence of hexokinase and glucose In a reaction mixture commonly used for investigations of oxidative phosphorylation (that is, no added pyridine nucleotide or coenzyme A, adenosine diphosphate in place of the triphosphate and added hexokinase and glucose), neither acetate nor β-hydroxybutyrate had any appreciable effect on the disappearance of isocitrate α-Ketoglutarate stimulated the disappearance substantially, and this stimulation was not affected by malonate It appears, then, that adenosine triphosphate is necessary for the stimulation of the oxidation of isocitrate by acetate or β-hydroxybutyrate, but not by α-ketoglutarate

It is known[6,7] that heart sarcosomes, previously depleted of pyridine nucleotide by incubation in phosphate, oxidize isocitrate in the presence of added pyridine nucleotide by the sequence

$$\text{Isocitrate} + \text{TPN}^+ \rightarrow \alpha\text{-ketoglutarate} + \text{CO}_2 + \text{H}^+ + \text{TPNH} \qquad (3)$$

$$\text{TPNH} + \text{DPN}^+ \rightleftharpoons \text{TPN}^+ + \text{DPNH} \qquad (4)$$

$$\text{DPNH} + \tfrac{1}{2}\text{O}_2 + \text{H}^+ \rightarrow \text{DPN}^+ + \text{H}_2\text{O} \qquad (5)$$

Klingenberg and Slenczka[8] have shown, however, that in rapidly respiring intact mitochondria the diphosphopyridine nucleotide is largely oxidized while the triphosphopyridine nucleotide is largely reduced, suggesting that the accessibility of intra-mitochondrial TPNH to DPN+ to one another or to the pyridine nucleotide transhydrogenase is limited This is probably the reason for the poor oxidation of isocitrate by isolated mitochondria

The stimulation of the oxidation of isocitrate by diphosphopyridine nucleotide can be simply explained by assuming that high concentrations of this compound are able to break the accessibility barrier and

Table 1

Molecule	C_4 substituent	Proton chemical shifts (τ-values)							Proton coupling constants (c p s)				
		2*a*	3*a*	3*b*	4*b*	5*a*	5*b*		2*a*–3*a*	2*a*–3*b*	4*b*–5*a*	4*b*–5*b*	
L-Proline		6 0	—†	—†	—†	6 78	6 78		7 1*	7 1*	6 3*	6 3*	
L-Hydroxyproline (II)	OH(4*a*)	5 83	7 77	8 05	5 52	6 83	6 69		7 66	10 14	1 22	4 09	3*a*–3*b* 14 06 5*a*–5*b* 12 69 3*a*–4*b* 1 41 3*a*–5*a* 1 28 3*b*–4*b* 4 31
Allohydroxy-L-proline (I)	OH(4*b*)	5 96	7 69	7 95		6 81	6 75	4*a* 5 6	10 48	3 84	(4*a*) 4 57	(4*a*) 0 94	3*a*–3*b* 14 23 5*a*–5*b* 12 50 3*a*–4*a* 4 71 3*b*–5*b* 1 37 3*b*–4*a* 2 09
Trans-4-methyl-proline (IV)	CH_3(4*a*)	5 98	—†	—†	—†	7 31	6 60	4CH_3 9 06	4 4*	8 6*	8 3*	6 7*	5*a*–5*b* 11 0*
cis-4-Methylproline (III)	CH_3(4*b*)	6 00	8 53	—‡		7 19	6 61	4CH_3 9 06 4*a*‡	9 0*	8 1*	8 6*	6 6*	5*a*–5*b* 10 8*
Hydroxymethyl-proline (V)	CH_2OH	5 99	8 37	—§	—§	6 98	6 66	4CH_2OH 6 57	8 5*	8 5*	7 8*	8 0*	5*a*–5*b* 11 6*

* Denotes first order values † Complex multiplet τ = 7 6–8 4 ‡ Complex multiplet τ = 7 4–7 9 § Complex multiplet τ = 7 3–7 9

the spectra (Figs 2*b* and *c*) of the *cis* and *trans*-4-methylprolines, provides an indication of its possible stereochemistry (After this part of the work was complete, we were informed by Prof F C Steward and K Biemann that they had reached a similar conclusion by mass spectrometry[11] Their results were considered to favour a *trans* configuration) Thus the 4-hydroxy-4-methyl structure (VI) is immediately eliminated, since no methyl peak is found, and the spectrum agrees very well with the 4-hydroxymethyl alternative (V) In particular, the doublet of intensity two protons at 6 57 is characteristic of a —CH—CH_2—O— fragment, and the peaks at 5 99, 7 6 (approximately) and 8 37, and 6 66 and 6 98 fall in the expected ranges for the H_2, H_{3a} and H_{3b} and H_{5a} and H_{5b} protons of the proline ring Thus all the main groups in the spectrum are consistent with the proposed formula, and define both the nature of the substituent and its position of attachment to the proline ring

The spectrum of (V) (Fig 2*a*) shows very marked similarities with that (Fig 2*b*) of the *cis*-4-methylproline (III) in both the structure of the H_2 peak and the relative chemical shifts of the H_3 protons, which are quite different (Figs 2*b* and *c*), for the two epimeric 4-methylprolines (III) and (IV) This may be considered some support for the assignment of a *cis* configuration to natural 4-hydroxymethylproline (V) (This result is unexpected since it is the *trans* isomer of 4-methylproline which occurs alongside 4-hydroxymethylproline in apple and pear juices [1]) However, it should be emphasized that to confirm this statement

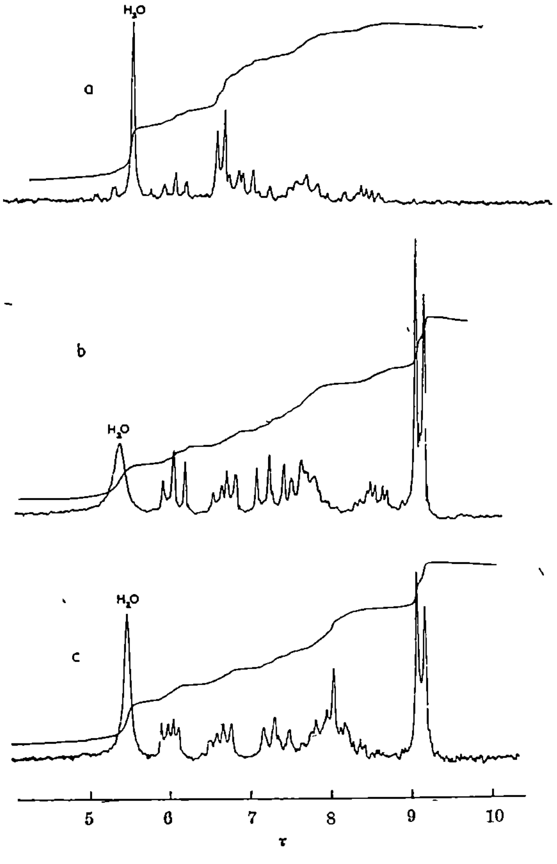
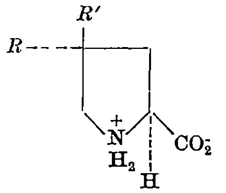


Fig 2 Proton resonance spectra (60 Mc/s) with integrals of (*a*) 4-hydroxymethylproline, (*b*) *cis*-4-methylproline and (*c*) *trans*-4-methylproline in deuterium oxide solution



I R=H, R'=OH
II R=OH, R'=H
III R=H, R'=Me
IV R=Me, R'=H
V R,R'=H,CH_2OH
VI R,R'=OH,Me

(The synthesis of *trans*-4-methyl-L-proline, which is identical with the natural amino-acid from apple and pear juices, has already been described[1]. A modification of this synthesis has now yielded *cis*-4-methyl-D-proline, enantiomeric with the amino-acid previously isolated from an antibiotic[5]. Full details of this synthetic work will be submitted for publication in the *Journal of the Chemical Society*.)

All the amino-acids were examined as zwitterions in deuterium oxide solution on a Varian spectrometer operating at 60 Mc./s., using dioxan as an internal reference (τ = 6.43). The results are summarized in Table 1 with the nomenclature that *a* and *b* refer to protons on the opposite and the same side respectively of the ring as the CO_2^- group.

The compounds all give complex spectra, some of which have not been fully analysed. The coupling constants obtained from these latter spectra are simply first-order values, that is, they are the actual spacings between particular peaks in the spectrum. Such an analysis only gives approximate values of these coupling constants, which may be in error by as much as 1 c.p.s. in some cases. However, the spectra of the two hydroxy-L-prolines were completely analysed[6], to give all the coupling constants between adjacent protons around the ring. The values of these coupling constants vary from 1 to 10 c.p.s. (Table 1). Both these compounds show, in addition, a long-range coupling over four bonds, between one 3-proton and one 5-proton. The variation of adjacent coupling constants has been explained[7] by means of equation (1)

$$J = K_1 \cos^2\varphi + K_2 \qquad (1)$$

relating the coupling constant J to the dihedral angle φ between the relevant C—H bonds, which in turn depends on the precise conformation of the molecule in solution. (The factor K_2, which was predicted to be very small, will be ignored henceforth.)

In each of the two compounds we have three pairs of coupling constants J_{2-3}'s, J_{3-4}'s and J_{4-5}'s. Each pair, using (1), will give the K_1 and φ for this particular fragment. Only two φ's are necessary to determine the conformation of the molecule. The value of the third pair of couplings thus serves as a test of the applicability of the Karplus equation (1) to the molecule considered.

In the case of the *trans* hydroxyproline the conformation shown in Fig. 1 satisfactorily explains all the observed constants for an angle of buckle of 50–60°. This is an envelope conformation, analogous to the C_s form of cyclopentane[8]. The Karplus equation (1) was derived assuming sp^3 hybridization of the carbon atoms concerned. For the above angle of buckle, the C—C—C angles decreased to around 100°. Thus both equation (1) and the angle of buckle may well be only approximate in this case. However, these results certainly suggest that the molecule exists in solution in a single conformation which is considerably more buckled than has been realized previously.

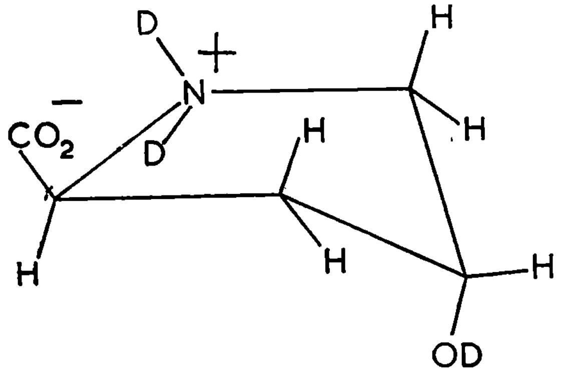


Fig. 1

In the conformation suggested here the OH group attains a pseudo axial configuration while the larger CO_2^- group attains a pseudo equatorial position. (The molecular plane should be considered more correctly as defined by centres 1, 2 and 5 than by centres 1, 2, 3 and 5 (ref. 8).) Presumably the influence of the larger substituent group is the dominant factor.

It is of interest to note that the conformation of the molecule in solution differs from that in the solid, in which a similar type of conformation is found but the angle of buckle is now only 20° (ref. 9).

In the case of the *cis* (allo) hydroxyproline, we now find that there is no single conformation which will predict, by applying equation (1), the observed coupling constants. One possible explanation of this is that the molecule exists in more than one conformation in solution. These conformers would be interchanging rapidly and the observed J's would then be averages of the J's of the particular conformers. Two factors tend to oppose this. Such an averaging process would normally tend to equalize all the coupling constants. Secondly, the long-range coupling would be either averaged out or found on both C_3 and C_5 protons. None of these predictions is observed.

One other possibility is that the Karplus equation (1) does not hold for this molecule, for example, a strong hydrogen bond between the OH and carboxyl groups may distort the C—C—C angles even more than in the *trans* hydroxyproline, in which case the Karplus equation may no longer apply. The formation of an internal lactone in derivatives of allohydroxyproline[10] does suggest that the ring can be considerably deformed.

Whatever the true reason for this discrepancy, two factors emerge from this investigation. First, the observed J's in the allohydroxyproline molecule strongly suggest that the molecule exists in solution in a considerably buckled conformation, or conformations. Secondly, the Karplus equation can give in certain cases information on the precise conformation of molecules in solution, which is extremely difficult to obtain by any other technique, but the results obtained by the application of this equation to other molecules, particularly those which may be expected to occur in a highly strained form, must be considered with some caution.

Although the remaining spectra considered here are too complex for complete analysis, they still provide some further information. In particular, the spectrum (Fig. 2*a*) of the naturally occurring proline derivative (V) or (VI) allows an unambiguous decision in favour of the former, and considered in relation to

Table 1. APPARENT MEAN DIAMETERS ($\bar{x}$) AND ESTIMATED MEAN DIAMETERS (D) FROM OPTICAL MEASUREMENTS OF MONODISPERSE POLYSTYRENE LATEXES

	Latex number				
	15*N*-8	*LS*-066-*A*	*LS*-067-*A*	*L*-3830-1	*L*-3830-19-*B*
N, total No. of particles	100	640	599	78	57
R, No. of rows	16	39	51	8	9
$\bar{x} \pm \text{S.E.}_{\bar{x}}$, m$\mu$	530 ± 4	804 ± 2	1,165 ± 2	1,721 ± 4	3,245 ± 3
$\Delta\bar{x}$, over-estimate, in per cent of $\bar{x}$	3·25	2·63	1·87	0·82	0·35
$D \pm \text{S.E.}_D$,* mμ	512 ± 6	783 ± 5	1,143 ± 6	1,710 ± 7	3,234 ± 11
Previous measurements					
D† ± S.E.$_D$, mμ	511 ± 6·4	814 ± 6·4	1,171 ± 9·7		
D‡ ± S.E.$_D$, mμ	511 ± 6·6	826 ± 22·6	1,166 ± 28·2		

* The 0·3 per cent standard error in the absolute calibration of the microscope stage has been included.

† Data of Bradford and Vanderhoff (ref. 1).

‡ Data of Bateman *et al.* (ref. 2).

where L_i is the measured length of the n_i particles in row i and N is the total number of particles which are measured, the sum being taken over all rows R. The standard error of the mean, S.E.$_{\bar{x}}$, is estimated by:

$$(\text{S.E.}_{\bar{x}})^2 = \frac{\Sigma \frac{1}{n_i} (L_i - n_i\bar{x})^2}{N(r-1)} \tag{3}$$

where r is the number of different row-lengths measured. The standard deviation σ_x of the values for apparent particle diameter is given by:

$$\sigma_x^2 = N(\text{S.E.}_x)^2 \tag{4}$$

For each latex, the error of measurement was estimated independently from repeated measurements on a pair of adjacent particles, and found to be about 80 mμ, although increasing slightly for the largest and smallest particles. These errors are about an order of magnitude greater than those obtained for the mean diameters, demonstrating the efficiency of the array method.

The array method leads to significantly lower values for the 0·8μ and the 1·1μ particles when compared with the electron microscope values[1]. This might be due to an increase in cross-sectional area because of flattening of the particles in the electron beam; such increases are observed for larger particles (Vanderhoff, J. W., personal communication). It is likely that refinement of this method through the use of photographic enlargements will yield even smaller mean diameters for these particles, since fault-lines now indiscernible may be recognized. It should be noted also that the measurement of particles in arrays leads to stratified sampling, disregarding the rare, very large or very small particles; thus, minor components of the distribution are rejected. But, in consequence, the reproducibility of this method is greater than would be expected under random sampling. The array method is, in principle, extendable to investigations of the variation of spherical viruses and macromolecules in crystalline arrays.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

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STRUCTURES AND CONFORMATIONS OF SOME NATURALLY OCCURRING DERIVATIVES OF PROLINE

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THE importance of proton magnetic resonance spectroscopy as an aid to the determination of the molecular structures of organic compounds is now well established. One purpose of this article is to show that, in addition, this technique may well prove to be of equal value in the determination of the conformations of molecules in solution.

The compounds examined in this investigation were all C_4 substituted derivatives of proline, namely, *cis* (allo) (I) and *trans*-hydroxyproline (II), *cis* (III) and *trans*-4-methylproline (IV), and a naturally occurring proline derivative for which the alternative structures 4-hydroxymethyl (V) or 4-hydroxy-4-methylproline (VI) have been suggested[3–5].

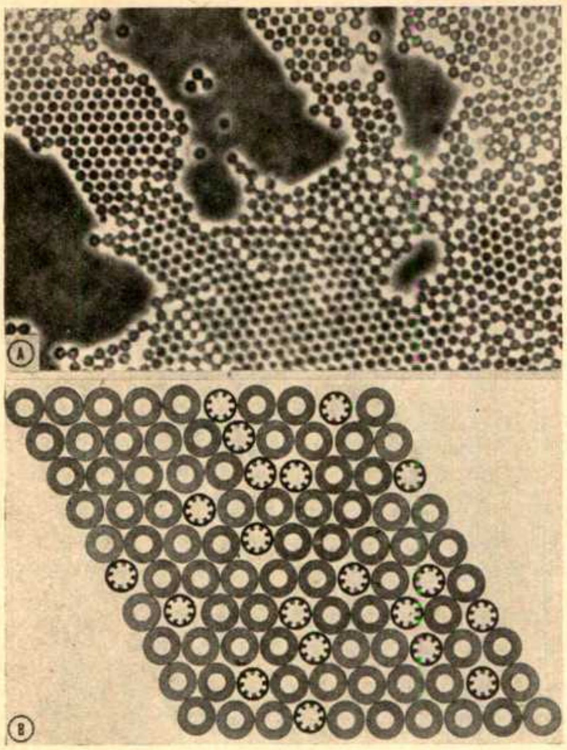

Fig. 1. *A*, arrays of 1·15μ polystyrene particles in oil. *B*, a standard two-component washer array

row is that errors arising from diffraction can be made negligible by choosing the line of symmetry between the diffraction images of the particles, or alternatively, measuring between the centres of two particles. The contribution of error of measurement is further reduced, of course, by spanning a number of particles in the row in a single measurement; variations in particle diameter tend to cancel one another, usually giving an improved estimate of the mean diameter. However, the cancellation is imperfect since a small particle may be only loosely encircled by its neighbours. Thus, a measurement which spans one of these small particles will give a result which over-estimates the true mean.

Simple models indicate that the over-estimate $\Delta\bar{x}$ of the mean diameter should be proportional to the standard deviation σ of the measured distribution of apparent particle diameters:

$$\Delta\bar{x} = k\,\sigma, \quad k = \text{constant} \qquad (1)$$

Thus, a knowledge of the value of k will allow an estimate, D, of the true mean diameter from the apparent mean diameter $\bar{x}$; that is, $D = \bar{x} - \Delta\bar{x}$. It should be possible to calculate k for the triangular arrays shown in Fig. 1, but to my knowledge this problem is unsolved.

The coefficient k was determined empirically from measurements with a vernier caliper of two-dimensional triangular arrays of machinists' washers of known diameters arranged in the rhombic pattern shown in Fig. 1*B*. Two-component arrays, as in Fig. 1*B*, were formed of eighty $\frac{1}{2}$-in. diameter washers with twenty slightly smaller washers added at random locations. The twenty rows of ten washers parallel to the edges of the rhombus were measured to obtain the apparent mean diameter $\bar{x}$, which was then compared with the known true mean to give $\Delta\bar{x}$. Fig. 2 displays the values obtained in this manner for $\Delta\bar{x}/\bar{x}$ and the corresponding values for $\sigma/\bar{x}$ for several two-component arrays, for one array constructed of three sizes of washers (three-component) and for six arrays formed from washers especially constructed to have a normal distribution of diameters (coefficient of variation = 2·36 per cent). These (array sample) values have been fitted with a straight line by least mean squares; the data are consistent with equation (1) giving $k = 0·461$ (S.E., 0·029). This value for k is directly applicable to the triangular arrays of spherical polystyrene particles even though these particles probably are tangent to a glass surface, removing their centres from a common plane. In this case the mean diameter is under-estimated approximately by the fraction $\frac{1}{4}(\sigma/\bar{x})^2$, which would have a value of about 0·01 per cent for the smallest three latexes in Table 1 according to the values of Bradford and Vanderhoff[1].

The formation of arrays from polystyrene latexes has been somewhat unpredictable. With the aid of a wire loop, a minute volume ($\sim$ 1 μl.) of a latex (kindly supplied by Dr. John Vanderhoff, Dow Chemical Co.) was added to a drop of microscope immersion oil (Cargille's; index of refraction, 1·515) on a microscope slide and mixed gently, and a cover-slip was added with moderate pressure. Close-packed triangular arrays of spheres in single layers were found in some of these preparations. Measurements of these arrays were made with a phase microscope ($\times$ 970) having an ocular reticle calibrated to 0·3 per cent. The results of these measurements, summarized in Table 1, appeared independent of the number of particles in the array, with the possible exception of the 0·5μ particles for which arrays of more than twenty particles were not obtainable.

For each latex the apparent mean diameter was determined from:

$$\bar{x} = \frac{\Sigma L_i}{N} \qquad (2)$$

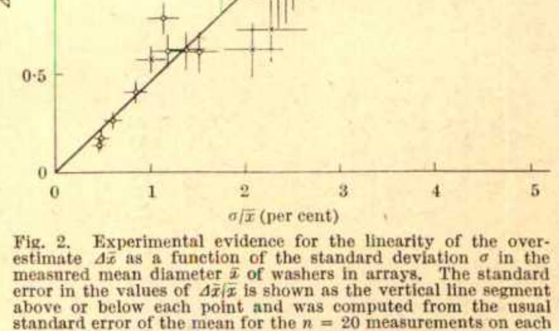

Fig. 2. Experimental evidence for the linearity of the over-estimate $\Delta\bar{x}$ as a function of the standard deviation σ in the measured mean diameter $\bar{x}$ of washers in arrays. The standard error in the values of $\Delta\bar{x}/\bar{x}$ is shown as the vertical line segment above or below each point and was computed from the usual standard error of the mean for the $n = 20$ measurements on each array. The corresponding horizontal line segments at either side of each point represent the estimated standard deviation of σ. The slope of the least mean square line is $k = 0·461$ (S.E., 0·029). ○, Two-component distribution; □, three-component distribution; $\times$, normal distribution. S.E.$_{\Delta\bar{x}} \simeq \frac{\sigma}{\sqrt{n}}$; $\sigma_\sigma \approx \frac{\sigma}{\sqrt{2n}}$

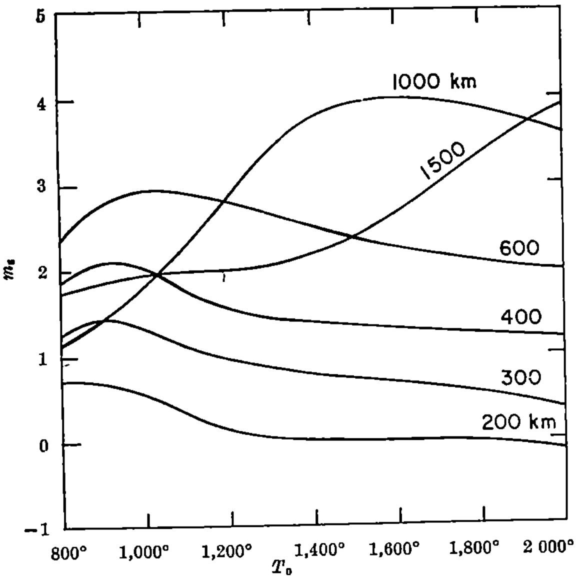

Fig 2 Curves showing the variation of m_0 (= d log ρ_0/d log F) with temperature in the night-time atmosphere for six representative heights

Curves of m_0 for six representative heights are shown in Fig 2 The increase of m_0 with height is evident in the range 200–600 km , at greater heights the picture is distorted by the increasing predominance of helium

The curves of Fig 2 are valid only for the anti-bulge point For any other point, where the temperature is $T = \alpha T_0$ and the density at a given height is ρ

$$m = \frac{\mathrm{d}\log\rho}{\mathrm{d}\log F} = \frac{T - 555\alpha}{T - 555}\, m_0 \qquad (4)$$

Since $\alpha > 1$, m is always smaller than m_0 for a given temperature Considering that the general tendency of m_0 is to decrease with increasing temperature, we can easily see that the parameter m is considerably smaller in the diurnal bulge, compared with its value in the night-time atmosphere

Table 1 Observed and Computed Values of m

Height	Satellite	Time	Observed m	Calculated m
350 km	1958 α	Day	0 75 ± 0 2	0 6
		Night	1 05 ± 0 2	1 1
650 km	1958 $\beta 2$	Day	1 2 ± 0 3	1 2
		Night	3 0 ± 0 4	3 0
1,300 km	1960 $\iota 1$	Day	2 0 ± 0 4	2 2

Priester's observed values of m are predicted almost exactly by Nicolet's model and equation (1), as can be seen from Table 1.

Lastly, Nicolet's model can correctly predict the observed increases of atmospheric density which accompany magnetic storms As was pointed out by me[5,6], these atmospheric perturbations are nearly independent of geomagnetic latitude and occur simultaneously all over the globe Accurate accelerations, as yet unpublished, computed by me in collaboration with Mr J Slowey, show that magnetic storms of comparable intensity give rise to larger drag perturbations when the background drag of the satellite is lower, either because of lower solar flux or because of greater angular distance of the perigee from the diurnal bulge This is what would occur in Nicolet's model atmosphere if as a consequence of the corpuscular storm the temperature of the upper atmosphere were raised by the same amount all over the globe. A fair quantitative representation of all the observed atmospheric perturbations can be given by assuming that the temperature of the upper atmosphere is augmented by an amount ΔT proportional to the geomagnetic planetary index a_p, according to the relation

$$\Delta T = 1\ 5^\circ\, a_p \qquad (5)$$

This equation represents quite well also a number of smaller atmospheric perturbations in March and April 1961, revealed by the ultra-sensitive satellite 1961 $\delta 1$ (the 12-ft balloon satellite)

Equations (1), (2) and (5) provide the means for computing temperatures of the upper atmosphere, for which Nicolet's model gives all other parameters All three equations are only rough outlines of the true relations between the respective variables and should be susceptible of considerable refinements in the future, also, other effects on the atmospheric temperature should be revealed in the more detailed analysis which is now being made It should be apparent, however, that Nicolet's model will provide an adequate basis for such an analysis, as had been expected by its author

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[2] Priester, W , and Martin, H A , *Mitt Univ Sternwarte Bonn*, No 29 (1960)
[3] Jacchia, L G , *J Geophys Res* , 65, 2775 (1960)
[4] Priester, W , *J Geophys Res* (in the press)
[5] Jacchia, L G , *Nature*, 183, 1662 (1959)
[6] Jacchia, L G , *Smithson Astrophys Obs* , *Spec Rep* 62 (1961)

OPTICAL CALIBRATION OF SOME MONODISPERSE POLYSTYRENE LATEXES IN ARRAYS

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The monodisperse polystyrene latexes, which have been used widely by electron microscopists as secondary standards, have been calibrated in a variety of ways, chiefly by electron microscopy of single particles[1] and light scattering from suspensions[2]. These methods are tedious, and their results have afforded little or no applicability to optical microscopy It seemed possible that arrangements of spherical monodisperse particles in arrays might permit rapid and precise determination of their mean diameters, since a number of particles could be included in a single measurement

The existence of an array of particles, as in Fig 1*A*, is evidence for their uniformity, their contiguity, and the linearity of their rows For microscopic particles, the greatest advantage of measuring particles in a

A WORKING MODEL FOR THE UPPER ATMOSPHERE

By LUIGI G. JACCHIA

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NICOLET[1] has recently published a tabulation of atmospheric densities at altitudes of 150–2,000 km., computed from a model based on diffusion equilibrium with known boundary conditions at 120 km. and with helium diffusion starting at 105 km. The tabulation is given for 12 asymptotic temperature values, ranging from 773° K. to 2,133° K. Assuming the validity of such a model, it is possible to compute upper-atmospheric temperatures directly from densities derived from satellite accelerations, and—since the variation of the density is related to the variation of the decimetric solar flux[2,3]—it appears possible to establish a correspondence between the solar flux and the asymptotic temperature of the upper atmosphere.

For a specific value of the solar flux the temperature in the upper atmosphere ranges from a maximum at the centre of the diurnal bulge[3] to a minimum in the dark hemisphere. Unless we know accurately how the temperature varies with the location of a point in the atmosphere with respect to the bulge, we cannot reduce the temperatures derived from satellite accelerations to a standard location. Since, however, the variation of temperature appears to be quite small within a radius of at least 50° around the anti-bulge point[3], that is, the point 180° from the centre of the bulge, we can take satellite accelerations obtained within that region and establish for them a correspondence between solar flux and temperature. Results from four satellites are plotted in Fig. 1. The good inner agreement of the individual points, irrespective of satellite height, shows that Nicolet's model appears quite satisfactory. The relation between the minimum night-time temperature T_0 and the solar flux F at 10·7 cm. appears to be linear within the observed accuracy, the straight line in the diagram corresponds to the equation

$$T_0 = 555° + 3° \; F \tag{1}$$

where T_0 is expressed in °K. and F in units of 10^{-22} W./m.^{2}/cycle/sec.

The accelerations of the four satellites from which this relation was derived extend over the interval February 1958–March 1961, during which the 10·7-cm. flux varied from a maximum close to 350 F units to a minimum of about 90, covering about 90 per cent of the expected range during the present 11-year solar cycle (the 1954 minimum was about 70, and it can be confidently expected that a similar reading will prevail in 1964–65). Atmospheric densities in the bulge can be represented with Nicolet's model, assuming that the temperature T_M at the centre of the bulge is in a constant ratio

$$T_M = 1{\cdot}35 \; T_0 \tag{2}$$

to the night-time temperature T_0.

The decay of the temperature from the centre of the bulge outward will require a more refined analysis which is not yet completed. In my 1960 model, I (ref. 3) assumed that the density at a given height varied as $\cos^6 \frac{\psi'}{2}$, where ψ' is the angular distance from the centre of the bulge. On the basis of Nicolet's model it appears that for height under 700 km. the bulge must become sharper with increasing height, and this is corroborated by the comparison of recently obtained accelerations of satellites 1958 α (perigee height 350 km.) and 1958 $\beta 2$ (perigee height 600 km.). In addition, the shape of the bulge must vary with the solar flux, only an analysis of temperatures through the bulge seems likely to yield satisfactory results.

In my 1960 atmospheric model[3] it was assumed that the atmospheric density ρ for any given height and any given ψ' is proportional to F^m, for simplicity it was assumed that m = constant = 1. Recently, Priester[4] found that m increases with height and is larger in the night-time atmosphere than in the sunlit hemisphere. It is interesting to note that this result can be deduced directly from Nicolet's model, if we assume that the relation between F and T_0 is given by equation (1). Calling ρ_0 the night-time density at a given height in the atmosphere, we shall have:

$$m_0 = \frac{d \log \rho_0}{d \log F} = \frac{T_0 - 555}{T_0} \; \frac{d \log \rho_0}{d \log T} \tag{3}$$

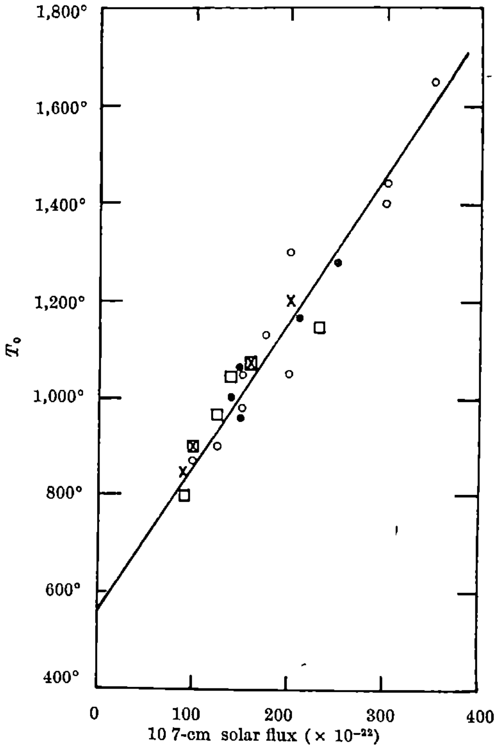

Fig. 1. Relation between the 10·7-cm. solar flux and the night-time temperature of the upper atmosphere, derived from four satellites using Nicolet's atmospheric model.
●, 1958 $\beta 2$; ×, 1959 $\alpha 1$; □, 1959 η; ○, 1958 α

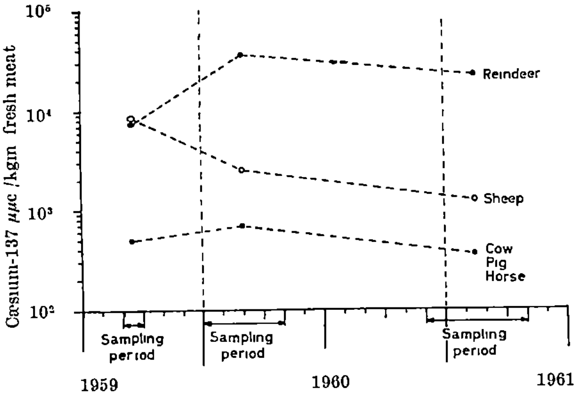

Fig 3

by a yearly precipitation of about 300 mm The total strontium-90 fall-out in Norway may be estimated from Fig 2 *B* to be approximately 5,500 c. at the end of June 1959, of which about 30 per cent may be dry fall-out. Adding 4 9 mc /km.^{2} found as the increment for Bodø from June 1959 to September 1960 (Fig 1 *D*, the mean annual precipitation at Bodø being near the average for Norway), the total strontium-90 fall-out at the end of September 1960 may be estimated to be about 7,000 c. The ratio of cæsium-137 in soil to strontium-90 in soil, both as estimated, is about 1·7

Cæsium-137 in meat was measured during 1959, 1960 and 1961 The results are

	1959	1960	1961	
Cow, pig, horse	2 samples	31 samples	38 samples	
Average value	500	700	400	μμc cæsium-137/kgm fresh meat
Maximum ,,	600	2,800	1,500	,, ,,
Minimum ,,	300	200	~0	,, ,,
Sheep	2 samples	12 samples	9 samples	
Average value	8,000	2,500	1,200	μμc cæsium-137/kgm fresh meat
Maximum ,,	11,000	9,000	3,000	,, ,,
Minimum ,,	5,000	50	100	,, ,,
Reindeer	2 samples	6 samples	48 samples	
Average value	7,700	35,000	22,000	μμc cæsium-137/kgm fresh meat
Maximum ,,	9,300	56,000	37,000	,, ,,
Minimum ,,	6,200	16,000	7,000	,, ,,

The results are plotted in Fig 3 The level found in the fall of 1959 is uncertain due to the very limited number of samples measured. During 1960–61 there appears to be a decrease of about 50 per cent in cæsium-137 concentration in all three groups of animals It appears that, on the average, the uptake of cæsium-137 by sheep is about five times higher than by cow, pig and horse The uptake by reindeer is about ten times higher than by sheep. As this difference is apparently not due to a difference in fall-out amounts, it seems reasonable to relate it to different grazing habits and different grazing field qualities Within each group of animals there is a difference from district to district that may be related partly to differences in amounts of fall-out and partly to differences in conditions of grazing. There does not appear, however, to be any large difference in concentration of cæsium-137 in reindeer from southern Norway and northern Norway The samples collected in 1961 were also analysed for potassium The average values are

Reindeer	4 4	gm potassium/kgm fresh meat
Sheep	3 2	,, ,,
Cow, pig horse	3 0	,, ,,

It appears that the potassium content in reindeer is about 40 per cent higher than in sheep, cows pigs and horses

Strontium-90 in animal bones from Norway has been measured by HASL, United States Atomic Energy Commission, New York Operations Office The results are

	1954 summer	1956 autumn	1958 autumn	
Cow	2 samples			
Average value	0 5			μμc strontium-90/gm calcium
Maximum ,,	0 8			,,
Minimum ,,	0 2			,,
Sheep	5 samples	14 samples		
Average value	4	17		μμc strontium-90/gm calcium
Maximum ,,	7	34		,,
Minimum ,,	2	6		,,
Reindeer		4 samples	10 samples	
Average value		100	150	μμc strontium-90/gm calcium
Maximum ,,		120	250	,,
Minimum ,,		40	110	,,

The four samples of reindeer bones collected in 1956 were analysed at the Atomic Energy Research Establishment, Harwell The reindeer bones collected in 1958 were also analysed for strontium-89 The results are

	Northern Norway	Southern Norway	
	4 samples	6 samples	
Average value	610	40	μμc strontium-89/gm calcium
Maximum ,,	650	94	,,
Minimum ,,	540	17	,,

The reindeer from southern and northern Norway were of comparable age and the samples collected at the same time The difference in concentration of strontium-89 may then indicate a difference in time of arrival of strontium-89, or different grazing conditions giving a difference in delay of uptake of strontium-89

In good agreement with the results for cæsium-137, it appears that the uptake of radioactive strontium by sheep is higher than that by cows, and the uptake by reindeer is higher than that by sheep The relatively high uptake by reindeer may be related to the special grazing habits of this animal Reindeer feed to a great extent on the slow-growing lichen The uptake of fall-out materials by lichen appears to be higher than by other vegetation

Three samples of lichen from northern Norway were measured during autumn, 1959 The results are

Sample	μμc cæsium-137/kgm lichen	mc cæsium-137/km^2
I	42,000	74
II	37,000	56
III	31,000	66
Average	36,000	65

From the yearly precipitation at the sampling place, the total amount of cæsium-137 deposited may be estimated from Fig 2 to be about 60 mc /km ^{2} at the end of 1959 It appears therefore that the efficiency of fall-out retention for lichen is very high

Summarizing, it seems that for internal radiation from fall-out materials the variations in uptake of radioisotopes by animals may be more important than the uneven geographical distribution of fall-out

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It appears that the maximum yearly cæsium-137 fall-out measured is approximately ten times the minimum. The cæsium-137 fall-out in 1960 was considerably lower than in the three foregoing years. The cumulative cæsium-137 fall-out at Bergen (60° 23′ N, 5° 21′ E) from September 1956 to June 1961 is shown in two-monthly periods by curve *A* in Fig. 1. The total deposition during this period is about 70 mc./km.^{2}. The relatively heavy cæsium-137 fall-out during the period September–October 1957 may be due chiefly to the Windscale accident. Strontium-90 in precipitation at Bodø (67° 17′ N, 14° 24′ E), and Tromsø (69° 40′ N, 18° 57′ E) has been measured by the Atomic Energy Research Establishment, Harwell, as part of a world-wide precipitation sampling programme[1]. The concentration in the total yearly precipitation for the past 3 years has been

	1958	1959	1960	
Bodø	6·2	17	3·8	μμc strontium-90/l. precipitation
Tromsø	5·4	14	2·5	,, ,,

The yearly fall-out was

	1958	1959	1960	
Bodø	7·1	18	2·6	mc strontium-90/km.^{2}
Tromsø	4·2	17	2·2	,, ,,

The cumulative strontium-90 fall-out at Bodø from April 1957 to December 1960 is shown in three-monthly periods by curve *C* in Fig. 1.

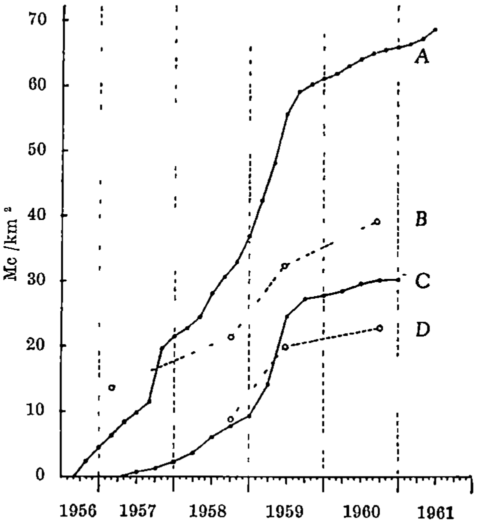

Fig. 1. *A*, Bergen cæsium-137 in precipitation, *B*, Bergen strontium-90 in soil, *C*, Bodø strontium-90 in precipitation, *D*, Bodø strontium-90 in soil

Cæsium-137 in soil was measured at several places in Norway in September 1960. Fig. 2 *A* shows the results plotted versus precipitation during the period from January 1, 1953, to the date of sampling. It appears that fall-out/mm precipitation decreases with increasing precipitation. As indicated in Fig. 2, this may be due to an amount of dry fall-out equal to the fall-out deposited by a yearly precipitation of approximately 400 mm. It follows from this that in Norway the dry fall-out may be about 30 per cent of the total fall-out, the mean annual precipitation in Norway being about 900 mm. This is in reasonably fair agreement with what has been found for Kjeller[2]. The total cæsium-137 fall-out in Norway, which has an area of 324,000 km.^{2}, may be estimated from Fig. 2 *A* to be about 12,000 c at the end of September 1960.

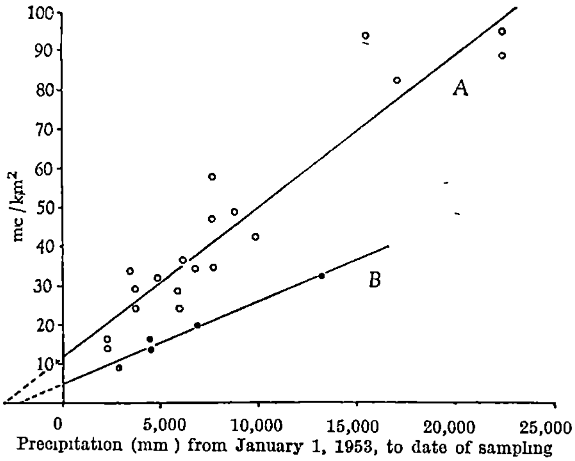

Fig. 2. *A*, cæsium-137 in soil, September 1960. *B*, strontium-90 in soil, June 1959

Strontium-90 in soil has been measured at several places in Norway by L. T. Alexander, as part of a world-wide soil-sampling programme[3]. The results for Bergen are shown by curve *B* in Fig. 1, and the results for Bodø by curve *D*. It appears from Fig. 1 that the strontium-90 fall-out at Bodø as deduced from soil measurements is lower than that measured in precipitation. The ratio is

	Oct. 1958 to June 1959	July 1959 to Sept. 1960	Oct. 1958 to Sept. 1960
Strontium-90 in soil / Strontium-90 in precipitation	0·65	0·88	0·71

The difference might indicate leaching out of the soil or re-suspension of deposited fall-out, but more probably it is an indication of the difficulty involved in obtaining a representative value of the amount of precipitation falling on the ground, especially where snow-drifting occurs during winter. The differences in the sampling and measuring methods may also be important factors. The results for Bergen, shown in Fig. 1, give the following ratio of cæsium-137 in precipitation to strontium-90 in soil

	March 1957 to Sept. 1958	Oct. 1958 to June 1959	July 1959 to Sept. 1960
Cæsium-137 in precipitation / Strontium-90 in soil	3·3	2·0	1·6

The high ratio during the period March 1957–September 1958 seems to agree with the suggestion that the cæsium-137 fall-out during the period September–October 1957 was due chiefly to the Windscale accident, the strontium-90 release of which has been given as less than 1 per cent of the cæsium-137 release[4]. If the cæsium-137 fall-out during this period is excluded, the ratio will be 2·2 instead of 3·3. In view of the different methods of sampling and measurements, the ratio of cæsium-137 to strontium-90 of about 2 seems to indicate that the soil data are in reasonably good agreement with the precipitation data. The measurements by the Atomic Energy Research Establishment, Harwell, give an average ratio of cæsium-137 to strontium-90 in fall-out nearer 1·7 (ref. 1).

Fig. 2 *B* shows strontium-90 in soil-samples collected during June 1959 plotted versus precipitation during the period January 1, 1953, to the date of sampling. In good agreement with the cæsium-137 results in Fig. 2 *A*, the strontium-90 results indicate an amount of dry fall-out equal to the fall-out deposited

for evaluation in the region of the spectrum (2640–4300 Å.) examined silver, aluminium, calcium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, sodium, nickel, lead, silicon, tin, titanium, vanadium, zinc and zirconium

Of these elements the only ones which the spectroscopic evidence proved to be significantly more concentrated in the glaze than in the fabric were silver, lead and tin. The evidence also suggested that the concentrations of copper and vanadium were higher in the glaze, but for these elements the evidence was not unequivocal The use of lead in pottery glazes is, of course, well known, and the presence of tin in medieval glazes has previously been recognized by Jope[6,7] As no reason for the deliberate addition of silver is apparent, it appears probable that this element occurs as an impurity in one of the other additives While lead is the obvious choice for the silver carrier, the relative concentrations of lead and silver fluctuate too widely in closely related glazes for this suggestion to be readily accepted The variation of silver content more closely follows that of the variation of tin, and this may indicate that some alloy or mineral containing both these elements has been added to the glazing material in addition to the galena normally accepted as the source of the lead Both solder and pewter have been suggested by Jope[7] as possible sources of tin in glazes from medieval pottery

The presence, and amount, of tin in the glaze has been used as a check on some of the accepted attributions of origin of the pottery sherds All the samples examined of glaze from Laverstock kilns have a high tin content However, the glazes of sherds found at Winchester and Bergen have low tin contents It appears therefore, that, although the Winchester and Bergen samples are visually similar to those from Laverstock, they are unlikely to have originated from the Laverstock kilns Of those we have examined the only English glaze with a comparable low tin content is that from the Tyler Hill kiln[8] near Canterbury

One group of English glazes and one group of Continental glazes contained no detectable tin This pottery, with the exception of a sample from Oslo, is of an earlier date (eleventh–twelfth century A D) than the remainder (late thirteenth–fourteenth century) The English group comprised three samples of 'Stamford ware', two from excavations at Thetford and one from Peveril Castle, Derbyshire The Continental group comprised four samples of 'Andenne ware', one from an Andenne kiln, one from Antwerp, one from London and one from Canterbury, and the sample from Oslo designated on archæological grounds as 'Nottingham ware' (Dunning, G C , private communication Mr Dunning suggests on typological grounds that this sample came from a kiln in that part of eastern England in the area Nottingham–Scarborough–King's Lynn) This spectroscopic similarity between the glazes of Stamford and Andenne wares is a striking confirmation of the theory put forward by Dunning[9] that the two wares developed from a common origin It is clear, however, from observed differences in the composition of the fabric materials that these samples could not have originated from the same kiln

Although all the Laverstock glazes had high tin contents, the relative amounts of tin and lead in the glaze varied significantly On calculating the ratio of the observed intensities of lead and tin lines in the glaze, this was found to vary from 1 1 to 4 7 Placing the ratios in numerical order arranges the kilns from which the glazes originated in an order very close to the chronological order assigned to these kilns on the basis of archæological evidence, the lowest tin content arising from the earliest kiln It is difficult to envisage any reasons why the tin content of the glazes should have been gradually increased, and it may well be that analyses on further samples will prove that this apparent systematic variation of the lead/tin ratio is fortuitous

Two further points of interest have been noted from the examination of the Laverstock glazes In all the kiln samples the calcium content of the glaze is higher than that of the fabric, suggesting that a calcium compound has been deliberately added, possibly to improve the adhesive properties of the glaze Some of the samples examined had an additional stripe decoration in a darker colour than the body glaze Comparative analyses of the two different coloured glazes from the same pot have revealed that this colour is solely due to the presence of enhanced concentrations of iron

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[2] Herteig, A E , *Med Arch* , 3, 177 (1959)
[3] Excavated by J W G Musty For interim reports see *Med Arch* , 2, 213 (1958), *Med Arch* , 3, 326 (1959)
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CÆSIUM-137 AND STRONTIUM-90 IN PRECIPITATION, SOIL AND ANIMALS IN NORWAY

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FROM fall-out measurements in Norway it seems that in this country the variations from one group of animals to another in uptake of radioisotopes may be greater than the variations in amounts of fall-out from one district to another. The geographical variations in fall-out are indicated by the amounts of cæsium-137 measured in precipitation at twelve places in Norway The concentration in the total yearly precipitation for the past 4 years has been.

	1957	1958	1959	1960	
Average value	5 7	8 9	15	3 2	μμc cæsium-137/l precipitation
Maximum ,,	7 2	11	18	4 4	,, ,,
Minimum ,,	2 7	6 9	9 8	2 5	,, ,,

The yearly fall-out was

	1957	1958	1959	1960	
Average value	6 1	7 6	11	2 3	mc. cæsium-137/$km.^2$
Maximum ,,	17	15	25	4 8	,, ,,
Minimum ,,	2 2	1 9	3 5	0 4	,, ,,

PRODUCTIVITY IN LETTERPRESS PRINTING

THE mounting cost of printing would alone give wider interest to the report on *Productivity in Letterpress Printing*, recently published by the Department of Scientific and Industrial Research*.

The report describes the results of an investigation made during 1956–57 by the Printing, Packaging and Allied Trades Research Association under a contract financed from Conditional Aid Funds and in which eighteen firms collaborated. The organization, methods and production of seventeen firms were examined in detail, and a detailed breakdown of machine time indicated that, on average, machines are only running for about 40 per cent of their manned time. Speed is accordingly not of prime importance: it is usually easier to increase production by reducing delays, make-ready times and other stoppages, and the average speed of new machines with a high potential running speed is little better than that of pre-war machines. What is needed in printing machines at present is the provision of devices to facilitate make-ready, control and adjustment.

The factors responsible for the low running time are discussed in the report, and while in some firms particular operations are inefficient, the greatest improvement can only result from a thorough examination of the printing process as a whole. Waiting time averaged 15 per cent and make-ready time 45 per cent of available time, and although the latter is a necessary and chargeable operation in printing, it can be substantially reduced by improvements in methods and organization. These usually could be made with little capital outlay but require an industrial engineering approach using work-flow principles, greater specialization of work by employees, quantitative measurement and mechanical aids for handling, and the like. At present, for example, so little method study is used that employees can earn bonuses on inefficient and even unnecessary operations, and conditions, as regards lay-out, in most machine rooms are described as chaotic.

The report is also critical of organization, production planning and storage in the industry, where also major changes in procedure are recommended. It is recognized that not all factors influencing efficiency are within the immediate control of printers, but there is scope for more standardization and greater co-operation between printers and other sectors of the industry. Better production planning and greater precision at each stage of the process could effect a real increase in production at lower costs, and a separate chapter in the report outlines the problems requiring further investigation. These chiefly call for operations research and kindred methods, very few purely technological problems came to light.

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* Department of Scientific and Industrial Research. *Productivity in Letterpress Printing*. Pp. viii+65. (London: H.M.S.O., 1961.) 5s. net.

A SPECTROSCOPIC SURVEY OF ENGLISH AND CONTINENTAL MEDIEVAL GLAZED POTTERY

By L. C. THOMAS and J. W. G. MUSTY

Chemical Defence Experimental Establishment, Porton Down, Nr. Salisbury, England

ONE of us (J. W. G. M.) recently claimed[1] that a medieval glazed jug from Bergen, found during the excavation of the Hanseatic Wharf there[2], had been made in the late thirteenth century kilns at Laverstock, near Salisbury, Wiltshire, England[3]. This and other attributions, based on a visual comparison of pottery sherds, have been checked by the spectroscopic examination of sherds from these sites and from a number of other English and Continental sites. While a range of sites has been covered, the number of samples from any site, other than Laverstock, has been limited. The variation in glaze composition found between samples from different kilns at Laverstock clearly indicates that more samples from each site must be examined for the full value of this technique to be realized. However, this preliminary survey has provided sufficient evidence to enable some conclusions to be drawn.

The pottery samples examined, other than those from Laverstock, were supplied by Mr. Gerald Dunning and included sherds from English kiln sites at Brill (Bucks.), Cheam (Surrey), Ham Green (Glos.) and Tyler Hill (Kent). Pottery found at Winchester and at Clarendon Palace, near Salisbury, was also examined as the kiln sites for these samples are unknown and Laverstock was thought to be one of the possible sources of supply. Examples of pottery attributed to foreign kilns, but found in Britain, included French polychrome ware from Glastonbury and Dover, French pottery found at Bristol, and the Belgian Andenne ware found at London and Canterbury. Samples of Andenne ware from Antwerp and from an Andenne kiln[4] were also examined. Samples found at Oslo and Bergen but attributed to British kilns were also included in this preliminary survey.

Small areas of the glaze were removed from the sherds by careful scraping with a tungsten carbide tipped masonry drill. Samples of the fabric were obtained from the unglazed side of the sherds as a control on the inevitable contamination of the glaze by the fabric. The samples thus obtained were ground in an agate mortar, packed into special 'cathode layer' graphite electrodes, and their spectra obtained and the line intensities evaluated using techniques previously described[5]. The results obtained will be reported in detail elsewhere. No attempt has been made to place these qualitative results on other than a semi-quantitative basis. Variations in the relative concentrations of elements have been assessed by variations in the relative intensities of lines of these elements in the same spectrum. The following elements had lines suitable

Dipicolinic Acid Content, Heat-Activation and the Concept of Dormancy in the Bacterial Endospore

Two distinct meanings have been applied to the use of the term 'dormancy' as applied to the bacterial endospore. First, the expression 'the dormant bacterial endospore' usually describes the apparent metabolic inactivity of the spore stage as compared with the vegetative cell. Secondly, the same term has been used to describe the fact that spore suspensions respond poorly or not at all to germination agents under conditions that permit rapid germination of aged[1] or heat-activated[2] spores. It is in the latter sense that the term dormancy is applied in this communication.

It is difficult to express quantitatively the dormant state of a spore preparation. Recent work suggests that the duration of heat-activation required for optimal germination can be used to measure the process. However, the duration of heat-activation necessary to induce optimal germination is not constant itself and can differ widely for different preparations of the same spore strain. The duration of heat-treatment necessary to induce optimal germination decreases with the age of the spore preparation[1]. L-Alanine-induced germination in *Bacillus cereus*[3] and glucose-induced germination in *Bacillus megaterium*[4] also indicate another relationship. With increasing concentrations of the germinating agents, less heat treatment is required for optimum germination.

In our work the requirement for heat-activation was further related to the chemical composition of the spore and the nature of the germinating agent employed.

Dipicolinic acid content and the germination-rate. The content of dipicolinic acid (DPA) in a spore can be varied by the conditions of growth and sporulation[5-7]. Also, it was observed that if higher concentrations of yeast extract were added to the '*G*' medium[5], the level of spore DPA could be increased from 7 to about 15 per cent of the dry weight. In the present work DPA contents of 2·2–15 per cent were employed.

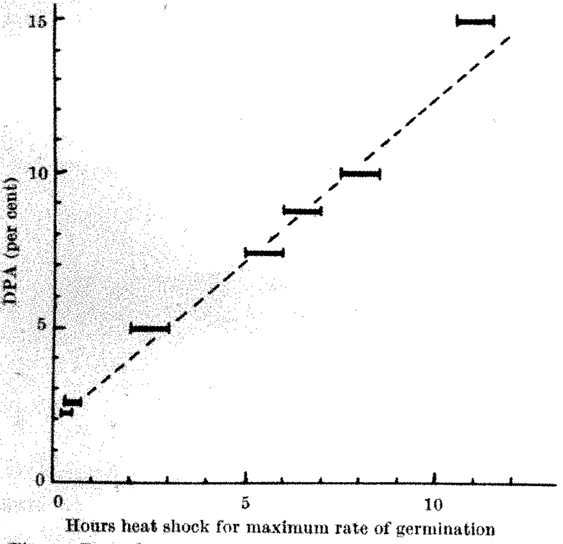

Fig. 1. Dependence of the duration of heat shock on intrasporal DPA concentration. Spores containing various concentrations of DPA were heat-shocked for various periods at 65° C., centrifuged, washed and resuspended in *M*/50 *tris* buffer *p*H 8 to an optical density of 0·40. Germination at 30° C. was initiated by the addition of 15 mgm./ml. L-alanine. The rate of germination was followed spectrophotometrically, as described by Woese *et al.* (ref. 8). The bars indicate the approximate time of heat-shock required to obtain maximum germination-rates

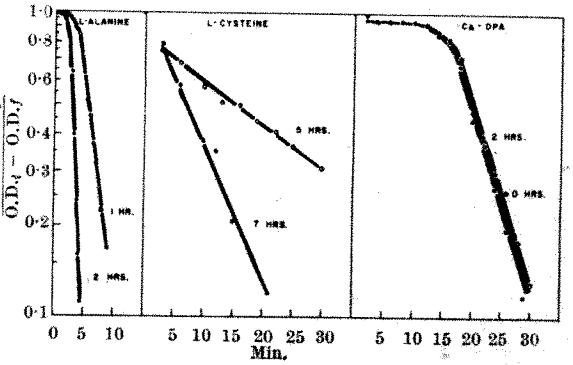

Fig. 2. Effect of heat-shock on the rate of germination in L-alanine, L-cysteine and CaDPA. See Fig. 1 for details of heat-shock and L-alanine-induced germination. L-Cysteine (15 mgm./ml.)-induced germination was followed at 30° C., whereas germination induced by $4 \cdot 8 \times 10^{-3}$ *M* calcium chloride, 4×10^{-3} *M* DPA was carried out at 20° C. in the presence of 0·2 per cent gelatine

When the amount of heat-activation necessary to give optimal L-alanine-induced germination was determined it was found that the extent of heat treatment necessary (65° C. in water) was related to the DPA content. The heat-shock requirements increased with increasing contents of DPA. These results are shown in Fig. 1. The germination-rates obtained with the various spore preparations after optimal heat treatment were constant when the same concentrations of L-alanine were used.

Relationship between the heat-shock requirement and the germinating agent. The effectiveness of L-cysteine as a germinating agent under various conditions of heat-shock and with spores with various DPA contents was identical to that observed with L-alanine. On the other hand, the effect of calcium DPA was quite different. As observed by Rieman and Ordal[9], equimolar Ca^{++} and DPA are effective germinating agents for bacterial spores. The rate of germination in the presence of CaDPA was not influenced by heat-shock treatment or by the endogenous level of DPA. These results, as well as the behaviour of L-alanine and L-cysteine towards heat-shock treatment, are shown in Fig. 2.

DPA release during heat-activation. Harrell and Mantini[10] observed a release of DPA during the heat-activation of spores of *B. cereus* strain *T*. The hypothesis is therefore posed that germination ensues only after the endogenous DPA content is first reduced to a critical threshold. The observations that CaDPA complexes with amino-acids and peptides[11] and the numerous suggestions that dormancy is maintained by endogenous levels of inhibitors[12] are easily explained on the basis of a critical DPA threshold hypothesis. To test this directly with regard to DPA itself, the amount of DPA released after sufficient heat-shock to permit optimal germination was measured in spore stocks with widely varying levels of DPA. The results are shown in Table 1. In all spore stocks examined there was no relationship between the amount of DPA released and germination. Optimal germination occurred with about a 10 per cent loss of DPA. It is possible, however, that several reservoirs of DPA exist, only a minor fraction of which controls dormancy. Before such a possibility can be investigated, more information on the state of spore DPA will be necessary.

The ability to measure quantitatively the degree of dormancy of a bacterial endospore enables one to

Table 1. RELEASE OF DPA FROM SPORE SUSPENSIONS DURING HEATING

DPA content of spore suspension (percentage dry weight)	Heat activation (hr.)	Percentage DPA released during heat activation
14·9	11	10
10·2	9	9
7·4	8	14
4·9	4	9

Spores were grown in modified *G* medium, and heat-shocked as described in Fig. 1. The spores were then centrifuged and the DPA content of the supernatant determined by the method of Jansen *et al.* (ref. 13).

study some of the principal factors regulating dormancy. First, what is the chemical nature of the mechanism regulating dormancy? Although this cannot be answered, the present experiments provide a suggestion that DPA may be a part of the system responsible for the dormant state. If the intrasporal concentration of DPA determines dormancy, the breaking of dormancy cannot be related merely to the removal of DPA. One possibility is that the breaking of dormancy involves an activation stage (heat-activation) and that the achievement of this stage requires the displacement of DPA (or a compound correlated with DPA) from some endogenous site(s).

Secondly, is the dormant state unique? As seen above, the degree of dormancy is dependent on the germinating substance used. For example, high DPA spores are dormant towards L-alanine, and L-cysteine, but not towards CaDPA. The concept of dormancy is, therefore, not a general one, characterizing the state of a given spore suspension, but is relative to the germinating agent.

This work was supported in part by a research grant from the Wisconsin Alumni Research Foundation and by a grant from the National Science Foundation (*B*–7150).

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Thermophilic Micro-organism of Uncertain Taxonomic Status from the Hot Springs of Tiberias (Israel)

IN the course of a survey of the fauna and flora of the hot springs of Tiberias[1], which attain a temperature of 60° C., a bacterium was isolated which shows a special mode of reproduction and apparently has not yet been described.

The mature cells are spherical, ovoid or pear-shaped, measuring 0·5–2μ. They are non-pigmented, non-spore forming and Gram-variable. They occur singly or as free-floating groups of two, three, four or even more individuals, in the form of rosettes and short chains (Fig. 1 *a*, *b*). Daughter cells are formed as buds (Fig. 1 *c*) and appear sometimes to be situated between two larger cells. A low percentage of the solitary forms are motile, these cells possessing a single polar flagellum (Fig. 2).

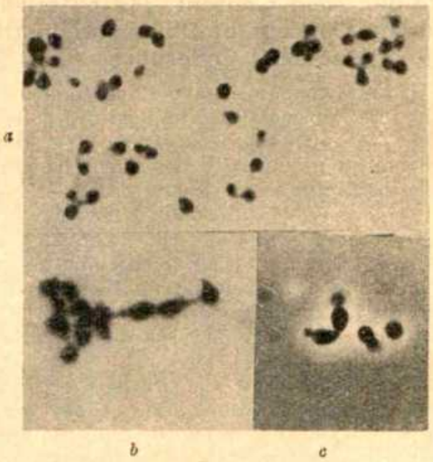

Fig. 1. Micrographs of bacteria. *a*, Single, pair and rosette forms; *b*, a chain form; *c*, budding bacteria. (× 1,500)

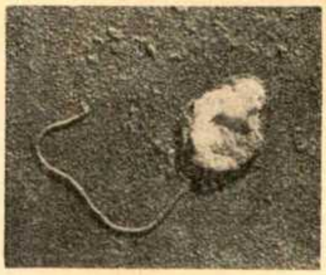

Fig. 2. A motile form possessing a single polar flagellum. Electron micrograph. (× 11,000)

Motility was observed occasionally in groups of two cells, and only in one case in a group of three.

The micro-organisms can be grown in autoclaved water from the hot springs, supplemented with 0·005 per cent yeast extract (Difco). No growth occurred when the concentration of the yeast extract was raised to more than 0·02 per cent. At 50° C. in the dark, the cultures achieved maximum cell density after one day, while at 37° C. they grow slowly and the peak is achieved only after several days. No multiplication occurs at 30° C. The organism is euryhaline and growth occurs over a range of 1–6 per cent (w/v) of total salt concentration; the concentration of minerals normally found in Tiberias hot springs is about 3 per cent[2]. Growth also occurred when spring water was replaced by sea water and supplemented with yeast extract as described here. The range of tolerance to sea water salinity is 0·5–8 per cent (w/v) total salinity. No growth was obtained on a solid agar medium. The highest concentration of agar ('Purified Bacto Agar, Difco') in which multiplication could be obtained was 0·2 per cent. The organism is aerobic as growth appears in a thin top layer of the semi-solid medium. The *p*H tolerance is wide, ranging from 6·2 to 9·5.

The taxonomic position of this micro-organism is as yet uncertain. It undoubtedly belongs to the budding bacteria included in the order Hyphomicrobiales[3]. Unfortunately, the taxonomy of the members of this order is uncertain as most descriptions are based on microscopic examination[4,5].

Further investigations on this interesting micro-organism are being carried out.

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CYTOLOGY

Chromosome Preparation from Fresh and Cultured Tissues using a Modification of the Drying Technique

TECHNIQUES for making satisfactory chromosome preparations have evolved rapidly during the past decade since Makino and Nishimura[1] and Sachs[2] adopted pre-treatment with hypotonic solutions and the squash method of preparation. Administration of colchicine increased the number of countable mitoses and facilitated analysis by arresting cell division in metaphase, producing shortening of chromosomes and divergence of sister chromatids.

The Feulgen squash method[3] has been used widely for chromosome preparations. Although adequate in many instances it has several disadvantages. (1) Firm and even pressure is not easy to achieve, and areas of insufficient or excessive squashing are difficult to avoid. (2) Nuclei with incomplete or broken chromosomes are found in an appreciable number of cases. Tough *et al.*[4] reported 22 per cent of incomplete groups in their countable material. This complicates analysis. (3) Valuable material may be lost during squashing around the edges of the coverslip—an important factor, if small quantities only are available for analysis.

The drying technique for chromosome preparation was introduced by Rothfels and Siminovitch[5]. Spreading of chromosomes is achieved by alteration of surface tension forces involved during drying on the wet slide. The method has been reported on favourably by Moorhead *et al.*[6] and Tough *et al.*[4], the latter reporting that out of all countable groups 96 per cent were unbroken in their preparations. The technique was, however, only suitable for relatively fragile nuclei such as that from cultured human leucocytes. In human bone marrow and animal material, that is, bone marrow, spleen, lymph glands, etc., squashing was usually necessary.

A method of achieving satisfactory chromosome preparations by the drying technique has been evolved which can be applied to both human and animal tissues. Besides human cultured leucocytes, good-quality preparations have been obtained from marrow and spleen of mice, rats and rabbits. The technique is reliable, relatively simple, incurs minimal loss of material and produces a high count of unbroken and well-spread chromosome groups.

After pre-treatment with 'Colcemid' either *in vitro* in the case of cultures, or *in vivo*, a cell suspension is prepared (solid tissues are homogenized) and incubated with 0·95 per cent sodium citrate solution at 37° C. for 30 min. The following scheme is then followed. The suspension is centrifuged at 200*g* for 5 min. in Kahn tubes and the supernatant fluid is removed. Fixative (3 parts absolute alcohol to one part glacial acetic acid) is added and changed after 5 min. without disturbing the layer of cells lying at the bottom of the tube. The preparation is kept in iced water or a refrigerator at 4° C. for 1 hr. The fixative is removed and chilled 45 per cent acetic acid is added. The tubes are kept in a refrigerator for 18–24 hr. The 45 per cent acetic acid is removed and chilled 60 per cent acetic acid is added and the preparation kept cool for 1 hr. Only then are the cells resuspended in the 60 per cent acetic acid, so as to make a concentration of approximately 15×10^6 cells per ml. A microscope slide is placed on a flat piece of solid carbon dioxide and kept in place until a white film of frozen condensation forms on its upper surface. The slide is lifted and held obliquely until thawing has just begun at the edges. Small drops of the suspension are then placed on the frozen surface. The drops must not freeze on the slide but should spread rapidly, the cells adhering to the surface of the slide. The slide is then immediately heated over a spirit lamp, very gently if the material was obtained from cultured human leucocytes or more strongly in the case of other cells. The degree of heating may involve some trial and error, but in the case of mouse spleen cells, for example, it should be more rapid, so that drying is accomplished within several seconds, the temperature of the slide being above that tolerated by the back of the hand. Staining is performed with aceto-orcein.

The method produced satisfactory spreading of chromosomes in both fresh and cultured material. It was equally successful in preparations from marrow and solid tissues. The number of countable groups of chromosomes was considerably higher than in corresponding preparations by the squash technique. A large number of countable and clearly spread mitoses was obtainable on one slide. In the case of chimæric spleens from 3–10-day-old *A* strain mice (obtained by injecting spleen cells from adult *CBA* mice into *A* neonates), using a concentration of 15×10^6 cells per ml., it was possible to prepare 5–10 slides from each spleen and 100 or more clearly countable mitoses were usually present on each slide. This represented 20–30 per cent of nuclei in which chromosomes were visible. Ninety-four per cent of countable cells had the modal number of chromosomes.

In the technique described by Moorhead *et al.*[6], the final suspending medium before spreading was the fixative. It was found that the fragility of the nuclei could be [illegible]creased by using 45 per cent acetic acid, and [illegible] by 60 per cent acetic acid. Although it w[illegible]irable to leave the cells in the 45 per cen[illegible]rolonged period of time (usually ov[illegible]th the 60 per cent acetic acid fo[illegible] avoided, otherwise excessive s[illegible]f the chromosomes occurred a[illegible]. The thin film of frozen c[illegible] produced by cooling the slide with dry ice afforded good spreading, and immediately afterwards, adherence of the cells to the glass surface. Subsequent heating was important to complete the spreading of more resistant nuclei during drying.

We are grateful to Prof. M. F. A. Woodruff for his encouragement and advice, and to Dr. Donald Michie for his helpful criticism. One of us (M. F.)

carried out the work during the tenure of a clinical research fellowship from the Medical Research Council.

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Culture of Human White Cells using Differential Leucocyte Separation

SEVERAL variations of white blood cell culture methods for the purpose of human chromosome study are now in use. All these are derived from the method of Hungerford *et al.*[1]. We have used the original and some of the modified procedures, particularly that of O. J. Miller (personal communication). In our experience with these techniques, about 75 per cent of cultures produce adequate numbers of mitotic figures for examination, while the remaining 25 per cent grow poorly, if at all. It was our impression that most failures occurred in specimens containing large numbers of polymorphonuclear leucocytes. On incubation, such cultures became excessively acid, and large clumps of degenerating polymorphs appeared.

While visiting the laboratory of K.-E. Fichtelius at the Histological Institute in Uppsala in 1957, one of us (K. H.) observed a method for removing polymorphonuclear leucocytes from white cell suspensions. The method was used for a metabolic examination of lymphocytes (Fichtelius, K.-E., and Hallander, H., personal communication). The procedure utilizes the phagocytic properties of polymorphonuclear cells by permitting them to ingest iron particles. The iron and the cells containing it are then removed by a magnet. Similar methods for different purposes have been used by several other investigators[2–4].

We have incorporated this procedure into our culture method, and have since performed more than 150 consecutive cultures with absence of growth in only one case. Our present technique produces good mitotic-rates (Fig. 1) and well-spread metaphase plates suitable for analysis (Fig. 2). The procedure is as follows.

Draw 10–20 c.c. of venous blood into a heparinized syringe and transfer to a sterile tube. Place the tube in an incubator at 37° at a 45° angle for [redacted] min., until the red cells sediment. Transfer to a sterile container having a flat su te with two volumes of culture m 99 with antibiotics). Add appr of very fine sterile iron powde r at 37° C. for 30–60 min. f container down. The sha 100 strokes per minute. strong magnet for 5 min., transfer the supernatant to a sterile tube, and place this tube on the magnet for an additional 5 min. Transfer the supernatant to culture vessels. After performing a cell count, dilute the cell suspension with culture medium to achieve a final concentration of 0·5–1·0 × 10^6 cells per ml. (These are now 95–99 per cent mononuclear cells.)

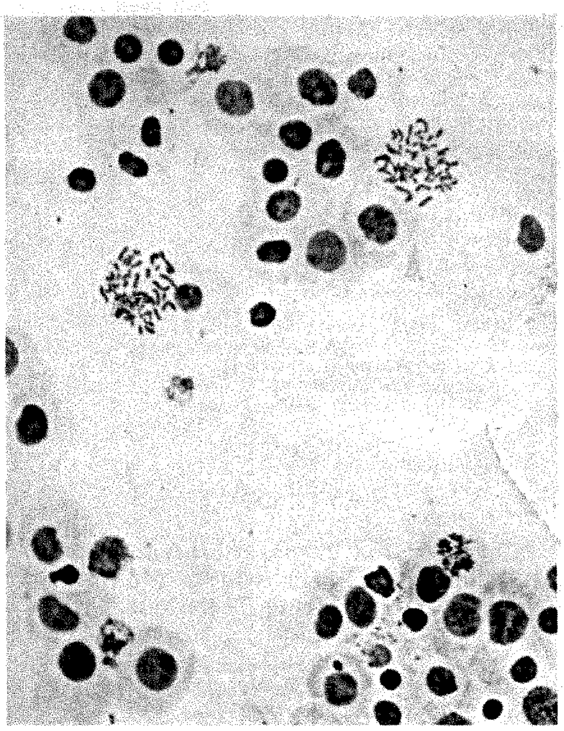

Fig. 1. Low-power view of growing mononuclear cells, showing mitotic figures swollen with hypotonic salt solution. Note nuclei of mononuclear cells in various stages of growth

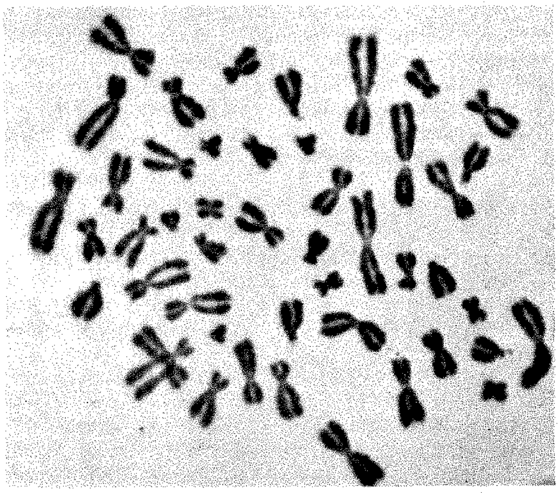

Fig. 2. High-power view of well-spread metaphase plate, showing chromosomes adequate for karyotype analysis

Add 0·2 ml. phytohæmagglutinin (Difco, type *M*) per 10 ml. of culture fluid and incubate at 37° C. for at least 72 hr. Add 0·1 ml. of 10^{-6} molar colchicine per 10 ml. of culture fluid. Incubate for an additional 2 hr. Examine a sterile sample of the culture under the phase microscope at this time. Particular attention is paid to clusters of mononuclear cells, which are examined for the presence of mitotic figures. If none is seen, incubate for an additional hour and repeat examination. When mitotic activity is observed, shake cultures well and centrifuge at 400 r.p.m. for 5 min. Suspend the cells in 1 per cent sodium citrate for 4 min. Add 1 drop of fixative (3 : 1 absolute ethyl alcohol–glacial acetic acid) and centrifuge at 200 r.p.m. for 2 min. Discard the supernatant. To the residue add the fixative very slowly with constant agitation to a volume of 1 ml. After 5 min., centrifuge at 400 r.p.m. for 5 min., resuspend in fresh fixative. (This may be repeated several times to improve chromosome spreading.) Place small droplets on a coverslip and air-dry by blowing. When dry, invert coverslip into a drop of 2 per cent acetic orcein on a slide and blot off excess stain for temporary preparation. For permanent preparations, immerse air-dried coverslips in stain for 1 min., dehydrate (tertiary butyl alcohol, 10 sec., twice; 1 : 1 tertiary butyl alcohol–xylene, 1 min.; xylene, 1 min., twice) and mount in 'Permount' or balsam. Such preparations are best examined by phase contrast. For bright-field examination, longer staining is required.

The technique outlined above depends for its success on the presence of a small amount of the substance phytohæmagglutinin, which appears to act as a mitotic stimulant[5]. Examination of growing cultures reveals that the mononuclear cells form large clusters, apparently as a result of the action of the phytohæmagglutinin, and it is only within these clusters that mitoses are found. That human mononuclear cells can be cultured *in vitro* without addition of phytohæmagglutinin is shown by the work of Timofejewsky in 1928 [6], where leucocytes from the buffy coat of centrifuged blood were grown in a plasma clot using autologous plasma. This suggests that part of the effectiveness of phytohæmagglutinin in causing leucocyte growth may be due to its ability to cause white cell clumping, providing an environment similar to that in a plasma clot.

This work was aided by grants from the American Heart Association, the U.S. Public Health Service (*H*–2202) and the New York City Health Research Council (*U*–1030).

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Two Types of Ribonucleoprotein in the Nucleolus of Intestinal Carcinoma of the Newt following Injection of Herring-Sperm Deoxyribonucleic Acid

RECENTLY I demonstrated two types of ribonucleoprotein in the nucleolus of tumour cells in the squamous-cell carcinoma of the lizard *Lacerta agilis*[1]. To establish whether a more general significance should be attached to this result I performed a corresponding investigation with the experimental carcinoma of the intestine of the newt *Triturus alpestris* following injection of herring-sperm deoxyribonucleic acid (eight days injection with 0.1 ml. of 5 per cent solution)[2]. After killing the animals on the 40th day of the experiment the nucleolar differentiation of the tumour cells was studied by the toluidine blue-molybdate method[3,4]. As in *Lacerta*[1], the ribonucleoprotein of the nuclear sap or parachromatin was coloured deep purple, the nucleolus partly assuming a green colour. Also in *Triturus* the existence of two types of ribonucleoprotein, if not actually demonstrated, had thus become highly probable. In the nucleoli a variable quantity of metachromatic ribonucleoprotein, as well as a number of very small vacuoles, appeared to be present, remaining unstained by the method described here. In the interphase cells the metachromatic ribonucleoprotein was most distinct, being in the form of small granules, hollow spheres or clusters of hollow spheres. Examination with a phase-contrast microscope showed that the nucleoli of the living tumour cells contained numerous very small vacuoles.

Comparing the nucleolus in the toluidine blue-molybdate stained sections with that in the living tumour cell it could be ascertained that the metachromatic ribonucleoprotein was situated around, or in, some very small vacuoles. After pre-treatment with ribonuclease or hot trichloroacetic acid[5] this nucleolar ribonucleoprotein was not coloured. In all phases of staining by the toluidine blue-molybdate method staining could be obtained in the same way as the nuclear parachromatin. Following Love and Bharadwaj[3,4] it can, therefore, in this intestinal tumour of *Triturus* also be called the nucleolar parachromatin. Just as in the squamous-cell carcinoma of the lizard and the Ehrlich ascites tumour[4], the tumour cells in *Triturus* often persisted throughout the mitosis, without being incorporated in the daughter nucleus. During mitosis the nucleolar parachromatin exhibited a different localization: in early prophase it was frequently situated at the rim of the nucleolus, to disappear from view during the later prophase, and become clearly visible again in the metaphase, anaphase and telophase as a vacuolated nucleolus green-coloured in the toluidine blue-molybdate sections.

Love[3] suggested that the nucleolar parachromatin is probably extruded from the nucleolus during the prophase to contribute to the accumulation of the granular parachromatin, which takes place in this period. The results obtained for the *Triturus* tumour may certainly be an argument for this reasoning. The earliest symptom of reformation of the nucleolus in the daughter nucleus was a metachromatic inclusion, afterwards acquiring an outer coating of ribonucleoprotein staining green in the toluidine blue-molybdate sections, which could be vacuolated as well as amorphous. This nucleolar differentiation of the tumour cells in *Triturus* is undoubtedly related to the special structures known for a long time already in the nucleolus.

The relationship of the two types of ribonucleoprotein in the nucleolus to the nucleolini[6-8] and the nucleonemata[8] could also be solved with the data obtained from the intestinal tumour of *Triturus*. Also in the tumour cells of *Triturus*, hollow and solid structures, resembling the true nucleolini, could be demonstrated after staining with iron hæmatoxylin. The physiochemical differences in ribonucleoprotein determining the differential staining by the toluidine blue-molybdate method may, therefore, also be responsible for some differences in the affinity for the hæmatoxylin. As these results are in good agreement with those obtained in the Ehrlich ascites tumour cells[4] and the squamous-cell carcinoma of *Lacerta*, it would seem that they are certainly of general importance.

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PSYCHIATRY

Erythrocyte Cholinesterase-levels in Mental Patients

RUBIN has published data[1a] on erythrocyte acetylcholinesterase which showed a bimodal distribution of this enzyme in schizophrenic patients This paper was criticized[1b] and the criticism answered[1c] Our work was an attempt to repeat Rubin's findings in larger groups of patients and normals

The assay procedure we used is based on the following reactions.

acetylthiocholine + erythrocyte acetylcholinesterase → acetate + thiocholine

thiocholine + dithiobisnitrobenzoate → yellow colour

The rate of formation of the yellow anion of 2-nitro-5-thiobenzoic acid is proportional to the enzyme activity This method is rapid and uses small amounts of blood (10 µl) and is useful for surveys of this type The plasma esterase was inhibited by addition of quinidine sulphate to the reaction mixture[3] The assays reported in this communication are therefore of the erythrocyte esterase Details of this method have been published[2]

Blood samples for the patient group were collected by the clinical laboratory staff from each patient on admission to this Institute At that time the patient's record number and the date of the blood withdrawal were noted A portion of the blood sample was assayed in our laboratories for erythrocyte acetylcholinesterase At the time the assay was performed, the clinical diagnosis was unknown to us After we had determined the erythrocyte acetylcholinesterase-levels of 64 patients, the diagnosis and status of these patients were studied A psychiatrist (E C) examined all the hospital records on members of this group The patients were then placed in the categories listed in Table 1 on the basis of the diagnosis recorded in the hospital record, supplemented by first-hand examination of the patients' chart The results we obtained are shown in Table 1

Table 1 ERYTHROCYTE ACETYLCHOLINESTERASE-LEVELS

Type	$\bar{x}$	σ	n
Normal *A*	1 08	0 18	75
Normal *B*	1 01	0 16	8
Schizophrenic, no drugs	0 98	0 12	16
Schizophrenic, on phenothiazine	1 02	0 22	6
Organic psychoses and epilepsies	0 98	0 42	11
Psychoneuroses	1 00	0 23	13
Depressions	1 12	0 15	10

$\bar{x}$, Observed mean-rate (moles substrate hydrolysed per min. per red blood cell × 10^{15})

σ, Standard deviation of the group

n, No. in each group

The group labelled Normal *A* is a sample of individuals who volunteered finger-tip blood during a display of medical research facilities held on this campus The equipment for assaying the erythrocyte acetylcholinesterase was on display and the assays were performed in public Persons whose blood was taken by our clinical laboratory and who were not patients (for example, physicians, nurses, staff, students, etc) are included in the classification Normal *B*

The results indicate that we cannot distinguish hospitalized mental patients from non-hospitalized, presumably normal persons, on the basis of mean erythrocyte acetylcholinesterase rates The group called 'psychoses and epilepsies' did show considerably greater variability than the other groups For this reason, we decided to follow the erythrocyte acetylcholinesterase rates for several days in these patients, to see if this difference was significant

In summary, an epileptic patient was hospitalized for more than a month During that period 28 erythrocyte acetylcholinesterase determinations were performed on his blood cells Some of these were shortly prior to, others shortly after, seizures; some were quite distant in time before or after seizures. In addition, on two occasions we were able to make determinations at regular intervals after a seizure In no case was there any obvious correlation of the duration or time of occurrence of the seizure with the observed erythrocyte acetylcholinesterase-level. Nor were his rates different from the normal (1 09 ± 0·12, Table 1) for the whole series In a smaller series from two other such patients similar negative findings were observed We believe, therefore, that the original observed large variability in this group of patients was an artefact of the small sample.

In re-examining Rubin's results it is clear that the major difference between his results and ours is the magnitude of the standard deviations observed. Our normals show about 15 per cent variation from the mean value In a series of 101 human adult subjects, Sabine[4] showed a standard deviation of 10 per cent about her mean value Sawitsky[5] reports a standard deviation of a group of 15 normal subjects which is 9 6 per cent of their mean activity Rubin, on the other hand, observed a 3 per cent variation Had his variation been as large as 10 per cent, his results would have been similar to ours

The number of verifiable physiological differences between mentally ill patients and normals is small and any new observations in this field would be of value in assessing the etiology and progress of the disease The difficulties of confirming the results of one investigation by another investigator in the field of mental health has been discussed by Kety[6] We have been unable to confirm Rubin's results

We wish to acknowledge the help and co-operation of Dr Robert B Anderson, Department of Neurology, University of California Medical Center

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[1] *a* Rubin, L S , *Science*, **128**, 254 (1958), *b*, Krech, D , Rosenzweig, M R , and Bennett, E L , *ibid* , **128**, 1176 (1958) *c*, Rubin, L S , *ibid* **128**, 1176 (1958)

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[6] Kety, S , *Science*, **129**, 1528 (1959)

FORTHCOMING EVENTS

Thursday, December 28

Royal Institution (at 21 Albemarle Street, London, W 1), at 3 p m —Sir Lawrence Bragg, F R S "Electricity" The One Hundred and Thirty-second Course of six lectures adapted to a Juvenile Auditory. Further lectures on December 30, January 2, 4, 6 and 9)

Thursday, December 28—Friday, December 29

World University Service (at B M A House, Tavistock Square, London, W C 1), at 10 30 a m on Thursday and 10 a m on Friday—Conference on "The Student and Mental Health"

Friday, December 29

Royal Society of Arts (at John Adam Street, Adelphi, London, W C 2), at 2 30 p m —Dr A D Booth "Machines that Think" (Mann Lecture)

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Physicist (preferably with experience in isotope techniques, including autoradiography and physical methods for microanalysis) in the Department of Preventive Dentistry, to engage in research into the mechanism of the action of fluorides in preventing dental caries, in co-operation with a research team including chemists and dental pathologists and histologists—Prof J L Hardwick, Turner Dental School, The University, Manchester 13 (December 28).

Librarian—The Secretary, University of East Anglia, City Hall, Norwich, Norfolk (December 30)

Junior Lecturer (interested in stratigraphy and sedimentation) in Geology—The Registrar, Trinity College, Dublin, Republic of Ireland (January 1)

Lecturer or Assistant Lecturer (medically or non-medically qualified) in the Department of Histology—The Registrar, The University, Liverpool, quoting Ref No CV/5/2/N (January 1)

Lecturer in Applied Mathematics, to take charge of the Electronic Computation Laboratory—The Registrar, University College, Singleton Park, Swansea (January 5)

Lecturer (medically qualified, and with special experience in histopathology) in Pathology—The Registrar, Trinity College, Dublin, Republic of Ireland (January 15)

Lecturer in Geology (Mineralogy and Petrology) at the University of New England, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 15)

Lecturer in Microbiology in the Department of Agriculture, University of Sydney—The Secretary Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 19)

Senior Lecturers (2) in Anatomy at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 19)

Assistant Lecturer in Botany—The Registrar, The University, Manchester 13 (January 20)

Senior Lecturer/Lecturer in Bacteriology at the University College, Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W C 1 (January 22)

Senior Lecturers or Lecturers (with at least an honours degree or equivalent qualifications, with experience and research interests in pure mathematics) in Mathematics at the University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W C 2, and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N S W , Australia (January 26)

Lecturer in Engineering, for duties which will include teaching and research in applied mechanics and one or more of the usual civil engineering subjects—The Secretary, The University, Aberdeen (January 31)

Lecturer in Physics—The Registrar, The University, Hull (January 31)

Reader in Physics at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office,) Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 31)

Research Fellows (2) in Hydrobiology, to work on fundamental problems in Lake Kariba at the Nuffield Kariba Research Station (University College of Rhodesia and Nyasaland)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 31)

Lecturer (with special qualifications or experience in inorganic chemistry or in some branch bearing on inorganic chemistry) in Chemistry at the University of Canterbury—The Registrar, University of Canterbury, P O Box 1471, Christchurch, New Zealand (February 28)

Robert Blair Fellowship in Applied Science and Technology tenable for one year of advanced study or research abroad—The Education Officer (WA 14/N/3316/12), County Hall, London, S E 1 (March 12)

Assistant Biochemist, Basic grade (preferably holding the A R I C , the Grad Mem R I C or B Sc (Hons) in chemistry), in the Group Laboratory at Crumpsall Hospital—The Hospital Administrator, Crumpsall Hospital, Manchester 8

Assistant Experimental Officer (with a pass degree, H N C , or equivalent, or, if under 22, G C E "A" level, O N C or equivalent) in the Chemistry Department, to help with research on the forms of combinations of magnesium, calcium and phosphate in cattle diets and ingesta, and on the development of the analytical methods used —The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref 61/15

Assistant Experimental Officer (with a pass degree, H N C or equivalent, or, if under 22, G C E "A" level, I N C or equivalent) in the Physics Department, for work on the flow and coagulation of blood—The Secretary, National Institute for Research in Dairying (University of Reading, Shinfield, Reading, quoting Ref 61/19

British Petroleum Research Student (with experience in microbiological techniques) in the Department of Chemical Engineering, for research on the conversion of petroleum hydrocarbons into fatty acids—The Secretary, University College London, Gower Street, London, W C 1

Graduate Assistants in Biochemistry—Dean, Faculty of Graduate Studies, University of Alberta, Edmonton, Alberta, Canada

Head of the Science Department, Head of the Mathematics Department, and Assistant Masters to teach Science, Mathematics and Biology—The Secretary, Atlantic College (U.K), Ltd , Denmark House, 71/72 Piccadilly, London, W 1

Physicist, for teaching and fundamental research—Head of the Department of Physics, Victoria College, Victoria, B C , Canada

Physics Laboratory Technician (man or woman, with experience of work in a teaching laboratory)—The Secretary, Royal Holloway College, Englefield Green, Surrey

Principal Lecturer in Cloth Manufacture and Applied Testing, a Senior Lecturer in Textile Physics, with particular emphasis on the structure of natural and man-made fibres, a Senior Lecturer in General Textiles and Testing Methods, a Senior Lecturer in Design and Colour, a Lecturer (physicist or mathematician) for statistical work in textile engineering, and a Lecturer in Physical Chemistry, for the application of chemistry in textile processing—The Registrar, Bradford Institute of Technology, Bradford 7

Principal Veterinary Research Officer (member of the Royal College of Veterinary Surgeons, or with an equivalent qualification) with the Federal Government of Nigeria—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W C 2, quoting Ref Z 5/3

Senior Lecturer in Automatic Control in the Department of Production Technology and Control Engineering—The Secretary, Northampton College of Advanced Technology, St John Street, London, E C 1

Specialist Officer (Plant Nematologist) (with a first- or second-class honours degree in natural science (botany or zoology) plus two years postgraduate experience) with the Federal Government of Nigeria—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W C 2 quoting Ref B 16/2

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(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Science or Sanity We Cannot Retain Both By Thomas Brennan Pp 5 (Roydon, Harlow Thomas Brennan, 7 Temple Mead, 1961) [410

Society for Analytical Chemistry Monograph No 1 Methods for the Analysis of Non-soapy Detergents (NSD) Products By G F Longman and J Hilton Pp ii+30 (London Society for Analytical Chemistry, 1961) 15*s*. [410

National Central Library 45th Annual Report of the Executive Committee, 1 March 1960 to 31 March 1961. Pp 24 (London National Central Library, 1961) [410

Ministry of Agriculture, Fisheries and Food National Agricultural Advisory Service Experimental Husbandry Farms and Experimental Horticulture Stations—Progress Report 1961 Pp v+55+4 plates (London H.M Stationery Office, 1961) 4*s* net [410

Universities Council for Adult Education The Universities and Adult Education a Report prepared by a Working Party of the Universities Council for Adult Education Pp 32 (Bristol Mr W E Salt, Hon Secretary, Universities Council for Adult Education, 1961) 2*s* 6*d* [410

University Grants Committee Returns from Universities and University Colleges in receipt of Treasury Grant, Academic Year 1959–60 Pp 76 (Cmnd 1489) (London H M Stationery Office, 1961) 4*s* 6*d* net [410

West of Scotland Agricultural College Research Bulletin No 29 Comparison of Production from Three Varieties of Tall Fescue and Three Varieties of Meadow Fescue By I V Hunt Pp 43 (Auchincruive, by Ayr West of Scotland Agricultural College, 1961) 1*s* [410

Titles of Dissertations approved for the Ph D , M Sc , and M Litt Degrees in the University of Cambridge during the Academical Year 1960–1961 Pp v+23 (Cambridge Board of Research Studies, The University, 1961) [410

Imperial College of Science and Technology (University of London) Calendar 1961–62 Pp xi+396 Prospectus, 1962–63 Pp viii+63+4 plates (London Imperial College of Science and Technology, 1961) [410

Society for the Promotion of Nature Reserves Handbook 1961 Forty-fourth Annual Report, year ending 31st March, 1961. Pp 44+2 plates (London Society for the Promotion of Nature Reserves, British Museum (Natural History), 1961) 10*s* [410

Proceedings of the Royal Irish Academy. Vol 61, Section A, No 8 Statistical Efficiency of Different Methods of Estimation of Coefficients in a Very Simple System of Equations By R C Geary Pp 67–76 1*s* 6*d* Vol 61, Section B, No 14 The Lee Basin, Part 2 The Drainage Pattern By S Farrington Pp 233–254+plate 13 2*s*. 6*d* Vol 61, Section B, No 15 The Dalradian Successions and their

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Colonial Office Fishery Publications, No 16 A Quantitative and Qualitative Study of some Indo-West-Pacific Plankton By J H Wickstead Pp iv+200+5 plates (London H M Stationery Office, 1961) 55*s* net [410

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Manchester Literary and Philosophical Society Memoirs and Proceedings, Vol 103, 1960–61 Pp 80+liii (Manchester Manchester Literary and Philosophical Society, 1961) 31*s* 6*d* [910

Department of Scientific and Industrial Research National Physical Laboratory Notes on Applied Science No 24 Photometric Standards and the Unit of Light By J S Preston Pp iv+32+4 plates (London H M Stationery Office, 1961) 3*s* net [910

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POLITICS AND EDUCATION IN GHANA

RECENTLY, three Cabinet Ministers of Ghana—Mr. Adamafio, Minister of Information and Broadcasting, Mr Duwuona-Hammond, Minister of Education, and Mr Boateng, Minister of the Interior—have issued warnings to the students of Ghana's two Universities concerning their "arrogance and anti-State activities" which, so the Ministers declare, have been inherited "from a colonial past". We believe that anyone, student or otherwise, who indulges in serious anti-State activities should be warned and, if necessary, punished; but there is all the difference between anti-State activity and open opposition to the political policy of those in power—at any rate in a democracy Moreover, to accuse the students, or anyone else, of arrogance strikes one as being somewhat naive—even childish

Recently, Osagyefo Dr Kwame Nkrumah, President of the State of Ghana, was installed as Chancellor of the University of Ghana, near Accra, and on November 29 he was also installed as Chancellor of the Kwame Nkrumah University of Science and Technology at Kumasi We understand there was an obvious lack of enthusiasm among the students during both these ceremonies, which angered the President Though one cannot conceive of such resentment being demonstrated in such countries as the United States or Great Britain, the President's annoyance can be readily understood, though to appoint one and the same man as Chancellor of the only two Universities in a country is unique and risky, and in this case the objective seems very clear, it bodes ill for any semblance of autonomy in either University

Wearing of academic gowns has been banned, and apparently stern measures, including the dismissal of some lecturers known to be "instilling in their students an attitude of disrespect for the President", are to be taken The first policy strikes one as being absurd, and seems to be aimed against any attempt to follow the custom of many universities in the more highly developed countries The second decision is more serious Students are notoriously interested in politics, and are surely old enough to think for themselves, which is the very basis of a university education. It is a student's responsibility to think, but if as a result of such thinking he threatens to be a bad citizen, then he should be dealt with accordingly by his immediate superiors, that is, the university authorities and not those outside the university who enjoy political power

All three Ministers have threatened that any students who "misbehave would be thrown out". This warning, too, should come from the university authorities and not from the State This appears to be an intolerable position for any university, for it seems that those responsible for the running and development of the university are to be deprived of an important part of their academic responsibility. It should be they, and only they, who should take to task their students for breaches of university discipline (including, if necessary, expulsion) and not any outsider, not even a Minister of State

The Minister of Information and Broadcasting in Ghana has announced that from now on the Council of the University of Science and Technology will be directed by the Government in the running of that institution so that the Government "might turn out men and women who have unflinching loyalty to the office and person of President Nkrumah". If this is to be taken literally, it savours too much of undesirable dictatorship This alarming statement implies at least three conditions (1) appointments to the staff must meet with the approval of the Government in power, (2) entry of students to that University will be conditional on (among other things) political grounds, (3) the University staff and their authority and the students will be subject to the whims and fancies of the Government

There can be no autonomy in such an institution, no freedom of thought, expression and action, no development of democratic ideals In fact, if the threats and warnings made by certain Ministers of State in Ghana are carried out, the University of Ghana, and certainly the University of Science and Technology, will not be universities at all

On December 4, Mr Duwuona-Hammond, the Minister of Education, addressed three hundred Ghana school teachers and told them that they must show loyalty to "the ideals of the State and of its chief architect, President Nkrumah" He proceeded to say "Nobody resents fair comment, but systematic, spiteful and irresponsible criticism calculated to undermine the Government cannot be accepted as sincere teaching" The second statement would be acceptable to all educationists, but it will be understandable, in view of the first statement, if they suspect why it was made

Ghana is an emergent country It has fairly and justifiably won its independence But it has yet much to learn, and during the past few decades the University of Ghana has been learning and developing with the help of academic men and women from the world's older universities—especially those of Great Britain In Britain, the universities are proud of their autonomy and guard it with justifiable jealousy, though, in spite of the fact that they are increasingly dependent on State funds, they suffer no threat of State interference in the way they are conducted and the manner in which they train their students. Moreover, much to their credit, successive Governments have shown no tendency to influence the universities so far as policy is concerned In fact, university students are encouraged to think for themselves, and, within reason, are entitled to their own political views and to express their opinions of Government and others in political power. Ghana would be wise to take note of this

ELECTRON MICROSCOPY

Proceedings of the European Regional Conference on Electron Microscopy

Delft, 1960 Edited by A L Houwink and B J Spit Vol 1 Pp i–xvi + 1–608 Vol 2 Pp xvii–xxvii + 609–1075 (Delft· Nederlandse Vereniging voor Electronenmicroscopie, 1961) 135 florins the two volumes

It is very appropriate that the 1960 Regional Conference on Electron Microscopy should have been held at Delft, as was the first Conference in 1949 Delft is in a very real sense the birthplace of microscopy, and it is a fitting tribute to Antony von Leeuwenhoek, possibly the most acute of all microscopists, and a happy gesture that the covers of these two volumes should be impressed with an image of one of his microscopes The twelve years which have elapsed since the first Conference have witnessed spectacular advances in this newer form of microscopy, both in technique and in the number of workers using the instrument It could scarcely have been imagined, for example, that stemming from the first ultra-thin section demonstrated at Delft by Brettschneider in 1949, the use of sectioning for electron microscopy would by 1960 have become so routine a matter as to be in regular use in a multitude of electron microscope laboratories Indeed, what impresses the reader of these volumes the most is both the range of materials which can be examined by this means and the extremely rapid, even explosive, burst of interest developed during these few years

The books comprise reports of 246 articles presented at the Conference, copiously illustrated by electron micrographs They are not therefore intended to be read, in the sense that text-books are read, but rather to be used as works of reference The first volume, containing 138 articles, deals in the main with instrumentation, including an article on specimen techniques, with crystal physics and with metallurgical and other industrial applications The second, of 108 articles, is devoted exclusively to biology The leading article in Volume 1 deals with electron optics, leading naturally to a discussion of developments both in electron microscopes and in methods of use The articles on solid-state physics (including crystal growth and lattice defects) are perhaps especially notable In Volume 2 the cytology of muscle, skin, bone, sense organs, spermatozoids, and viruses all receive due attention It is somewhat disappointing to see that while animal cytology is represented by a hundred articles, plant cytology accounts for only eight

These two books together cover much of the field as explored in 1960 Even a cursory glance through the list of contents, however, reveals the absence of some names already famous in the field of electron microscopy and some lines of research, both well established and developing, which are not represented This is clearly a fault neither of the organizers of the Conference nor of the editors, and explanation is surely to be sought on other and more fundamental lines One contributory factor is that conferences on electron microscopy by their very nature meet only part of the needs of electron microscopists Few workers are specifically interested in the instrument itself or even in their own electron micrographs alone Rather are they interested in the results of electron microscopy as illuminating their own chosen fields and as contributing to the solution of problems often already stated before the advent of this new technique and of interest also to non-microscopists They therefore both receive and confer more benefit by discussions in conferences arranged by their own professional bodies, before audiences acquainted with and sympathetic to their problems, than by reports to those with whom the only interest in common is the instrument itself While it is clearly satisfactory to electron microscopists that they should form societies, and hold symposia which attract large numbers of articles, and while it is clearly desirable that electron microscopists should meet from time to time, it is not self-evident that the best interests of the separate sciences are achieved in this way

The books are beautifully produced on excellent paper and the illustrations could in most cases scarcely have been better Bibliographies are attached to each article, and each volume carries a complete author index Both the publishers and the editors are to be complimented on this work It is notable, moreover, that in these days of publication delays, the books have appeared in less than one year from the date of the Conference, it is not easy to see how this herculean task has been performed and the editors are to be highly commended on it

Anyone who wishes to acquaint himself with the situation in electron microscopy up to 1960 could do no better than to read these two volumes.

R D Preston

FLORA OF THE ZAMBEZI BASIN

Flora Zambesiaca

Mozambique, Federation of Rhodesia and Nyasaland, Bechuanaland Protectorate, Vol 1, Part 2 Edited by A W Exell and H Wild Pp vi + 337–581 (London Crown Agents for Oversea Governments and Administrations, 1961 Published on behalf of the Governments of Portugal, The Federation of Rhodesia and Nyasaland, and the United Kingdom) 25s

The second part of the first volume of *Flora Zambesiaca* has made its welcome appearance The part completes Volume 1, for which an index and title-page are provided It covers the flowering plants from family 21, Caryophyllaceae to family 30, Sterculiaceae arranged in the Bentham and Hooker sequence The joint editors are Mr A W Exell of the British Museum (Natural History) and Dr H Wild of the Federal Herbarium, Salisbury, Southern Rhodesia This part, as in the case of the first part, is an excellent example of botanical co-operation, for it contains contributions by botanists working at the British Museum (Natural History), the Federal Herbarium, Salisbury, Southern Rhodesia, the Botanical Laboratory of the Free University, Brussels, the National Herbarium, Pretoria, and the Royal Botanic Gardens, Kew, while the Portuguese Government is represented both on the Managing Committee and the editorial board of this Flora The Flora covers the Federation of Rhodesia and Nyasaland, Mozambique and Bechuanaland Protectorate The part under review contains accounts of 10 families, consisting of 55 genera and 265 species The largest family is the Malvaceae with 16 genera and 111 species in the area while the smallest is the Theaceae with one monotypic genus *Ficalhoa*, alone The plants included range from small annual herbs with inconspicuous flowers such as *Bergia* (in the Elatinaceae) to large forest trees, 30 m high, such as *Sterculia subviolacea* (in the Sterculiaceae), and the massive baobab, known scientifically as *Adansonia digitata* (in the Bombacaceae), so familiar to travellers in the drier parts of eastern and southern Africa.

The diagnostic keys, provided to distinguish the genera, species and in some cases the varieties, show signs of having been carefully prepared, and should be of great assistance to the user. The 46 plates, drawn by eight different artists, are of a high standard and add greatly to the helpfulness of the book, while the coloured frontispiece, depicting *Hibiscus rhodanthus*, is a pleasing addition.

The treatments adopted are in some cases interesting. The Hypericaceae merged with the tropical family Guttiferae will surprise botanists familiar with the temperate genus *Hypericum* alone. The placing of *Ficalhoa* in the Theaceae is now rather widely supported, as one has long felt unhappy about its being in the Ericaceae. The acceptance of the Illecebraceae as a family distinct from Caryophyllaceae shows how much the personal opinions of botanists enter into taxonomic decisions. Only recently, in another African regional Flora, these two families were treated as one.

At the generic level, *Hibiscus esculentus*, the young fruits of which are the well-known tropical vegetable okra, is placed in the genus *Abelmoschus*, while the tree widely spread throughout the area and hitherto known as *Thespesia garckeana* finds itself in the unfamiliar genus *Azanza*.

When one comes to species, one finds that a more conservative view has been taken, and a number of familiar names appear in synonymy. This tendency to accept a broader view of a species is very welcome and can at least partly be attributed to the taxonomists knowing the plants in the field and learning at first hand how greatly climatic, seasonal and edaphic conditions can modify them. It will undoubtedly make the Flora easier for the public to use.

The production is excellent, and the authors and editors are to be congratulated on the high standard of this much-needed work.

E. MILNE-REDHEAD

A BOTANICAL PANOPTICON

A New Illustrated British Flora

By Dr. Roger W. Butcher. Part 1: Lycopodiaceae to Salicaceae. Pp. viii + 1016. (London: Leonard Hill (Books), Ltd., 1961.) 84s. net.

THERE was a time when every aspiring student of British plants first groped his way through "Bentham and Hooker" and then, with growing confidence, broached the more esoteric contents of "Butcher and Strudwick". The fact that it was generally considered unnecessary to mention the precise titles of either work was proof not only of the general popularity of the *Handbook of the British Flora* and *Further Illustrations of British Plants*, but also of the almost legendary fame of their respective authors. Dr. Butcher showed keen psychological insight in naming his book *Further Illustrations*, for, to be quite honest, most beginners and amateurs valued pictures more highly than text, and many professional botanists, now ageing and erudite, would, under pressure, confess that it was the genius of Walter Fitch and the urbane lucidity of Florence Strudwick, rather than the arid exactitudes of botanical prose that guided their first steps along the rocky road of taxonomy.

Is the same true nowadays? Do young naturalists still turn to Fitch and Strudwick for assistance? Or has modern youth inherited such bumps of scientific understanding that it can imbibe Clapham, Tutin and Warburg as mother's milk and advance to erudition via chromosomes and histograms? I do not think so—nor evidently does Dr. Butcher—for his *New Illustrated British Flora* differs in no material respect from his earlier book on British plants. It is *Further Illustrations* expanded to include the entire range of native British phanerogams and vascular cryptogams, as well as many of the more widely established colonists, denizens and aliens. Almost the only concession to the 'new look' in British botany is the departure from the Bentham and Hooker sequence of families in favour of the Clapham, Tutin and Warburg scheme, and even here Dr. Butcher accepts the latest of phylogenetic vagaries with such ill-concealed reluctance that one wonders what irresistible forces compelled him to take this course.

The sad fact that Florence Strudwick did not live to prepare the illustrations for this new work has resulted in a loss of uniformity in the style and quality of the illustrations, though it should be added that at least two of the ten artists employed have attained Miss Strudwick's immaculate clarity and firmness of line, and that, on the whole, the 885 drawings in this first volume are perfectly satisfactory. The same, alas, cannot be said for the reproduction, perhaps in the case of those drawings transferred from *Further Illustrations* because the blocks have deteriorated with age, but less easily explained in other cases. Too many of the illustrations, at least in the review copy, are unduly black and murky, while a few are pallid to the point of anæmia. Something has surely gone wrong with *Equisetum hyemale* (Fig. 10) which, to judge from the scale, appears to be more slender than *E. trachyodon* (Fig. 11)—or should the illustrations be interchanged? The flowers of *Barbarea vulgaris* (Fig. 209) also appear considerably larger than those of *B. verna* (Fig. 212), which is neither in accordance with the facts nor with the accompanying legends.

Such minor slips and blemishes are, however, virtually inevitable in a detailed work of this magnitude. It is more general matters, such as the author's choice of subjects for inclusion in the volume, that are likely to intrigue the critics. Of course one recognizes the futility of wasting space on every fleeting casual and rubbish-tip waif that postures as a British plant, but it is difficult to follow the logic which admits *Hypericum canadense* and excludes *Cerastium brachypetalum*, which allows *Viola kitaibeliana* and disallows *Ranunculus muricatus*, and when one comes to scrutinize the so-called 'critical groups', even the most sanguine must begin to despair. Are there really ten species of *Thalictrum* in Britain—or for that matter six (or seven) *Cochlearias*? It would be unfair to expect Dr. Butcher to solve, at one blow, all the problems which have overtaxed the mental equipment of generations of taxonomists, but one cannot help feeling that if these, and a hundred similar problems in *Juniperus, Caltha, Erophila, Arabis, Viola, Polygala, Sagina, Salicornia*, etc., were to be given serious attention, not by one botanist, but by a group of botanists, prepared to follow up every possible clue, then elucidation would follow. Perhaps not, for it may be that our whole system of taxonomy and nomenclature is too crude and static to encompass the restless mutatings of living things, but at least the effort would be praiseworthy. Besides, it is demoralizing for any scientist to have to keep on repeating what he does not wholly believe.

It is sad to find that Dr. Butcher has joined forces with those who require a popular name for every unpopular plant: one senses political undertones in 'Red German Catchfly' (*Lychnis viscaria*), redressed perhaps by the fact that *Rumex orientalis* has escaped to become the 'Western Dock' and—it was bound to happen sooner or later—one blushes at the frankly clinical implications of Dickie's Bladder Fern. How fortunate that *Cystopteris dickieana* is such a very rare plant!

Finally, it must be pointed out that the Achlamydeae, Group VI (p. 37) in the mammoth identification key, do not have "both calyx and corolla present".

R. D. Meikle

THE MITES AND TONGUE-WORMS OF AFRICAN VERTEBRATES

The Arthropod Parasites of Vertebrates in Africa South of the Sahara, (Ethiopian Region)

Vol. 1 (Chelicerata). Edited by F. Zumpt. (Publications of the South African Institute for Medical Research, No. 1, Vol. 9.) Pp. vi+457. (Johannesburg: South African Institute for Medical Research, 1961.) R. 6.00.

The "onlie begetter" of this handsome volume was undoubtedly the *Check List* of the late G. A. H. Bedford, published during 1927–36, a fact which is acknowledged in the first sentence of its introduction; it would perhaps have added a further touch of graciousness if it had been made clear that the very form of the present compilation is that inaugurated by Bedford—keys, figures, method of specific citation, sections, notes and even the general typography. Bedford's achievement was a great one, and to admit it to be so does not in the least diminish one's admiration for the extreme care and competence of all the authors of this, Bedford's successor.

Three decades of research in this field have increased the numerical total of Bedford's *Check List* many times and the present volume adds numerous new and excellent figures and completely new keys. The extreme care which has been taken by all the authors can be seen in the treatment of 'difficult' genera, for example, *Haemolaelaps*. A conservative systematics is evident everywhere, and if some acarologists feel that occasionally this conservative constraint may obscure some important relationships (as in some feather-mite genera), none, at least on the eastern side of the Atlantic, but will welcome the consequently rational treatment such as is accorded the Trombiculidae, in which family the fluidity of present-day generic concepts is frankly recognized and reflected.

The general introductory discussion of arthropod parasitism is succinct and reliable, and, within the obviously set limits which have been imposed, gives a useful survey of the subject for the purposes of the general student; but it leaves unanswered, of course, several problems such as why, for example, there are different patterns in the reduction of the biological cycle of the Acari. In this connexion it might be mentioned that the extremely complicated matter of the evolution of parasitism in the Trombidiformes is clearly if rapidly sketched, but one imagines that there will be several workers who will share my resistance to the suggestion that the Ereynetidae have evolved from "Trombicula-like" ancestors.

It seems ungrateful, given here so much, to ask more, but one could have wished for a system of cross-references between the bibliographical citations and the records. This would have made for easy validation of the latter, and although the specialist can perhaps often make an intelligent guess, the non-specialist has the labour of working systematically through all the entries. *Optat ephippia bos piger, optat arare caballus.*

F. A. Turk

FIFTY YEARS OF STEDMAN

Stedman's Medical Dictionary

Twentieth edition, completely revised. Pp. xlvii+1680+31 plates. (London: Baillière, Tindall and Cox, Ltd., 1961.) 110s.

Any published work which has run into its twentieth edition has proved its worth. In 1911, Thomas Lathrop Stedman compiled the first edition of his medical dictionary which, compared with the latest, was relatively so simple that he needed no collaborators.

When the present work was projected, a team of experts in the various phases of medical lexicography was brought together to act not as advisory consultants but as working partners. These twenty-three consulting editors include such names as Prof. A. I. Lansing, C. D. Leake, and N. B. Taylor, and are supported by outstanding colleagues in the fields of dentistry and pharmacology. Together they have scrutinized each entry in the nineteenth edition, re-defined many thousands of them and inserted a vast number of new ones. Each entry carries with it, if necessary, a reference to the appropriate anatomical or pharmacological nomenclature, while tables are used for many sorts of entries, for example, methods, operations, diseases, anatomical structures and pharmacological terms.

One difference between this and other medical dictionaries is that it carries many dental terms; the volume will be equally useful to dental practitioners and students as to members of the medical profession. Many entries also relate to veterinary science. Obsolete words and expressions, duplications and repetitions have been eliminated while terms relating to botany, homœopathy, eclectic medicine, life insurance and electricity are also omitted unless they have medical applications.

Such wholesale alteration has meant a resetting of the entire work. The publishers have consequently used a new type face and arrangement which make the entries much easier to find and read. The pages are larger and there are many more of them. Pronunciations are given only where the editors consider English-speaking readers might have difficulties. With the vastly improved illustrations, the high-quality paper and the sturdy binding, *Stedman's Medical Dictionary* in its twentieth edition is so new that it resembles the first one only in bearing the name of Stedman. Although prepared in the United States, the producers had all English-speaking readers in mind during the production and the dictionary can be recommended to all who read English as remarkable value for money.

K. M. Hawkins

Handbuch der Papierchromatographie

Herausgegeben von I M Hais und K Macek Band 2 Bibliographie und Anwendungen Pp xxiv+726 (Jena Gustav Fischer Verlag, 1960) 44 D M

IN their introduction, the editors state that as it was difficult to incorporate a comprehensive bibliography on paper chromatography in their first volume on principles and techniques, they decided to do this separately in a second volume This book contains more than 10,000 references (about four times as many as in Volume 1) and the arrangement follows roughly the same pattern as that of the subject-matter of the first volume. The references cover the period up to 1956, each is numbered and each title is given in full if the original language is English, French or German, otherwise the English or German translations as they occur in the recognized abstracts in these languages are given The literature is covered very thoroughly, and there is probably no publication of any importance or interest about fundamentals or applications which has been missed The systematic sectional arrangement, cross-references and comprehensive indexes of subjects and authors make for easy location of references to any desired topic in which paper chromatography has played a part

R CONSDEN

Précis de Climatologie

Par Prof Ch P Péguy Pp iv+348+3 planches (Paris Masson et Cie , 1961) Broché 42 N F , Cartonne toile 52 N F

THE studies on various aspects of physical geography and related sciences published since 1958 by Masson et Cie have gained wide acceptance as soundly based, timely and authoritative and the *Précis de Climatologie* maintains well the standards set by earlier volumes of the series Prof Péguy approaches his topic on a global scale, and is also actively concerned to demonstrate the practical applications of climate and weather to human and vegetal life His method is first to discuss the elementary physical principles involved in climate: atmospheric radiation and circulation, temperature, humidity, and air mass characteristics, then to show the interrelations of these elements both on a local scale and for Earth as a whole; and next, to consider the effects of these elements on soils, and plant and animal life (especially human) Lastly, present-day theories are reviewed with, on occasion, evaluation by the author of differing approaches to a particular problem—for example, by Sorre and Koppen in the matter of thermal zones

This treatment has the important advantage of subordinating theory and basic principles to interrelationships and effects within the natural environment, and thus Prof Péguy's study is useful not only for geographers but also for the agronomist, botanist, planner and engineer concerned with problems of land development Recent advances and trends within the subject can be treated at some length as a logical culmination frontal and air-mass analysis, evapo-transpiration, rainfall dispersion, intensity, and efficacy, and the concept of water-budgets There are also sections on climate classification and fluctuation

With such an approach there can, however, be considerable risk of over-simplification and superficiality, especially when allied to a concise, near-didactic style of presentation This the author in general avoids, due to extensive use of numerical examples, graphs, and a rigid statistical treatment of material Too often in climatological treatises there is an insufficient factual basis—this is by no means the case here although in one or two rare instances (for example, treatment of geostrophic forces and discussion of frontal surfaces) greater detail and clarity might have been attained One might also have expected more than four pages on micro- and local-climates, especially in view of the exhaustive discussion of such matters as angle of incidence of the solar beam

These, however, are very small matters when viewed alongside the many and solid merits of Prof Péguy's work Here in one volume we have a spread of modern climatological topics so far not brought together (at least in English), with a wide range of examples from the work of research workers both in Europe and America, completed in many instances by a review of future research possibilities The work is a distinct advance in the subject, and is a demonstration of geographical techniques applied soundly to scientific material

W B FISHER

Wunderwelt Kakteen

Von Curt Backeberg Pp 242 (176 Abbildungen) (Jena Gustav Fischer Verlag, 1961) 19 50 D M

THIS is one of the most entertaining books that the reviewer has read for a very long time Dr. Backeberg is possibly the world's foremost authority on the classification of the Cactaceae and has published six large volumes on the taxonomy of the numerous species, magnificently illustrated and containing a wealth of critical judgment But Dr Backeberg is not concerned with taxonomy in the book that lies before us Here we are introduced to the Cactaceae, not in the arid fashion of a botanical treatise, but most entertainingly to what he calls the "miracle world" of these extraordinary plants The first miracle is how they manage to exist in some of the most arid and inhospitable parts of the Earth The second miracle is the amazing variety of shape, size and characteristics developed A glance at the family members shows that in size the species vary from huge specimens of several tons weight to minute marble-size prickly spheres, in shape they may be cylindrical, spherical, erect, simple or branched, the surface may be smooth, wavy or furrowed, with or without a fearsome armament of spines, the flowers, whether large or small, night- or day-flowering, are, by any standards, beautiful, the flesh of some species yields food and drink, and the notorious drug mescalin can be obtained from another

Many additional uses are mentioned, one macabre, some utilitarian, some humorous The Aztecs slaughtered their human sacrifices on a bed made from the cut stems of one of the larger specimens The frame-work of a hut can be made from the stems of other species "Cactus fiends" in California decorate their clothing with cacti while "Miss Cactus" wears a spiny crown These prickly plants are a boon to the caricaturist, for the man who sat down on a cactus can scarcely fail to raise a smile Romanticists have even written poetry about them, and such is their attraction that "out and out" fiends will even descend to barefaced theft to acquire a rare specimen

The book is beautifully illustrated, beautifully printed, a credit to Dr Backeberg and, let us add, worthy of the Cactaceae

N L BOR

SMITHSON TENNANT, 1761-1815

By Dr. A. E. WALES
Principal, Burnley Municipal College

SMITHSON TENNANT was born at Selby, Yorkshire, on November 30, 1761, and christened in the Abbey Church there on December 3. His father, the Rev. Calvert Tennant, was the younger son of Edward Tennant of Mount Parke Farm, Wensley, and a Fellow of St. John's College, Cambridge. His mother was Mary, the only surviving daughter of William Daunt, apothecary of Selby. The Rev. Calvert Tennant's sister, Alice, married George Dinsdale of Nappa Hall, Wensleydale, and her daughter Alice married Christopher Pryme of Hull. The son of this marriage was George Pryme (1781–1868), who held the professorship of political economy at Cambridge, while Tennant was the professor of chemistry there (1813–15).

Tennant lost his father when he was ten years old, and his mother, by a fall from her horse while they were out riding in 1781, when he was twenty. During the intervening period, he attended the grammar schools of Scorton, Tadcaster and Beverley, in all of which he was fortunate to come under the influence of progressive and enlightened headmasters. It was during this period that the foundations of his later life as a natural philosopher were laid, for in addition to a good grounding in the classics, mathematics and French, the early interest in science which resulted from studying his grandfather Daunt's books was further encouraged by attendance in Tadcaster at the lecture of a Mr. Walker, an itinerant lecturer on popular science, and by the full use which he made of the excellent library at Beverley Grammar School, in which he found and eagerly studied a copy of Newton's *Treatise on Optics*.

His mother encouraged his growing love for science, and in 1781 plans were made for him to live as a pupil with Joseph Priestley. These, however, fell through and instead, just after his mother's fatal accident, Tennant entered the University of Edinburgh ostensibly as a medical student, but in reality so that he could study chemistry under Black. His notebooks of Black's lectures on chemistry, those of Munro Secundus on anatomy and surgery, and of Walker on natural history are still in existence. At Edinburgh, Tennant came into intimate relationship not only with Black but also with Hutton the geologist, Playfair the mathematician and Webb Seymour and his friends, the Horners, and it may be that Tennant performed experiments under the direction of Black and Hutton designed to support the latter's theory of rock formation. Tennant's stay in Edinburgh and especially his close association with Black profoundly influenced his attitude to science, confirming him in his choice of chemistry as the sphere of his life's work and leading him to accompany Black about 1790 as among the very first British scientists to accept in full the implications of the antiphlogistic theory.

Tennant left Edinburgh in 1781 and entered the University of Cambridge, again as a medical student, first as a pensioner at Christ's College and then, in 1782, as a Fellow Commoner of Emmanuel College. In an age when Fellow Commoners were noted more for their profligacy than for studious habits, Tennant's dedication to the pursuit of scientific inquiry is outstanding, especially as he was now in complete control of his own fortunes. Tennant's close friends at Cambridge included Sir Busick Harwood, who occupied the chair of anatomy during 1785–1814 and was Downing professor of medicine during 1800–14, Isaac Milner, who became the first Jacksonian professor in 1793 and to whom Tennant lent his notes of Black's lectures on chemistry, Dr. Edward Waring, the Lucasian professor of mathematics, Whitehall Whitehall Davies, a close family friend of his boyhood, Thomas Smith, later of Easton Grey and friend of Ricardo and Malthus and John Whishaw, later the "Pope of Holland House", confidant of the most influential of Whig politicians and Tennant's lifelong correspondent. Both Milner and Waring sponsored his election to the fellowship of the Royal Society in 1785 when he was twenty-four, and all of them remained his close friends throughout his life. In 1788, he "kept his Acts" for the degree of M.B., and a historian of the period who knew him in College remarks that this Act and that for his M.D. in 1796 were "of the highest order though marked by great eccentricity".

In addition to his preoccupation with items of chemical research, Tennant, while at Cambridge, began his foreign travels. In 1784 he visited Denmark and Sweden with the object of becoming personally acquainted with Scheele, Gahn and Berzelius. The visit stimulated his interest in geology and mineralogy, especially as he learnt from Gahn the use of the blowpipe in mineral analysis and soon became an acknowledged expert. In 1786 he paid a visit to France, Holland and the Lowlands, where he met Berthollet, Delametherie and very probably Lavoisier. This tour enlarged and deepened his interest in political economy, a subject which fascinated him for the rest of his life. A further European tour in 1793 took him to Switzerland, where he met Gibbon, then living out his last years at Lausanne. Returning via Italy, Germany and France, he remarked that most of the German chemists he had met appeared still to be preoccupied with the search for the 'philosopher's stone'. About this time, Tennant was asked to stand as a candidate for the Jacksonian professorship at Cambridge, but withdrew his name later. F. J. H. Wollaston was eventually elected.

Shortly after taking his degree in 1788, Tennant left Cambridge to reside in London at 4, Garden Court, Temple, and began to engage in chemical research, to attend meetings of learned societies and to build up a wider and wider circle of friends and acquaintances among the scientists, political economists and political reformers of the day. He never practised medicine, although he seems to have been very much attracted to the works and opinions of Sydenham and the chemical aspects of disease. His personal fortune, his love of independence and hatred of restraint, his sensitive nature and benevolent personality stifled in him any desire to undertake the drudgery of the life of a practising physician, and added to this there was the growing success attending his private researches. Between 1791 and 1797 he read papers before the Royal Society on the decomposition of fixed air, the nature of diamond and the action of nitre on gold and platina, all showing his growing mastery of research methods and in concept and design and showing, too, the strong influences of Black and the Edinburgh school of chemistry, the new ideas of chemical philosophy originating in the French school under Lavoisier and bearing, within the results, the seeds of greater triumphs in the future.

At this period of his life, Tennant's situation was indeed an enviable one. Rich enough to follow his own inclinations, he was at peace with himself because of his decision to devote himself to chemical research, was well established in London as a scientist of repute, and by the charm and benevolence of his personality was the centre of a widening circle of intellectual and influential friends. John Whishaw had come to live near him and through his friendship Tennant moved among the members of the Whig party at Holland House, while Francis Horner lived very near him in the Temple. The only ill-fortune was a gradual decline in his health, and even this turned out for the best, for, on being urged to cultivate more interests, he took up horse riding, in which he had not indulged since his mother's tragic death. This new form of exercise, which he continued to practise for the rest of his life, not only restored his health, but also started him off on a new venture. He became so interested in farming that, after experimenting for a year or two with a few acres of land near Epworth in Lincolnshire, he purchased 500 acres of land newly allocated under the Enclosure Act at Shipham in Somerset. On this land, Tennant built a small country house in which he lived every summer and entertained his friends. His interest in farming was not in growing crops, but in conducting agricultural experiments, and his experiences led to the publication of his paper on the different sorts of lime used in agriculture with special reference to the deleterious effect on crops of magnesium limestone.

So, from about 1800 onwards, Tennant's life moved within two circles, each with its own friends and acquaintances but at times overlapping, one predominantly scientific and the other centred around politics and political economy. The latter included Thomas Smith, and his home at Easton Grey in Wiltshire, which was within easy travelling distance of Tennant's country house. He was a constant visitor at Easton Grey, where he was held in the greatest personal esteem and where he became friendly with Ricardo and Malthus. The political circle also included Whishaw, Warburton, Sharp, Rogers, Dumont, Sir Samuel Romilly, Sir J. Mackintosh, Lord Brougham and Francis Horner, and others who moved among the Whigs of Holland House and belonged to a select dining club called the 'King of Clubs'. Here the talk was of reform and theories of political economy, and Tennant's interest was so aroused that he offered to found a chair of political economy at Cambridge and contemplated writing a treatise which would embody his views on the subject with reference both to Britain and to other European countries.

This increasing preoccupation with political economy and reform did not, however, affect in any way his scientific activities. His paper on the composition of emery published in 1802 gives evidence of his continuing experimental work, but a more important feature of his scientific life is the deepening of his friendship with W. H. Wollaston, which blossomed into active collaboration and partnership during the next ten years. The friendship between the two men began at Cambridge, where Wollaston was a Pensioner and Scholar at Gonville and Caius College from 1782, graduating M.B. in 1787. He, too, attended Milner's lectures on chemistry and was taught the use of the blowpipe in mineral analysis by Tennant himself. During 1787–97 Wollaston was practising medicine in Huntingdon and Bury St. Edmunds, but when he went to London permanently in 1797, in order to pursue the calling of physician, the friendship was renewed and became intimate enough for Wollaston to act as Tennant's assistant during his experiments on the analysis of the diamond in 1797. It may be that this fruitful collaboration was a real factor, as well as the well-known incident at St. George's Hospital, in influencing Wollaston in his decision to give up a career in medicine for one devoted to chemical research. Indeed, there is evidence that Tennant's influence on Wollaston at this period of his life was very profound, for Warburton wrote in his biography of Wollaston that "Wollaston held Tennant's knowledge of the sciences in profound admiration and a few years after this time expressed his despair of ever becoming Tennant's equal as a Chemist".

From at least 1800 until his death in 1815, Tennant was both a financial and a scientific partner of Wollaston, a collaboration which brought fame to both men and success in many spheres of scientific achievement. The most spectacular result of the collaboration was achieved by the successful outcome of the series of experiments, initiated by a further study of Tennant's paper on the action of nitre on platina, which resulted in Tennant's discovery and extraction of osmium and iridium from crude platina, and Wollaston's discovery and extraction of palladium and rhodium. For this discovery Tennant was awarded the Copley Medal of the Royal Society, and Wollaston went on to develop his well-known method of producing malleable platinum and so pure platinum sheet. There is some evidence to suggest that this method may have owed something to Tennant's friendship with Black at Edinburgh. The practical result, however, was that the two men proceeded to make and sell the first platinum stills for use in the manufacture and purification of sulphuric acid. A further successful venture which lasted until 1811 was the manufacture of oxalic acid and oxalates, tartaric and citric acids from argol and lemon juice.

To this partnership, Tennant contributed money, a wealth of scientific acumen and knowledge and some practical skill, but it was Wollaston who contributed more and more of all three as time went on. Indeed, during the partnership, Tennant began increasingly to show something of that trait in his character on which all his friends have commented—the exhibition of a sense of a lack of purpose, a carelessness sometimes amounting almost to indolence which seemed to take possession of him and seemed to them to be akin to that shown by Dr. Black himself. The friendship, however, remained firm and close, because Tennant and Wollaston visited Ireland together in 1805. Tennant repeated the visit in 1806, but this time with W. G. Browne, the celebrated African traveller and explorer, another whose friendship Tennant valued highly until Browne's assassination near Tabriz in 1813. This friendship led to a similar one with the explorer Burkhardt, who died in 1809.

In addition to being a Fellow of the Royal Society and a member of the Royal Institution, Tennant was a founder member of the Askesian Society (1799), renamed the Geological Society in 1806 In 1811, Tennant contributed a short note to the Society on native concrete boracic acid, and in 1813, when he was a vice-president, he delivered a lecture to the Society on the principles of mineralogy. in which he expressed his great indebtedness to the views expressed by Berzelius, who had stayed with him at his farm in 1812

The success of this lecture, and those which Tennant was accustomed to give on chemistry and mineralogy to quite large gatherings of his friends in his rooms at Garden Court, together with his outstanding scientific achievements, convinced his friends of his suitability as a candidate for the professorship of chemistry at Cambridge, and in 1813 he was elected to the chair, following Farish, who had been elected to the Jacksonian professorship

During the remaining two years of his life, Tennant divided his time between Cambridge, London and his farm at Shipham Letters in existence show a ripening friendship between Tennant and Berzelius after the latter's visit to England in 1812, and they throw light, too, on his friendship with the scientists Thomas Young, Marcet, Dr McMichael and Sir James Sebright

Tennant was involved in an interesting experience which may have had later important repercussions An explosion occurred in a gas-holder belonging to the Gas Light Co in 1813 In 1814, a committee from the Royal Society was charged with investigating the matter, and, Tennant, who was a member of the Council of the Society, and another (Wollaston ?), after a great deal of experimental work, showed that a flame will not pass through thin tubes and ignite an explosive mixture. Davy repeated these experiments in the following year and from the results discovered the use of gauze in the manufacture of the safety lamp Dr Marcet, writing to Berzelius in 1816, implies that Davy knew of Tennant's previous work but Davy always denied this

Tennant's election as professor of chemistry at Cambridge gave great satisfaction to all concerned and had a stimulating effect on Tennant himself Because of his scientific reputation and ability, and his personal qualities, it was confidently expected that he would establish and build up a notable school of chemistry in the University, and, but for his untimely death, there is every reason to think that this would have happened. His first course of lectures was attended by large audiences who were entertained and instructed by a masterly exposition of chemical principles illustrated by many experiments both of his own devising and also supplied by his many scientific friends. Among his first students were Charles Babbage and J W H Herschel During his short tenure of the professorship, Tennant revived an old interest in galvanism and built a much-improved voltaic battery embodying several unique features which he used with great success in his lectures He also developed a new method of manufacturing potassium and published the results of his very first piece of experimental research on double distillation It is now known that on the eve of his last Continental tour in 1814 he had completed two further pieces of research, one on the use of oxalic acid in the formation of sugar from starch and the other on the existence, extraction and purification of iodine from marine sources. He had also come to the conclusion that aragonite and calcite were dimorphic states of calcium carbonate, and he contemplated the publication of what would have been an important step in crystallography.

In September 1814 Tennant sailed for France, with Marcet and Wollaston, to enjoy a Continental holiday After touring southern France, meeting Berthellot and Berard in Marseilles and staying with scientific friends in Paris, he reached Boulogne on his way home on February 22, 1815 Strong winds prevented a boat from leaving the harbour and Tennant, accompanied by Baron Bulow, went riding on the sands While inspecting a small fort, a bridge over a deep ditch gave way under Tennant's horse, and as a result of this accident Tennant died in Boulogne hospital and was buried in Boulogne cemetery

So far as is known, no likeness of Tennant exists, but it is said that in his features he closely resembled the philosopher Locke He never married and seemed quite indifferent to female society Of his influence on the scientific events of his day, there can be no doubt of its prime importance, not only because of his own personal contributions, but also because of his influence on the work of other scientists, notably Davy and Wollaston—an influence which does not seem to have been adequately recognized or appreciated Of his character and personality, some words written by Brande and some by Dyer may be quoted Brande wrote in his *Manual of Chemistry* "Mr Smithson Tennant was a profound philosopher and a matchless companion His learning was without pedantry, and his wit without sarcasm He was deep but always clear, gentle but never dull. To those who knew him not, it is scarcely possible to offer an adequate representation of his singularly pleasing and enlightened character by those who enjoyed his acquaintance and partook of his social hours His extent of knowledge, his happy and unrivalled talent for conversation, his harmless but brilliant flashes of merriment and all his amiable peculiarities can never be forgotten" Dyer, who knew him at Cambridge, wrote "It is pleasing to pay respect to a person whose modesty surpassed his industry, whose love of science was greater than his love of fame and to whom nothing perhaps was wanting to have rendered him one of the most distinguished men of his time except better health and more systematic habits of study"

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GENERAL NATURE OF THE GENETIC CODE FOR PROTEINS

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THERE is now a mass of indirect evidence which suggests that the amino-acid sequence along the polypeptide chain of a protein is determined by the sequence of the bases along some particular part of the nucleic acid of the genetic material. Since there are twenty common amino-acids found throughout Nature, but only four common bases, it has often been surmised that the sequence of the four bases is in some way a code for the sequence of the amino-acids. In this article we report genetic experiments which, together with the work of others, suggest that the genetic code is of the following general type:

(*a*) A group of three bases (or, less likely, a multiple of three bases) codes one amino-acid.

(*b*) The code is not of the overlapping type (see Fig. 1).

(*c*) The sequence of the bases is read from a fixed starting point. This determines how the long sequences of bases are to be correctly read off as triplets. There are no special 'commas' to show how to select the right triplets. If the starting point is displaced by one base, then the reading into triplets is displaced, and thus becomes incorrect.

(*d*) The code is probably 'degenerate', that is, in general, one particular amino-acid can be coded by one of several triplets of bases.

The Reading of the Code

The evidence that the genetic code is not overlapping (see Fig. 1) does not come from our work but from that of Wittmann[1] and of Tsugita and Fraenkel-Conrat[2] on the mutants of tobacco mosaic virus produced by nitrous acid. In an overlapping triplet code, an alteration to one base will in general change three adjacent amino-acids in the polypeptide chain. Their work on the alterations produced in the protein of the virus show that usually only one amino-acid at a time is changed as a result of treating the ribonucleic acid (RNA) of the virus with nitrous acid. In the rarer cases where two amino-acids are altered (owing presumably to two separate deaminations by the nitrous acid on one piece of RNA), the altered amino-acids are not in adjacent positions in the polypeptide chain.

Brenner[3] had previously shown that, if the code were universal (that is, the same throughout Nature), then all overlapping triplet codes were impossible. Moreover, all the abnormal human hæmoglobins studied in detail[4] show only single amino-acid changes. The newer experimental results essentially rule out all simple codes of the overlapping type.

If the code is not overlapping, then there must be some arrangement to show how to select the correct triplets (or quadruplets, or whatever it may be) along the continuous sequence of bases. One obvious suggestion is that, say, every fourth base is a 'comma'. Another idea is that certain triplets make 'sense', whereas others make 'nonsense', as in the comma-free codes of Crick, Griffith and Orgel[5]. Alternatively, the correct choice may be made by starting at a fixed point and working along the sequence of bases three (or four, or whatever) at a time. It is this possibility which we now favour.

Experimental Results

Our genetic experiments have been carried out on the *B* cistron of the r_{II} region of the bacteriophage *T*4, which attacks strains of *Escherichia coli*. This is the system so brilliantly exploited by Benzer[6,7]. The r_{II} region consists of two adjacent genes, or 'cistrons', called cistron *A* and cistron *B*. The wild-type phage will grow on both *E. coli B* (here called *B*) and on *E. coli K*12 (λ) (here called *K*), but a phage which has lost the function of either gene will not grow on *K*. Such a phage produces an *r* plaque on *B*. Many point mutations of the genes are known which behave in this way. Deletions of part of the region are also found. Other mutations, known as 'leaky', show partial function; that is, they will grow on *K* but their plaque-type on *B* is not truly wild. We report here our work on the mutant *P* 13 (now re-named *FC* 0) in the *B*1 segment of the *B* cistron. This mutant was originally produced by the action of proflavin[8].

We[9] have previously argued that acridines such as proflavin act as mutagens because they add or delete a base or bases. The most striking evidence in favour of this is that mutants produced by acridines are seldom 'leaky', they are almost always completely lacking in the function of the gene. Since our note was published, experimental data from two sources have been added to our previous evidence: (1) we have examined a set of 126 r_{II} mutants made with acridine yellow, of these only 6 are leaky (typically about half the mutants made with base analogues are leaky), (2) Streisinger[10] has found that whereas mutants of the lysozyme of phage *T*4 produced by base-analogues are usually leaky, all lysozyme mutants produced by proflavin are negative, that is, the function is completely lacking.

If an acridine mutant is produced by, say, adding a base, it should revert to 'wild-type' by deleting a base. Our work on revertants of *FC* 0 shows that it usually

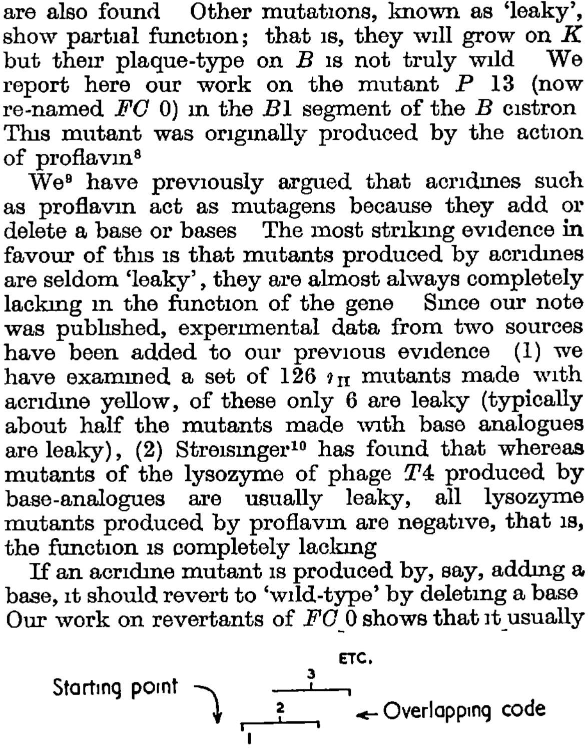

Fig. 1. To show the difference between an overlapping code and a non-overlapping code. The short vertical lines represent the bases of the nucleic acid. The case illustrated is for a triplet code.

reverts not by reversing the original mutation but by producing a second mutation at a nearby point on the genetic map. That is, by a 'suppressor' in the same gene. In one case (or possibly two cases) it may have reverted back to true wild, but in at least 18 other cases the 'wild type' produced was really a double mutant with a 'wild' phenotype. Other workers[11] have found a similar phenomenon with r_{II} mutants, and Jinks[12] has made a detailed analysis of suppressors in the h_{III} gene.

The genetic map of these 18 suppressors of *FC* 0 is shown in Fig. 2, line *a*. It will be seen that they all fall in the *B*1 segment of the gene, though not all of them are very close to *FC* 0. They scatter over a region about, say, one-tenth the size of the *B* cistron. Not all are at different sites. We have found eight sites in all, but most of them fall into or near two close clusters of sites.

In all cases the suppressor was a non-leaky *r*. That is, it gave an *r* plaque on *B* and would not grow on *K*. This is the phenotype shown by a complete deletion of the gene, and shows that the function is lacking. The only possible exception was one case where the suppressor appeared to back-mutate so fast that we could not study it.

Each suppressor, as we have said, fails to grow on *K*. Reversion of each can therefore be studied by the same procedure used for *FC* 0. In a few cases these mutants apparently revert to the original wild-type, but usually they revert by forming a double mutant. Fig. 2, lines *b*–*g*, shows the mutants produced as suppressors of these suppressors. Again all these new suppressors are non-leaky *r* mutants, and all map within the *B*1 segment for one site in the *B*2 segment.

Once again we have repeated the process on two of the new suppressors, with the same general results, as shown in Fig. 2, lines *i* and *j*.

All these mutants, except the original *FC* 0, occurred spontaneously. We have, however, produced one set (as suppressors of *FC* 7) using acridine yellow as a mutagen. The spectrum of suppressors we get (see Fig. 2, line *h*) is crudely similar to the spontaneous spectrum, and all the mutants are non-leaky *r*'s. We have also tested a (small) selection of all our mutants and shown that their reversion-rates are increased by acridine yellow.

Thus in all we have about eighty independent *r* mutants, all suppressors of *FC* 0, or suppressors of suppressors, or suppressors of suppressors of suppressors. They all fall within a limited region of the gene and they are all non-leaky *r* mutants.

The double mutants (which contain a mutation plus its suppressor) which plate on *K* have a variety of plaque types on *B*. Some are indistinguishable from wild, some can be distinguished from wild with difficulty, while others are easily distinguishable and produce plaques rather like *r*.

We have checked in a few cases that the phenomenon is quite distinct from 'complementation', since the two mutants which separately are phenotypically *r*, and together are wild or pseudo-wild,

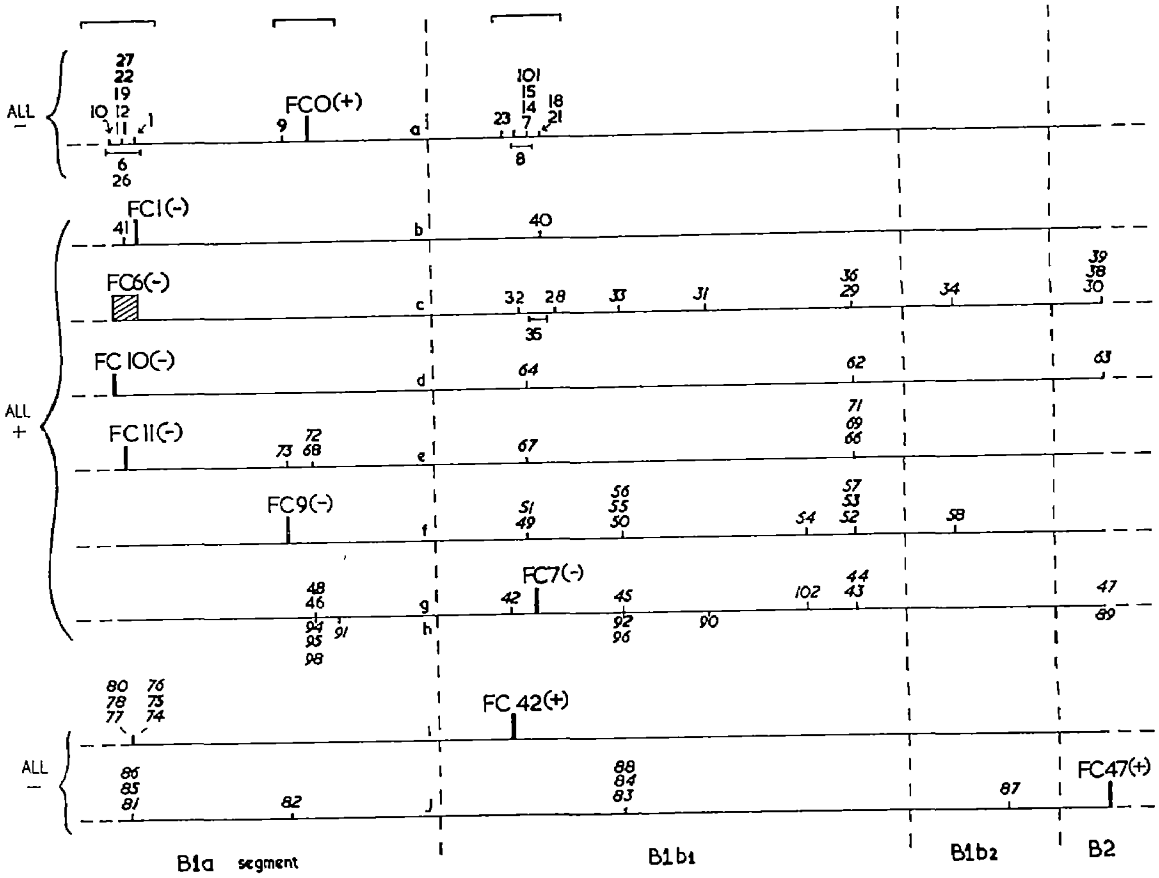

Fig. 2. A tentative map—only very roughly to scale—of the left-hand end of the *B* cistron, showing the position of the *FC* family of mutants. The order of sites within the regions covered by brackets (at the top of the figure) is not known. Mutants in italics have only been located approximately. Each line represents the suppressors picked up from one mutant, namely, that marked on the line in bold figures

must be put together in the same piece of genetic material A simultaneous infection of *K* by the two mutants in separate viruses will not do

The Explanation in Outline

Our explanation of all these facts is based on the theory set out at the beginning of this article. Although we have no direct evidence that the *B* cistron produces a polypeptide chain (probably through an RNA intermediate), in what follows we shall assume this to be so To fix ideas, we imagine that the string of nucleotide bases is read, triplet by triplet, from a starting point on the left of the *B* cistron We now suppose that, for example, the mutant *FC* 0 was produced by the insertion of an additional base in the wild-type sequence. Then this addition of a base at the *FC* 0 site will mean that the reading of all the triplets to the right of *FC* 0 will be shifted along one base, and will therefore be incorrect Thus the amino-acid sequence of the protein which the *B* cistron is presumed to produce will be completely altered from that point onwards This explains why the function of the gene is lacking To simplify the explanation, we now postulate that a suppressor of *FC* 0 (for example, *FC* 1) is formed by deleting a base Thus when the *FC* 1 mutation is present by itself, all triplets to the right of *FC* 1 will be read incorrectly and thus the function will be absent However, when both mutations are present in the same piece of DNA, as in the pseudo-wild double mutant *FC* (0 + 1), then although the reading of triplets between *FC* 0 and *FC* 1 will be altered, the original reading will be restored to the rest of the gene This could explain why such double mutants do not always have a true wild phenotype but are often pseudo-wild, since on our theory a small length of their amino-acid sequence is different from that of the wild-type.

For convenience we have designated our original mutant *FC* 0 by the symbol + (this choice is a pure convention at this stage) which we have so far considered as the addition of a single base The suppressors of *FC* 0 have therefore been designated – The suppressors of these suppressors have in the same way been labelled as +, and the suppressors of these last sets have again been labelled – (see Fig 2)

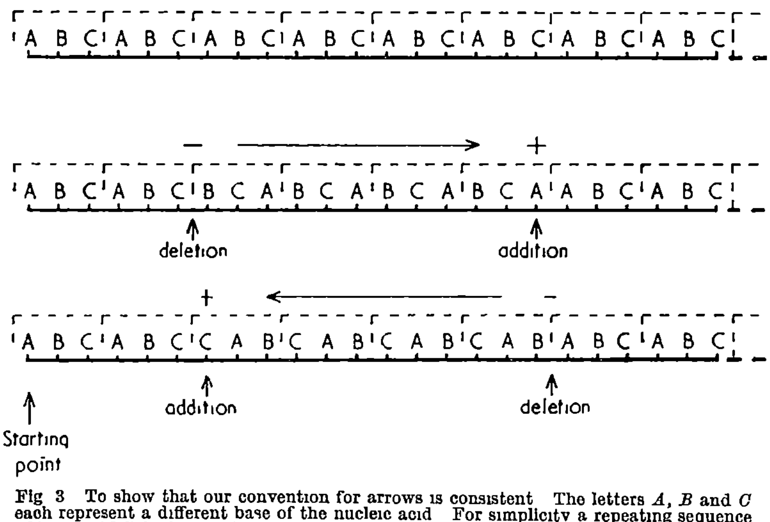
A B C A B C A B C A B C A B C A B C A B C A B C
–
+
A B C A B C B C A B C A B C A B C A A B C A B C
deletion
addition
+
–
A B C A B C C A B C A B C A B C A B A B C A B C
addition
deletion
Starting point

Fig 3 To show that our convention for arrows is consistent The letters *A*, *B* and *C* each represent a different base of the nucleic acid For simplicity a repeating sequence of bases, *ABC*, is shown (This would code for a polypeptide for which every amino-acid was the same) A triplet code is assumed The dotted lines represent the imaginary 'reading frame' implying that the sequence is read in sets of three starting on the left

Double Mutants

We can now ask What is the character of any double mutant we like to form by putting together in the same gene any pair of mutants from our set of about eighty ? Obviously, in some cases we already know the answer, since some combinations of a + with a – were formed in order to isolate the mutants But, by definition, no pair consisting of one + with another + has been obtained in this way, and there are many combinations of + with – not so far tested

Now our theory clearly predicts that all combinations of the type + with + (or – with –) should give an *r* phenotype and not plate on *K* We have put together 14 such pairs of mutants in the cases listed in Table 1 and found this prediction confirmed.

Table 1. DOUBLE MUTANTS HAVING THE *r* PHENOTYPE

– With –	+ With +	
FC (1 + 21)	*FC* (0 + 58)	*FC* (40 + 57)
FC (23 + 21)	*FC* (0 + 38)	*FC* (40 + 58)
FC (1 + 23)	*FC* (0 + 40)	*FC* (40 + 55)
FC (1 + 9)	*FC* (0 + 55)	*FC* (40 + 54)
	FC (0 + 54)	*FC* (40 + 38)

At first sight one would expect that all combinations of the type (+ with –) would be wild or pseudo-wild, but the situation is a little more intricate than that, and must be considered more closely. This springs from the obvious fact that if the code is made of triplets, any long sequence of bases can be read correctly in one way, but incorrectly (by starting at the wrong point) in two different ways, depending whether the 'reading frame' is shifted one place to the right or one place to the left

If we symbolize a shift, by one place, of the reading frame in one direction by → and in the opposite direction by ←, then we can establish the convention that our + is always at the head of the arrow, and our – at the tail This is illustrated in Fig 3

We must now ask Why do our suppressors not extend over the whole of the gene ? The simplest postulate to make is that the shift of the reading frame produces some triplets the reading of which is 'unacceptable', for example, they may be 'nonsense', or stand for 'end the chain', or be unacceptable in some other way due to the complications of protein structure. This means that a suppressor of, say, *FC* 0 must be within a region such that no 'unacceptable' triplet is produced by the shift in the reading frame between *FC* 0 and its suppressor. But, clearly, since for any sequence there are *two* possible misreadings, we might expect that the 'unacceptable' triplets produced by a → shift would occur in different places on the map from those produced by a ← shift.

Examination of the spectra of suppressors (in each case putting in the arrows → or ←) suggests that while the → shift is acceptable anywhere within our region (though not outside it) the shift ←, starting from points near *FC* 0, is acceptable over only a more limited stretch. This is shown in Fig 4. Somewhere in the left part of our region, between *FC* 0 or *FC* 9 and the *FC* 1 group, there must be one or more unacceptable triplets when a ← shift is made; similarly for the region to the right of the *FC* 21 cluster Thus we predict that a combination of a + with a − will be wild or pseudo-wild if it involves a → shift, but that such pairs involving a ← shift will be phenotypically *r* if the arrow crosses one or more of the forbidden places, since then an unacceptable triplet will be produced

Table 2 DOUBLE MUTANTS OF THE TYPE (+ WITH −)

+ / −	*FC* 41	*FC* 0	*FC* 40	*FC* 42	*FC* 58*	*FC* 63	*FC* 38
FC 1	*W*	*W*	*W*		W		W
FC 86		W	W	W	W	W	
FC 9	*r*	*W*	W	W	*W*		W
FC 82	*r*		W	W	W	W	
FC 21	*r*	*W*			W		W
FC 88	*r*	*r*			W	W	
FC 87	*r*	*r*	*r*	*r*			W

W, wild or pseudo-wild phenotype, *W*, wild or pseudo-wild combination used to isolate the suppressor, *r*, *r* phenotype

* Double mutants formed with *FC* 58 (or with *FC* 34) give sharp plaques on *K*.

We have tested this prediction in the 28 cases shown in Table 2 We expected 19 of these to be wild, or pseudo-wild, and 9 of them to have the *r* phenotype In all cases our prediction was correct We regard this as a striking confirmation of our theory It may be of interest that the theory was constructed before these particular experimental results were obtained

Rigorous Statement of the Theory

So far we have spoken as if the evidence supported a triplet code, but this was simply for illustration Exactly the same results would be obtained if the code operated with groups of, say, 5 bases Moreover, our symbols + and − must not be taken to mean literally the addition or subtraction of a single base

It is easy to see that our symbolism is more exactly as follows

+ represents $+m$, modulo n
− represents $-m$, modulo n

where n (a positive integer) is the coding ratio (that is, the number of bases which code one amino-acid) and m is any integral number of bases, positive or negative

It can also be seen that our choice of reading direction is arbitrary, and that the same results (to a first approximation) would be obtained in whichever direction the genetic material was read, that is, whether the starting point is on the right or the left of the gene, as conventionally drawn

Triple Mutants and the Coding Ratio

The somewhat abstract description given above is necessary for generality, but fortunately we have convincing evidence that the coding ratio is in fact 3 or a multiple of 3

This we have obtained by constructing triple mutants of the form (+ with + with +) or (− with − with −) One must be careful not to make shifts across the ‘unacceptable’ regions for the ← shifts, but these we can avoid by a proper choice of mutants

Table 3 TRIPLE MUTANTS HAVING A WILD OR PSEUDO-WILD PHENOTYPE

FC (0 + 40 + 38)
FC (0 + 40 + 58)
FC (0 + 40 + 57)
FC (0 + 40 + 54)
FC (0 + 40 + 55)
FC (1 + 21 + 23)

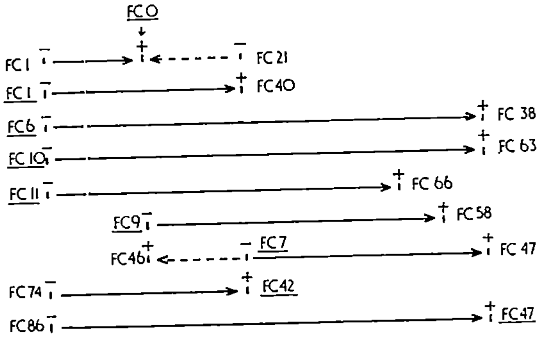

Fig 4 A simplified version of the genetic map of Fig 2 Each line corresponds to the suppressor from one mutant, here underlined The arrows show the range over which suppressors have so far been found, the extreme mutants being named on the map Arrows to the right are shown solid, arrows to the left dotted

We have so far examined the six cases listed in Table 3 and in all cases the triples are wild or pseudo-wild.

The rather striking nature of this result can be seen by considering one of them, for example, the triple (*FC* 0 with *FC* 40 with *FC* 38) These three mutants are, by themselves, all of like type (+). We can say this not merely from the way in which they were obtained, but because each of them, when combined with our mutant *FC* 9 (—), gives the wild, or pseudo-wild phenotype However, either singly or together in pairs they have an *r* phenotype, and will not grow on *K* That is, the function of the gene is absent Nevertheless, the combination of all three in the same gene partly restores the function and produces a pseudo-wild phage which grows on *K*

This is exactly what one would expect, in favourable cases, if the coding ratio were 3 or a multiple of 3

Our ability to find the coding ratio thus depends on the fact that, in at least one of our *composite* mutants which are ‘wild’, at least one amino-acid must have been added to or deleted from the polypeptide chain without disturbing the function of the gene-product too greatly

This is a very fortunate situation The fact that we can make these changes and can study so large a region probably comes about because this part of the protein is not essential for its function That this is so has already been suggested by Champe and Benzer[13] in their work on complementation in the r_{II} region By a special test (combined infection on *K*, followed by plating on *B*) it is possible to examine the function of the *A* cistron and the *B* cistron separately. A particular deletion, 1589 (see Fig. 5) covers the right-hand end of the *A* cistron and part of the left-hand end of the *B* cistron Although 1589 abolishes the *A* function, they showed that it allows the *B* function to be expressed to a considerable extent The region of the *B* cistron deleted by 1589 is that into which all our *FC* mutants fall

Joining two Genes Together

We have used this deletion to re-inforce our idea that the sequence is read in groups from a fixed starting point Normally, an alteration confined to the *A* cistron (be it a deletion, an acridine mutant, or any other mutant) does not prevent the expression of the *B* cistron Conversely, no alteration within the *B* cistron prevents the function of the *A* cistron This implies that there may be a region between the two cistrons which separates them and allows their functions to be expressed individually

We argued that the deletion 1589 will have lost this separating region and that therefore the two (partly damaged) cistrons should have been joined together Experiments show this to be the case, for now an alteration to the left-hand end of the *A* cistron, if combined with deletion 1589, can prevent the *B* function from appearing This is shown in Fig 5 Either the mutant *P*43 or *X*142 (both of which revert strongly with acridines) will prevent the *B* function when the two cistrons are joined, although both of these mutants are in the *A* cistron This is also true of *X*142 *S*1, a suppressor of *X*142 (Fig 5, case *b*) However, the double mutant (*X*142 with *X*142 *S*1), of the type (+ with −), which by itself is pseudo-wild, still has the *B* function when combined with 1589 (Fig 5, case *c*) We have also tested in this way the 10 deletions listed by Benzer[7], which fall wholely to the left of 1589 Of these, three (386, 168 and 221) prevent the *B* function (Fig 5 case *f*), whereas the other seven show it (Fig 5, case *e*). We surmise that each of these seven has lost a number of bases which is a multiple of 3 There are theoretical reasons for expecting that deletions may not be random in length, but will more often have lost a number of bases equal to an integral multiple of the coding ratio

It would not surprise us if it were eventually shown that deletion 1589 produces a protein which consists of part of the protein from the *A* cistron and part of that from the *B* cistron, joined together in the same polypeptide chain, and having to some extent the function of the undamaged *B* protein

Is the Coding Ratio 3 or 6 ?

It remains to show that the coding ratio is probably 3, rather than a multiple of 3 Previous rather rough extimates[10,14] of the coding ratio (which are admittedly very unreliable) might suggest that the coding ratio is not far from 6 This would imply, on our theory, that the alteration in *FC* 0 was not to one base, but to two bases (or, more correctly, to an even number of bases)

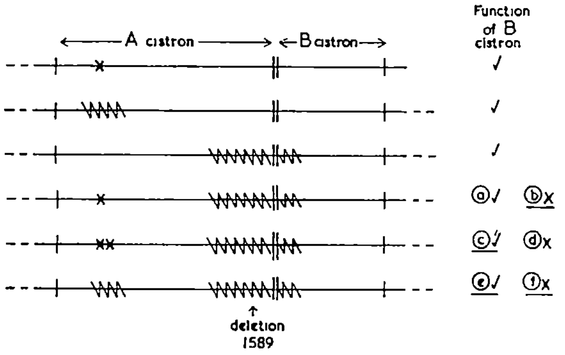

Fig 5 Summary of the results with deletion 1589 The first two lines show that without 1589 a mutation or a deletion in the *A* cistron does not prevent the *B* cistron from functioning Deletion 1589 (line 3) also allows the *B* cistron to function The other cases, in some of which an alteration in the *A* cistron prevents the function of the *B* cistron (when 1589 is also present), are discussed in the text They have been labelled (*a*), (*b*), etc , for convenience of reference, although cases (*a*) and (*d*) are not discussed in this paper ✓ implies function, × implies no function

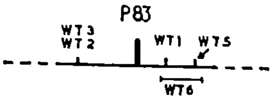

Fig 6 Genetic map of *P* 83 and its suppressors, *WT* 1, etc The region falls within segment *B*9*a* near the right-hand end of the *B* cistron It is not yet known which way round the map is in relation to the other figures

We have some additional evidence which suggests that this is unlikely First, in our set of 126 mutants produced by acridine yellow (referred to earlier) we have four independent mutants which fall at or close to the *FC* 9 site By a suitable choice of partners, we have been able to show that two are + and two are − Secondly, we have two mutants (*X*146 and *X*225), produced by hydrazine[15], which fall on or near the site *FC* 30 These we have been able to show are both of type −

Thus unless both acridines and hydrazine usually delete (or add) an even number of bases, this evidence supports a coding ratio of 3 However, as the action of these mutagens is not understood in detail, we cannot be certain that the coding ratio is not 6, although 3 seems more likely

We have preliminary results which show that other acridine mutants often revert by means of close suppressors, but it is too sketchy to report here A tentative map of some suppressors of *P* 83, a mutant at the other end of the *B* cistron, in segment *B* 9*a*, is shown in Fig. 6. They occur within a shorter region than the suppressors of *FC* 0, covering a distance of about one-twentieth of the *B* cistron The double mutant *WT* (2 + 5) has the *r* phenotype, as expected

Is the Code Degenerate?

If the code is a triplet code, there are 64 (4 × 4 × 4) possible triplets Our results suggest that it is unlikely that only 20 of these represent the 20 amino-acids and that the remaining 44 are nonsense If this were the case, the region over which suppressors of the *FC* 0 family occur (perhaps a quarter of the *B* cistron) should be very much smaller than we observe, since a shift of frame should then, by chance, produce a nonsense reading at a much closer distance This argument depends on the size of the protein which we have assumed the *B* cistron to produce We do not know this, but the length of the cistron suggests that the protein may contain about 200 amino-acids Thus the code is probably 'degenerate', that is, in general more than one triplet codes for each amino-acid. It is well known that if this were so, one could also account for the major dilemma of the coding problem, namely, that while the base composition of the DNA can be very different in different micro-organisms, the amino-acid composition of their proteins only changes by a moderate amount[16]. However, exactly how many triplets code amino-acids and how many have other functions we are unable to say

Future Developments

Our theory leads to one very clear prediction Suppose one could examine the amino-acid sequence of the 'pseudo-wild' protein produced by one of our double mutants of the (+ with −) type Conventional theory suggests that since the gene is only altered in two places, only two amino-acids would be changed Our theory, on the other hand, predicts that a string of amino-acids would be altered, covering the region of the polypeptide chain corresponding to the region on the gene between the two mutants. A good protein on which to test this hypothesis is the lysozyme of the phage, at present being studied chemically by Dreyer[17] and genetically by Streisinger[10].

At the recent Biochemical Congress at Moscow, the audience of Symposium I was startled by the announcement of Nirenberg that he and Matthaei[18] had produced polyphenylalanine (that is, a polypeptide all the residues of which are phenylalanine) by adding polyuridylic acid (that is, an RNA the bases of which are all uracil) to a cell-free system which can synthesize protein This implies that a sequence of uracils codes for phenylalanine, and our work suggests that it is probably a triplet of uracils

It is possible by various devices, either chemical or enzymatic, to synthesize polyribonucleotides with defined or partly defined sequences If these, too, will produce specific polypeptides, the coding problem is wide open for experimental attack, and in fact many laboratories, including our own, are already working on the problem. If the coding ratio is indeed 3, as our results suggest, and if the code is the same throughout Nature, then the genetic code may well be solved within a year

We thank Dr Alice Orgel for certain mutants and for the use of data from her thesis, Dr Leslie Orgel for many useful discussions, and Dr Seymour Benzer for supplying us with certain deletions We are particularly grateful to Prof C F. A Pantin for allowing us to use a room in the Zoological Museum, Cambridge, in which the bulk of this work was done

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SCIENCE AND WORLD AFFAIRS

THE Seventh Pugwash Conference on Science and World Affairs was held at Stowe, Vermont, during September 5–9 Forty-one scientists from twelve countries attended the Conference*

This Conference had as its theme "International Co-operation in Pure and Applied Science". The previous conferences have been chiefly concerned with ways of preventing the misuse of science in the wholesale destruction of mankind In this Conference at Stowe, constructive international co-operation in science was discussed, because it is a way to create trust between nations, a trust which develops from common interests and from experience in working together

Science misused by nations to foster their competitive interests as world powers makes possible the destruction of mankind Science used co-operatively by all nations for the increase of human knowledge and the improvement of man's productive capacity can give all men on Earth a satisfactory and worthwhile life Scientists bear a responsibility both to foster the constructive use of science and to help in preventing its destructive use

The deliberations of the Conference were carried out in plenary sessions and in meetings of working groups Similar suggestions for co-operative research activities arose independently from different working groups, and this is reflected in several places in the statement This is a welcome indication of the essential unity in science The discussions were carried on in a spirit of friendly co-operation, and full agreement was reached by the entire Conference on the suggestions enumerated here

(I) Co-operation in the Earth Sciences

The planet Earth is the common abode of all humans. They have a common interest, both intellectual and practical, in increasing the knowledge of the structure and dynamics of the Earth

The following proposals were made by the Conference

(*A*) *A survey of the entire ocean in three dimensions*

(1) *The ocean floor* An international programme was proposed to develop a detailed map of the floor of the world ocean, including sub-bottom reflecting layers

(2) *Waters of the ocean* An international programme should be devised to survey and map the three-dimensional distribution of temperatures, salinity, density, dissolved oxygen, and nutrient salts, under average conditions, of the ocean and synoptic surveys to develop the broad picture of seasonal and shorter-period changes in more limited areas, as well as the study of the interactions among the major bodies of water in the ocean

(3) *Ocean life* An international survey and mapping showing the major biological provinces of the ocean and determination of the fertility of the waters at all levels in the food chain and the standing crop of food materials available for human use should be undertaken

(*B*) *Earth's crust and mantle Deep drilling programme* The objective of drilling through the

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* Sir John Crawford (Australia), Prof Hans Thirring (Austria), Prof C Pavan (Brazil), Prof G Nadjakov (Bulgaria), Prof G Burkhardt (Federal Republic of Germany), Sir Edward Bullard, Prof A Haddow, Sir Ben Lockspeiser, Prof J Rotblat (Great Britain), Prof F B Straub (Hungary), Dr G Bernardini (Italy), Prof T Toyoda (Japan), Prof B V A Roling (Netherlands), Academician A A Blagonravov, Academician N N Bogolubov, Academician M M Dubinin, Prof V M Khvostov, Academician N M Sissakian, Prof N A Talensky, Academician I E Tamm, Academician A V Topchiev (U S S R), Prof Harrison Brown, Dr William Consolazio, Prof Paul Doty, Prof Bentley Glass, Prof C O'D Iselin, Dr Martin Kaplan, Prof Chauncey Leake, Prof Linus Pauling, Prof Jay Orear, Prof W Pickering, Mr Gerald Piel, Prof I Rabi, Prof Eugene Rabinowitch, Dr Roger Revelle, Prof Alexander Rich, Prof Walter Rosenblith, Dr Eugene Staley, Dr Alvin Weinberg, Prof Eugene Wigner, Prof J R Zacharias (United States)

Earth's crust to the mantle at selected points around the globe presents many unsolved technical problems which call for international collaboration It was agreed, therefore, that the calling of an international conference on these problems is an urgent first step.

(*C*) *Total environmental forecasting* An international conference should be called to consider and to organize the establishment of a world-wide network of radio-telemetering observational buoys This system of buoys would render continuous reports on atmospheric conditions and so contribute to the completion of the world weather map It would also continuously monitor the energy and water vapour energy exchange between the ocean and the atmosphere, and would observe the changes in the flow of ocean currents in three dimensions. Over a sufficiently long time-period, such a network of buoys would help to assign reliable values to the rate of overturn of the ocean as a whole, a key problem in both climatological forecasts and in the safe disposal of radioactive wastes at sea The hydrographic offices of the major maritime nations, as well as the World Meteorological Organization, should be encouraged to stimulate such a programme.

(*D*) *Resources* (1) *Fresh water* The rising world population and the increasing concentration of that population in metropolitan centres is already pressing on the water resources easily available for direct human consumption, for industrial purposes, and for agriculture It was agreed that an international conference should be called to consider the organization of an international hydrological decade for the study of the many unknowns that surround this ominous development

(2) *Living resources of the ocean* Acre per acre the oceans to-day sustain at least as large a plant crop on the average as does the land, yet man now gets only about 1 per cent of his food requirements from the sea The oceans, therefore, offer a means for the rapid solution of the protein deficiency afflicting two-thirds of the world population It is apparent that these resources can best be exploited to this end through international co-operation The ultimate aim should be to elevate the fishing industry from a hunting industry to an agricultural technology

(3) *Mineral resources of the ocean floor* Recent investigations of the ocean floor show a vast reserve of minerals, especially nickel, cobalt, copper and manganese By means of bottom-photography organized on a world-wide basis, the potentiality of this reserve may be more fully assessed The necessary observational programme could be organized by the Scientific Commission for Oceanographic Research.

(4) *Natural catastrophes* Earthquakes, tsunamis, volcanic eruptions, hurricanes and tornadoes constitute the principal hazards of this kind

With respect to the first three, a better international seismological network, including better instrumentation and distribution of stations, is required More detailed studies of hurricanes and tornadoes would reveal whether or not man can exert some control over these phenomena

To implement these programmes, it is recommended that in so far as possible existing international organizations be the means of furthering these programmes

(II) Co-operation in Space Research

Though the Conference felt that there should be close co-operation in space research it should be realized that complete co-operation in this field, as in some others, will become possible only when the arms race is ended, international tensions are reduced, complete and general disarmament becomes a reality, and the need for secrecy disappears But certain advances in the presently established co-operation in space are possible now, and certain others could be studied now with the hope of realization in the not too distant future

(*A*) First, an increase in the exchange of scientific information in areas such as the physics of space and the effects of the space environment on life is recommended It is further recommended that periodic international symposia be devoted to such subjects.

(*B*) The exchange of methods, and of information on instruments, for scientific space studies which have no military importance is also recommended.

(*C*) The orderly assignment and use of radio frequencies in space should be encouraged Strong support should be given to the initiative in this direction taken by the International Telecommunications Union, and it is to be hoped that a final solution can be reached within two years.

(*D*) The expansion of existing systems of satellite tracking stations is recommended Bilateral agreements between different nations, including (within the limits of military security) an agreement between the United States and the U S S R , on the common use of tracking stations; such a bilateral agreement could serve as the first step in this direction.

(*E*) International agreement should be reached on a co-ordinated programme for the use of rocket and satellite during the Quiet Sun Year (planned for the second half of 1962)

(*F*) Similar agreement should be reached on co-operation in the use of rockets and satellites in the projected world magnetic survey. This agreement should include arrangements for simultaneous observation from satellites launched into different orbits by different nations. This co-operation could be organized in the framework of the Committee on Space Research

(*G*) The desirability of international world-wide systems of communications satellites and of meteorological satellites is endorsed by the Conference, since these would clearly be in the interests of all mankind It is realized that certain difficulties now stand in the way, but it is to be hoped that the Governments of the United States and the U.S.S R , as well as of other nations embarking on rocket and satellite programmes, will undertake a common study of the ways to overcome them

(*H*) It is recommended that co-operation should be established in the instrumental study of the Moon, and also that the basic principles of the International Antarctic Treaty be applied to the Moon and other cosmic bodies

(*I*) The calling of an international conference or symposium to consider how to avoid the biological and radioactive contamination of extra-terrestrial bodies is strongly urged

(III) Co-operation in the Life Sciences

Among the many fields of biology in which international co-operation is possible, some are particularly well suited by their nature and importance for combined efforts These, which relate especially to the promotion of human welfare, have been given primary concern by the Conference

(*A*) *Biological aspects of food resources* One of the most important problems facing humanity is that of assuring an adequate supply of food Adequate biological information now exists to enhance considerably food production in low-yield areas. This can be done most effectively by the development of regional agricultural experiment stations, which can deal with the problem of developing agricultural methods suitable to the local terrain, as well as the long-term problem of breeding plants and animals which are able to thrive in the specific locality These local agricultural experiment stations should be co-ordinated with an international centre

It is recommended that existing marine biological laboratories be united into an international system perhaps under the International Union of Biological Sciences, to ensure permanent support and increase in number

(*B*) *Preservation and promotion of health* The health sciences offer one of the most rewarding meeting grounds for international co-operation in science Increased international co-operation and financial support are essential for the realization of important advances in such fields as cancer, cardiovascular disease, immunology. infectious diseases, mental health, environmental sanitation, problems of ageing, nutrition, human genetics, and others

International institutes of health devoted to these problems should be established in one carefully chosen place to serve as a world centre of medical research Smaller subsidiary institutes oriented towards more specialized problems or regional needs (space medicine, medical entomology, tropical medicine) should be established in different countries

(*C*) *The environment and its modification by man* The exponential growth of human populations and the accompanying industrial, agricultural and scientific activities have given rise to a number of serious problems, including pollution of air and water resources, which are of considerable biological importance Especially grave are the problems involving contamination of air, soil and water with radioactive substances Included here, as well as the more obvious problem of fall-out from nuclear explosions, there is the matter of the safe disposal of radioactive wastes More international attention should also be directed toward the problems of chemical pollution of air and water International conferences on these topics should be encouraged

The extinction of many plant and animal species seems possible There are large forest and game reserves in the newly formed African countries which are seriously threatened to-day because of a shortage of funds and trained personnel. This problem has been considered by a committee on ecology in the International Council of Scientific Unions A system of world-wide institutes should be established for preserving indigenous strains of plants, animals and micro-organisms

(*D*) '*Endless frontiers*' There has been a great development recently in our understanding of the structure and function of biological macromolecules and the central role of the nucleic acids and the mechanism which relates the nucleic acids to the protein molecules These developments have a significant bearing on the problems of cancer as well as the broader fields of molecular evolution and the origin of life Intense interest in this field has developed among scientists in all countries, and it may be possible to capitalize on this enthusiasm by developing an intercontinental institute of molecular biology.

Both the United States and the U S S R have announced that they are planning to carry out manned exploration of space It is not unreasonable to suggest that some of the biological developmental work be carried out in common A considerable saving of time and money would ensue from joint research projects in this area In addition, some of the instruments which man uses in space for his scientific investigations could be included in this co-operative programme Joint precautions must be exerted to prevent the contamination of extra-terrestrial bodies by terrestrial organisms

At the present time an International Biological Programme is under discussion by the International Council of Scientific Unions Many of the projects described above may be included in the Programme, which if carried out broadly and effectively, would have considerable scientific value as well as a favourable impact on public opinion

(IV) Co-operation in the Physical Sciences

Modern physical science has in many of its aspects become very big and expensive It therefore lends itself particularly well to intercontinental co-operation in which the costs are shared and the results are made available to all mankind

Four specific areas of physical science were identified as being ripe for vigorous action on an intercontinental basis These areas are the following

(*A*) *High-energy physics* Co-operation could centre around the establishment of a laboratory the main research tool of which would be an accelerator of not less than 300 × 10^9 eV and of a design which would achieve success in the shortest possible time.

(*B*) *Controlled thermonuclear and plasma research* In the field of controlled thermonuclear research there has been much effective exchange of information and scientists. This development is particularly significant since this research, prior to 1955, was secret It is urged that such collaboration be broadened, in particular, that the world's thermonuclear laboratories remain open to scientists of all nations who can contribute to this interesting and potentially important field of research

Although a new, very large thermonuclear device is probably not needed immediately, still there is a large field of general research in plasma physics which could well be advanced by the establishment of an intercontinental laboratory

(*C*) *Ultra-heavy element chemistry.* Two different devices are needed for such investigations high-flux reactors, and heavy-ion, high-current cyclotrons.

It is recommended that an intercontinental centre devoted to investigation of the properties of the ultra-heavy elements be established The centre probably should be equipped with the most powerful available heavy-ion cyclotron and with equipment for handling the materials The ultra high-flux reactor (10^{16} neutrons/cm ^{2}/sec), because of its hazard, probably should be located at a different, more isolated site

(*D*) *Large-scale computers* The development of the large modern electronic computer with its enormous memory and high speeds represents one of the most significant scientific events of the past two decades The future development of these computing machines, with larger memories and higher speeds by orders of magnitudes, would be of immense value to science Such computing machines will cost perhaps as much as 100 × 10^6 dollars

The development of such computers would be a suitable project for international co-operation The utilization of such a machine will advance not only mathematics but also all the physical sciences and the biological sciences, particularly the unravelling of the structure of macromolecules It would also find great utility in economics and other social sciences

(*E*) *A globular cluster of big science centres* It is believed that the separate big science laboratories in high-energy physics, heavy-element chemistry, macromolecular biology, health research, and possibly thermonuclear research, will prosper better if they are reasonably close together than if they are completely isolated from each other. The intercontinental computing centre will be more viable and will be a better centre if it were the nucleus of such a cluster.

The Conference urged that the intercontinental scientific laboratories be located in relative geographical proximity and that they be served by the intercontinental computing centre Such a centre, comprising much of what is called 'big science', would represent a capital investment of about 5×10^9 dollars It is believed that the astute location of such a striking epitome of science—the most characteristic theme of our modern civilization—could have extraordinarily great significance in improving the tone of the present political situation

(V) Co-operation in Assistance to Developing Nations

Assistance to developing countries is a duty and necessity for all countries This aid should be rendered so that it would not impair the independence of any country

Greater support for international co-operation in assistance to developing nations is needed Such co-operation could help to reduce world tensions, to strengthen peace, and to further disarmament Disarmament would in turn improve the climate for international co-operation in this and other fields and make available additional funds which could and would be used to increase the assistance to developing nations Clearly, the greatest co-operation and the release of maximum funds for the assistance programme could be achieved by complete disarmament

Scientists have a definite part to play in the development of assistance programmes and special emphasis should be placed on this subject in subsequent conferences Such a conference should include a large participation of representatives from the developing nations

The recommendation from the Economic and Social Council that a conference on science and technology in application to the problems of new nations should be held next summer in Geneva under United Nations sponsorship is to be welcomed

A subject of interest to scientists is that of natural resources of different areas and of their population trends Unprejudiced quantitative study of the latter topic should be carried out on an international, co-operative basis Another topic for similar co-operative research is world nutrition, including the study of reasons for widespread occurrence of avoidable malnutrition in many parts of the world. The problems of energy supply, particularly in areas in which the demand for energy is diffused rather than concentrated in large industrial centres, calls for a similar study It is suggested that preliminary study groups on these problems be organized prior to the above-mentioned conference

An international study should also be made of the advisability and practicability of establishing an international fund to which individual scientists from countries in which local funds are insufficient for this purpose could apply for assistance in their scientific research

(VI) Exchange of Scientists and Scientific Information

By its very nature and tradition science is a universal enterprise The rapid exchange of information mutual visits of scientists and their working as guests in the laboratories of other scientists constitute the main pathways of scientific collaboration among scientists all over the world It is to be noted that there has been substantial progress in recent years in the area of scientific exchange Yet it is to be regretted that there exists a number of difficulties which interfere in major ways with the further broadening of scientific collaboration and exchange

(*A*) *Exchange of scientists* The planned exchange of scientific personnel initiated by the Bronk–Nesmeyanov and similar agreements should be considerably increased The visits should be extended over periods sufficient for the completion of research projects In addition to planned exchange, the framework of these agreements should allow for and encourage invitations to scientists in the country in which they are to visit, and for the invited scientists to be able to accept such invitations Application of the *quid pro quo* principle to visits under the agreement (that is the exchange of one solid-state physicist for another solid-state physicist, etc) has tended to hamper fruitful exchange, and it is urged that such regulations be set aside in future agreements.

The role of Government bureaucracies in the administration of these agreements should be minimized To implement this recommendation, governments should be urged to expedite visas and passports for scientists since past and present failures in this respect have seriously hampered scientific exchange The scientific organizations of various nations should consider ways to facilitate the travel of pre- and post-doctoral fellows across national boundaries to study for adequate periods of time (one year or more) at research centres and under teachers where their training and scientific maturation can be best enhanced

(*B*) *Exchange of information* Noting the obstacle to the exchange of scientific information presented by the mounting volume of current publication, which is increasing exponentially and doubling about every decade, the situation calls for radical measures of rationalization Among potentially useful measures are the following to review and co-ordinate the character and content of journals published in all countries with the view of reducing the number of journals which a scientist must follow to keep abreast of work in his discipline, to institute standard formats for the presentation of scientific papers, to formulate a standard system for the annotation of the contents of published papers suitable for coding and manipulation by machines for the storage and retrieval of information, to institute regional depots under international co-ordination to store complete experimental records and other documentation in support of the brief published papers; to make such material rapidly available to interested scientists; to consolidate the abstracting services now carried on independently in many countries, a measure that could reduce present duplication of effort by a conservatively estimated factor of three

The publication of international review journals of two types as follows is recommended interdisciplinary review journals written in relatively non-technical language for the benefit of scientists in different disciplines, and more specialized review journals which would keep scientists working in a given area abreast of the work going on in the same or related areas all over the world.

All Governments are recommended to open their postal systems to the untrammelled flow of scientific publications whatever their country of origin or destination

LAND USE IN ARID REGIONS

THE arid zone programme of the United Nations Educational, Scientific and Cultural Organization, one of the Organization's three major projects, has yielded several valuable reviews of research and proceedings of symposia, mostly devoted to hydrology and ecology The latest in the series, a volume of some 350,000 words reviewing the history of land use in arid regions, is of more general interest* It deals with all the main dry lands with two main exceptions, those surrounding Abyssinia and those of South America in post-Columbian times

F K Hare explains the widespread aridity in sub-tropical latitudes in terms of the divergent wind-flow at low levels related to high pressure at 2–3 km, and considers it unlikely that any past climatic epoch can have been free of tropical aridity He finds it inconceivable that man can significantly alter the régime, the energy transformations involved are on so large a scale Thus the flooding of desert depressions, for example, would have only very modest effects on neighbouring areas

A summary by K W Butzer of what is known about climatic change in arid lands since the Pliocene sets the regional histories in a longer time perspective Full aridity was achieved in most arid lands some 15,000 years ago Since then there has been no progressive desiccation, only minor fluctuations of climate Of especial interest are those fluctuations which might possibly have affected the environment of man when he was first beginning to manage crops and animals in south-west Asia ten thousand years ago and in Mexico possibly not much later

Changes in climate over the past two or three thousand years are scarcely detectable with certainty until the fluctuation of the past three centuries This has involved more than the spectacular rapid advance and current retreat of Alpine glaciers, for it is becoming clear that semi-arid lands in many parts of the world have been receiving less rain in recent decades than in the latter half of the nineteenth century The dry trend continues, and the further outlook is unsettled

Prof Dudley Stamp, the editor of the volume, points out that a knowledge of the climate is not enough to allow a reconstruction of the past vegetation cover The effects of burning and of a more prolific fauna have also to be taken into account Ecologists and archæologists working together in the field are beginning to produce fairly reliable pictures of ancient landscapes, and R. O Whyte, in his contribution on south-east Asia, sees much promise in this type of co-operative research

South-west Asia is in many ways the most important of the regions and at the same time it produces the greatest difficulties in comprehension because of the mass of information available from widely scattered sites and conflicting interpretations of evidence Whyte's review is very stimulating but not at all easy to digest Egypt is of its nature simpler, and the material presented by G Hamdan will be more familiar to many readers V A Kovda's survey of the Russian plains, the Caucasus and Central Asia (plus a few pages on irrigation in China) naturally lays great stress on the revolutionary effects of introducing socialist methods of production to the steppe The scale of operations is indeed impressive Kovda notes the steady increase in the frequency of *sukhovei*, dry dusty winds, but asserts that their effects will be mitigated by new forests and irrigation projects which, he says, are expected to increase precipitation

J Despois, writing on North Africa, agrees with Butzer that there is no good evidence that the climate there was much more favourable to farming in the Roman period than in later times The decline in agriculture can mainly be attributed to eleventh-century and later invasions of Bedouin Arabs from the east Even their destructiveness seems to have been far exceeded by those other nomadic herdsmen, the Mongols, who descended on western Asia two centuries later From both, recovery was slow, and in Cyrenaica and parts of Iraq and Iran is still incomplete

Nomadism is a rational response to those seasonal and year-to-year variations in grazing and surface water typical of arid regions R F Logan describes flocks in the United States being driven in double-decker trucks to summer grazing in upland meadows under Forest Service permits Everywhere some State control of nomadism seems to be inevitable Although, as Th Monod and Ch Toupet note in the Saharan region, an irreversible process of "sedenterization" has set in since pacification, progress in this direction is likely to be too slow to satisfy administrators and conservationists

Innovations bring old problems Perennial irrigation can lead to salt accumulation and waterlogging, as in the Punjab, and to bilharzia and other diseases Improved water supplies from deep wells can accentuate overgrazing The balance is delicate in arid lands and the regulating mechanism must work efficiently or disaster comes swiftly. This seems to be the main lesson to be learned from the past

For the future, the possibilities of industry based on local minerals, with space-cooling and a sunny climate to attract labour, are becoming apparent from recent developments in Arizona and Central Asia The process of urbanization is not new to the arid lands, it probably began there, and may well go further there than anywhere else in the world

A T GROVE

*United Nations Educational, Scientific and Cultural Organization Arid Zone Research No 17 *A History of Land Use in Arid Regions* Edited by L Dudley Stamp Pp 388 (Paris: Unesco, London H M S O, 1961) 37 N F, 52*s* 6*d*, 10 50 dollars

OBITUARIES

Dr. B. L. Goodlet, O B.E

THE death occurred on October 27 of Dr Brian Laidlaw Goodlet, deputy chairman of the Brush Electrical Engineering Co , and, until recently, its managing director

Brian Goodlet was born in St Petersburg in 1903 of British parents who had settled in Russia in the service of the Government, and was educated at the Imperial School in St Petersburg He escaped from Russia during the Revolution and received his further training with Vickers, Ltd , and Metropolitan-Vickers, first at the Attercliffe Works, Sheffield, studying in the Applied Science Department of the University of Sheffield, where he gained the associateship in electrical engineering, and then as a special trainee in the Trafford Park Works In 1924 he was appointed to the staff in charge of the first High-voltage Laboratory, and there he introduced to the Company high-frequency and impulse voltage testing of electrical products, and made important contributions to the study of transmission and bushing insulators, during which time he designed the second High-voltage Laboratory equipped for a million volts He also designed the very small high-voltage generator which the Metropolitan-Vickers Co gave to the Cavendish Laboratory to assist me in the first attempts to accelerate particles to very high energy for transmutation experiments, and then designed the special high-voltage transformer of extremely small dimensions which was used by Cockcroft and Walton in their 1932 experiments There were no other high-voltage transformers in those days which could have fitted into the small rooms of the Cavendish Laboratory, and we owe Goodlet a great debt for these expert designs without them Britain might not have been ahead of the large American laboratories

It was through his contacts with the Cavendish Laboratory that he decided to take the Engineering Tripos in Cambridge, leaving Manchester at the age of twenty-seven with a generous grant from the Company On his return to Trafford Park he made a detailed study of extra high-voltage turbo-alternators, and also wrote his classic paper on lightning

In 1937 Goodlet was appointed to the chair of electrical engineering in Cape Town, but he returned to Britain in 1939 to take up the chair in Birmingham He offered his services to the Admiralty, but, because of his former connexion with Russia, he was not accepted, so he applied to return to the still vacant chair in South Africa, where he immediately joined the South African naval work on the degaussing of ships in Cape Town Harbour, ultimately gaining the rank of commander in the South African Navy After a short time he was sent to England to work with the British Admiralty as chief scientist at Rosyth, where he made distinguished contributions to work on underwater explosions, before joining the Far East Naval Task Force in Ceylon under Lord Mountbatten He was made O B E for his war services In 1946 he returned to Cape Town, where he built up a vigorous electrical engineering department with almost pitiable funds, gathering around him several engineers from the Metropolitan-Vickers Co and starting a lively research school, but this had scarcely begun to bear fruit when he returned to England again in 1950, this time as deputy chief engineer of the Atomic Energy Establishment at Harwell His text-book on basic electrotechnics—the result of his post-war work in South Africa—has become well established in British universities for second- and third-year honours engineering students

Goodlet began in Harwell with a small team, including Mr R V Moore, to investigate in detail the feasibility of a power-producing nuclear reactor system, referred to as *Pippa*, using natural uranium The team selected graphite as moderator, carbon dioxide as coolant gas and a vertical arrangement of fuel rods, and by 1952 all the main features of the design had been established it was then handed over to the Production Division to produce Calder Hall It is a tribute to Goodlet's engineering judgment that no major changes of design were necessary (His paper, "Nuclear Reactors for Power Generation", to the Institution of Mechanical Engineers, was awarded the Thomas Hawksley Gold Medal)

During 1952–56 he was the chairman of the Naval Education Advisory Committee of the Admiralty the Director of the Naval Education Service advises me that he made an ideal chairman and was greatly respected by his colleagues During Goodlet's period of office, the Committee advised on a number of important matters, including cadet entry into the Royal Navy, officer training, the technical training of junior ratings, the conditions of service of civilian teaching staff at the Royal Naval Colleges, the rehabilitation of the Dockyard Technical Colleges and the organization of facilities for informing the Service of modern developments in science and technology. His untiring interest and wise counsel in all aspects of naval education were of the utmost value to the Director of the Naval Education Service

By 1956, Goodlet felt that his work at the Atomic Energy Research Establishment was finished, and he joined the Brush Electrical Co , Loughborough, as chief engineer and director, later becoming its managing director Mr G B R Feilden tells me that Goodlet was confronted by a series of major breakdowns of 30-MW double-rotation turbines which had been designed before Goodlet joined the Company The breakdowns were caused by vibration, and the solution to the problem required the evolution of entirely new measuring techniques Goodlet was responsible for solving this problem, which was fortunately achieved successfully, largely due to his own perseverance, shortly before his death He submitted his published work to Cambridge and was awarded the Sc D in 1958

Perseverance and thoroughness characterized all his work like the great American engineer, Charles Proteus Steinmetz, he was never satisfied until he understood the fundamentals of any problem which confronted him, and he was fondly nicknamed 'Prot' by his closest friends because of this similarity He invariably wrote out the basic facts and deduced the solution in beautiful handwriting, and his notebooks were a joy to peruse His perseverance was equally manifest in his hobbies, and he enjoyed going up Table Mountain the hardest way He was extremely interested in military strategy and it was fascinating to hear him quote Napoleon's campaigns, he even wrote a mathematical paper on the ballistics of weapons used in classical times I was present when Rutherford, returning from the British Association meeting in South Africa, recited the exploits of Cetywayo, Goodlet, to Rutherford's great delight, continued the story with intimate details of the part played by the Zulu's lieutenants

Goodlet is survived by a widow, two sons, and two daughters T E ALLIBONE

Dr A K. Das

INDIA lost a meteorologist of note and a leading astronomer of this century with the death on February 18, 1961, of Anil Kumar Das. He was best known for his work at Kodaikanal Astrophysical Observatory, of which he was the head for fourteen years At the time of his death, however, he was acting temporarily as director of the Nazamiah Observatory, Hyderabad, and as professor of astronomy at Osmania University But although best known for his astrophysical researches, Dr Das published a number of papers on purely meteorological topics, mainly during the early part of his thirty-years service in the India Meteorological Department

After completing his education to postgraduate level in India, Das continued his studies in Paris under Fabry and later also worked with Max Born and Augenheister He returned to India in 1930 to commence his meteorological work, but in 1934 he went to England to study the temperature of the Sun by spectro-photographic means at the Solar Physics Observatory, Cambridge This virtually marks the beginning of his career in astronomy His many papers on solar physics thereafter appeared at regular intervals almost up to the date of his death

Das had the intuitive foresight, great energy and determination of the great research worker, but he was also an administrator of considerable ability Indeed, his greatest achievements probably lie in the improvements he introduced at Kodaikanal, by the installation of new equipment and the initiation of new fields of research Largely thanks to Das, this Observatory is now carrying out researches and making observations, using the finest equipment, in many branches of geophysics and solar physics It is a pity that he could not live long enough to fulfil his last ambition to work with his new instruments during the last years of his life

NEWS and VIEWS

Botany in University College, Dublin

Prof J Doyle

PROF JOSEPH DOYLE has retired from the chair of botany in University College, Dublin, after nearly fifty years service He entered the College on a mathematical scholarship, later he became interested in biology through the pathway of medicine Following a period of postgraduate research, part of which was spent in Germany, he was appointed assistant in biology in 1913, at this time, he and two others comprised the staff of the Biology Department In 1924 he was appointed professor of botany, and undertook the development of the first Department of Botany in the College His main research interest is represented by a long series of papers on conifer embryology, on which subject he is internationally recognized as an authority He was awarded the Boyle Medal of the Royal Dublin Society in recognition of this work The degree of D Sc was conferred on him in 1930 At the Dublin meeting of the British Association for the Advancement of Science in 1957, he was president of Section K (Botany) In addition to developing the Department of Botany, Prof Doyle rendered distinguished administrative service to his College As dean of the Faculty of Science he showed considerable organizing power, for many years he was a member of the Governing Body of the College and of its Finance Committee He has helped greatly in furthering the scientific and cultural life of Dublin He is a member of the Council of the Royal Dublin Society, secretary of the Royal Irish Academy, chairman of the Praegar Committee for Field Natural History, and a trustee of the National Library of Ireland Recently he has been actively engaged in planning new Science Buildings for University College, Dublin His many interests will ensure for him rewarding occupation in his retirement

Prof Phyllis M Clinch

DR PHYLLIS M CLINCH has been appointed to succeed Prof J Doyle Dr Clinch, in fact, majored in chemistry in University College, Dublin, with first-class honours and first place at her primary degree, but, botany being her second subject, she took an immediate interest in plant biochemistry and her earlier research was done in the Department of Botany on problems of seasonal metabolic changes in conifers In this and in all her subsequent work her early intensive training in basic chemistry proved a most valuable asset After a period of study under Prof V H Blackman in the Imperial College and Prof Guilliermond in Paris, she was appointed senior research assistant in plant virus diseases in the Department of Plant Pathology, University College, Dublin Here she played a major part in the team that produced so much important work on plant viruses, especially the analyses of viruses affecting the potato plant The value of the work from this Irish School is too well known to need comment here, suffice it to say that Dr Clinch's share in it has gained her a well-merited international reputation in the plant virus world In 1950 she accepted an invitation to join the staff of the Botany Department, University College, as senior lecturer with the primary purpose of developing courses and research in plant biochemistry and physiology along modern lines The very successful organization of this entailed, unfortunately, a temporary break in her work on viruses The break, however, was only temporary, and in recent years she has initiated a programme of research into the biochemical and physiological effects of virus infection on the metabolism of the infected plants themselves It is to be hoped that the administrative and other duties which will now naturally fall on her as head of a department will not unduly hamper progress in this promising line of research

Control of Government Expenditure on Research and Development

IN opening a debate on November 30 when the House of Commons was asked to take note of the First, Second and Third Reports from the Committee of Public Accounts in the Session 1960–61 and of the Special Report from that Committee, Mr. H. Wilson referred to the centenary of the establishment of the Committee in April 1861 and to the steady development of its work. The debate covered a fairly wide field, but the two chief points of scientific interest are to be found in the reply of the Financial Secretary to the Treasury, Sir Edward Boyle. Sir Edward, referring to research and development practice, endorsed the Treasury Minute that it is Government practice to rely largely on private industry when it is a matter of design studies for complicated new weapons and equipment, subject to the checks which Government establishments can operate, and that research, development and production are integral parts of a continuous process to which the Ministry of Aviation's research establishments contribute by virtue of their specialized knowledge and facilities. Sir Edward also said that the major part of the Atomic Energy Authority's net cost of operations—nearly £60 million in 1959–60—represents research and development effort in both the civil and military fields, and to publish the civil figures alone would make it easy to estimate the level of military research. Decisions on all matters concerned with the disclosure of information are taken after regular periodical reviews, and the importance of this issue to the Public Accounts Committee was realized. In principle, the Authority hoped to recover its research and development expenditure on civil reactor systems.

Medical Men in Britain

IN a debate in the House of Lords on November 29 on the shortage of medical men, Lord Taylor stressed the difficulty which non-teaching hospitals in Britain are experiencing in filling appointments. In replying for the Government, Lord Newton said that after reviewing the data and calculations of the Willink report, the Government is satisfied that the prospective demand for medical services will justify a rise in the university intake of pre-clinical students from Great Britain of 10 per cent above the 1,760 recommended by the Willink Committee. The intake in July 1961 was 1,788. The University Grants Committee has been asked to consider with the universities the implications of the Government's conclusion. Steps are being taken to improve the statistical information so far as reasonably possible. Lord Newton said that the Government intends to watch the figures constantly, including those of output from the medical schools, rather than attempt to make firm estimates at wide intervals of time. Lord Taylor had asserted that the shortage is now showing itself in the teaching hospitals also, and that the hospital service in Britain would have collapsed but for the influx of some 3,000–4,000 junior doctors from India and Pakistan. Lord Newton admitted that in March 1960 more than one-third of the posts in the middle and lower grades were occupied by doctors born outside Britain, but he emphasized the value of these doctors and the debt to their services. He said that in 1960 the average number of patients on a doctor's list was 2,287, about 150 fewer than it was in 1952, and that the improvement of the distribution of doctors was of special concern to the Medical Practices Committee constituted under the National Health Service Act.

Accelerators for Low-energy Nuclear Physics

RECENT advances in the theoretical description of the atomic nucleus, together with recent design improvements in cyclotrons and Van de Graaff accelerators have stimulated a special U.S. National Science Foundation programme for establishing and improving low-energy nuclear physics research facilities. The theoretical advances have resulted in a need for refined measurements of energy-levels, spin assignments, and other characteristics of the nucleus so that the synthesis of present theoretical models can be further advanced. Many of these refined measurements are now possible through extension of the range of Van de Graaff accelerators giving particles with energies of up to 20 MeV., and improvements in cyclotron design to permit well-controlled beams up to 40 MeV. Until about five years ago Van de Graaff accelerators were restricted to energies less than 8 MeV. Recently, the Foundation has made a group of six grants, totalling 4,161,500 dollars, in furtherance of the new programme. These six accelerators are expected to play an important part in the nation's effort in nuclear structure physics. Grants made so far in this special programme are as follows: University of Chicago, 296,700 dollars for a 4-MeV. Van de Graaff machine to be used by Dr. S. K. Allison and his Group; Michigan State University, 700,000 dollars toward a 40-MeV. cyclotron for the use of Dr. Henry G. Blosser's research team; University of Pittsburgh, 977,600 dollars toward an 18-MeV. three-stage Van de Graaff machine for research under the direction of Dr. Bernard L. Cohen; University of Washington (Seattle), 1,240,000 dollars toward a 22-MeV. three-stage Van de Graaff machine for the Physics Department, of which Dr. Ronald Geballe is executive officer; Ohio State University, 499,200 dollars for a 5·5-MeV. Van de Graaff machine for the Department of Dr. H. H. Nielsen; State University of Iowa (Iowa City), 498,000 dollars for a 5·5-MeV. Van de Graaff machine for the Department under Dr. James A. Van Allen.

The Royal Commission for the Exhibition of 1851

THE eleventh report of the Commissioners for the Exhibition of 1851 covers the activities of the Royal Commission since its tenth report was issued in May 1951 (Pp. 32. (Cmnd. 1502.) London: H.M.S.O., 1961. 2*s*. 6*d*. net). The general pattern of the Science Research Scholarships has remained unchanged, but the list of universities invited to participate has been extended and the value of the awards increased to £550 a year for Overseas Scholars and £800 a year for senior students, exclusive of allowances. In 1958 the National Research Council of Canada offered to each 1851 Scholar from Canada a bonus of £250 a year from its own funds, which has been particularly useful to married Scholars. Similar arrangements have been made, with various qualifications, by authorities in Australia, South Africa, India, Pakistan and New Zealand. Since 1952 the Commission has co-operated with the Council of the Royal Society in the administration of the Rutherford Scholarship. The value of the Postgraduate Scholarship in Naval Architecture was in 1958 increased to £600 a year. In view of the re-allocation of so much of the property on the Kensington Gore Estate in 1956, it was agreed with the Imperial College of Science and Technology and Government departments concerned to arrange a new lease to the College for 999 years from midsummer 1956 at a rental commencing at £5,000 and rising over ten years to £25,000 a year

The Commission's office at 1 Lowther Gardens provides accommodation also for the London headquarters of the British School at Rome, to which the Commission now contributes £8,000 a year, and for the Institute of Physics and the Physical Society Of the expenditure of £324,759 during the ten years January 1, 1951–December 31, 1960, £244,822 was on direct educational grants A new *Record of the Science Research Scholars of the Royal Commission for the Exhibition of 1851, 1891–1960,* has now been compiled, and it may be noted that of the 905 Scholars, 99, including 34 from overseas, became Fellows of the Royal Society, of which two were elected president, eight won Nobel Prizes (Pp iv + 107 London Royal Commission for the Exhibition of 1851, 1961 12*s* 6*d*) The *Record* lists separately Science Research Scholars appointed during 1891–1921, Overseas Scholars appointed since 1922 and Senior Students appointed since 1922 There are indexes to Scholars and to the Institutions from which they came

Experimental Eye Research

THE scientific literature on the eye and vision is dispersed in journals representing many different disciplines, and visual scientists have for long deplored the fact that they had no journal of their own Now they are to have at least two For the appearance last June of *Vision Research* (Pergamon Press) (see *Nature,* 191, 650, 1961) is followed by the present debut of *Experimental Eye Research* (Academic Press). Congratulations are due to Academic Press for the fine quality of *Experimental Eye Research,* the first number of which appeared in September (1, No 1; September 1961 Pp 97 Published quarterly Vol 1, approx 400 pages (London: Academic Press, Inc (London), Ltd ; New York: Academic Press, Inc , 1961) 115*s* , 16 dollars) The object of this quarterly journal is the publication (in English) of original papers on the anatomy, physiology, biochemistry and biophysics of the eye "Contributions on such subjects as the production and circulation of ocular fluids, the fine structure, biochemistry, biophysics and metabolism of the eye tissues, the photochemical processes related to visual function, and the neuromuscular mechanisms in the control of eye movements will be preferred " The editors-in-chief are Dr E A Balazs, of Harvard Medical School, and Dr Hugh Davson, of University College, London They are supported by a group of leading workers forming an editorial board of five and an advisory board of nine A study of the first issue reveals a high standard of editorial policy and also confirms the initial impression that the main interests of the publication are the minute anatomy and vegetative physiology of the eye Since the interests of the 'rival' publication, *Vision Research,* are centred more on the physiology of vision, there is less overlap in the scopes of the two journals than might appear

Engineering and Television

Two publications of the British Broadcasting Corporation issued to mark the twenty-fifth anniversary of B B C Television direct particular attention to the work done by its engineering division during the period under review The first, *Twenty-five Years of B B C Television,* is one of its periodic *Monographs* and has been prepared by one of the original members of the B B C , Sir Harold Bishop, now director of engineering (No. 39 Pp 41 (14 plates) London British Broadcasting Corporation, 1961 5*s*) After a survey of the events and developments which led to the start of the B B C 's high-definition television service, the monograph describes the main engineering developments during the past quarter of a century Particular attention is directed to those techniques which have been originated by the B B C , or which are utilized in a specially noteworthy manner in the services of the B B C. There is also a brief review of the B B C 's contributions to probable future developments, including colour television and a change of standards The second publication, *B B C Television a British Engineering Achievement,* is written in a more popular style, and gives a narrative account of how television works, the early growth of the services and what engineering services and techniques are involved to bring television into the home The brochure is well written, with good illustrations and, like so many B B C publications, is remarkably cheap (Second edition, revised Published on the 25th Anniversary of B B C Television Pp 64 London: British Broadcasting Corporation, 1961 3*s* 6*d*) It should find its way to bookstalls as well as libraries in schools and other places

Astronomical and Nautical Almanacs

Two ephemerides have recently been published for the year 1962, one by the U S Government and the other by the Indian Government. *The American Ephemeris and Nautical Almanac* is unchanged with regard to the presentation of material (Pp vii + 514 Washington, D C Government Printing Office, 1960 4 dollars) *The Indian Ephemeris and Nautical Almanac* (Pp xxviii + 460 Delhi Manager of Publications. 1961 Rs 14 00, 22*s*) is closely similar to the American *Ephemeris,* though more restricted in scope, but in addition contains a section on the Indian Calendar. The 1962 edition contains four new sections: (1) ephemeris for physical observations of the Sun; (2) ephemeris for physical observations of the Moon, (3) centre of mass of the solar system, and (4) local circumstances of occultations of bright stars and planets visible in India

Technical Publications of the Oak Ridge National Laboratory

THE publication *ORNL* 61-6-27 contains a list of papers published in journals and presented at meetings, reports issued, and theses completed, during 1960, by the members of the staff of the Oak Ridge National Laboratory, Oak Ridge, Tennessee (Pp v + 107 Oak Ridge, Tennessee Oak Ridge National Laboratory, 1961) Reprints of many of the articles can be obtained on request either from the Laboratory, or of papers presented at scientific meetings from the authors concerned There is an author index, and the various articles are grouped within the sections according to subject, biology, chemistry, physics, etc The prices of the *ORNL* reports are given, and the reports, except those indicated as 'not for sale'. can be obtained through the Office of Technical Services, Department of Commerce, Washington 25, D C

National Conference of Standards Laboratories

To help meet the needs of science and industry for improved uniformity and greater precision in calibration and measurements in all areas of the physical sciences, engineering and technology, a new organization—the National Conference of Standards Laboratories—was established on September 15, 1961, by representatives concerned with standards activities in Government, industry, education, and professional societies of the United States A major activity of the National Conference will be to arrange a continuing series of standards laboratory conferences The General Committee, headed by Lloyd B Wilson, of the Sperry Gyroscope Co , has arranged for the first conference in this series to be held during August 1962 at the National Bureau of Standards Boulder Laboratories, Boulder, Colorado Another major activity being considered is the establishment of a voluntary system of measurement agreement checks to demonstrate competence and continued uniformity among standards laboratories Further information can be obtained from Dr H W Lance, Electronics Calibration Center, Boulder Laboratories, National Bureau of Standards, Boulder, Colorado.

Conference on Measurement and Control Technology

The Society of Instrument Technology and the Royal Dutch Institute of Engineers are organizing a joint conference to be held during April 10–13, 1962, in Amsterdam The programme is being arranged to encourage discussion and exchange of information on the present state and future trends of measurement and control technology in Britain and Holland Six technical papers will be presented and discussed covering new measurement methods such as direct reading spectrography, X-ray fluorescence chromatography and optical measuring techniques, descriptions of working automation projects in the biscuit industry and sinking mine shafts, and control in manufacturing industry While most of the places will be reserved for members of the Society of Instrument Technology, it may be possible to entertain a few applications from non-members Further information can be obtained from Mr I V Idelson, Mullard Equipment, Ltd, Manor Royal, Crawley, Sussex

University News : Birmingham

The following appointments have recently been announced Dr J S Brimacombe, to be lecturer in the Department of Chemistry, Dr D E N Davies, to be lecturer in the Department of Electrical Engineering, Dr F Rieband, to be lecturer in the Department of Mechanical Engineering, E D R Shearman, to be senior lecturer in electromagnetism in the Department of Electrical Engineering

Leeds

In a statement to a meeting of the Court of the University of Leeds on November 15, when the annual report was presented, the vice-chancellor, Sir Charles Morris, referring to the increase in the number of students by 1,400 in five years, said that in the past year it had increased by 386 to 5,175, and it was probable that this order of increase would continue during the forthcoming quinquennium and that the student population in 1966 would be 6,900 The building programme was based on an increase of this order, but there was some risk that the provision of residential accommodation might seriously fall behind needs in the immediate future The vice-chancellor also referred at some length to the new courses which the University was offering leading to an honours degree covering study in depth in two of the traditional subjects These courses were meeting with immediate response, and twenty-one students were expected to graduate this year in the 'combined' courses in arts, the most popular combinations so far being economics and history, English and history, geography and history, and psychology and sociology The Science Faculty was starting such courses next October and a director of combined studies was being appointed to guide the students following these courses and advise on combinations of subjects While he did not think the 'special honours' courses would disappear or lose prestige, he thought it would be increasingly accepted that at the standard of first degree at honours-level, two subjects could effectively be studied together in depth

Sussex

The following appointments have been made Dr D B Scott, at present reader in mathematics at King's College, London, to be professor of mathematics, Dr. E R A Peeling, at present senior lecturer in chemistry in the University of Leicester, to be senior lecturer in chemistry

Announcements

Sir Harry Melville, secretary of the Department of Scientific and Industrial Research, has been made a member of the Council for Scientific and Industrial Research in recognition of his "notable services to D S I R " The appointment is personal to Sir Harry and does not mean that future secretaries of the Department of Scientific and Industrial Research will automatically become members of the Research Council

A symposium on the "Methodology of Plant Eco-Physiology" will be held in the Botanical Institute, 5 rue Auguste Broussonnet, Montpellier, France, during April 7–12, 1962 Further information can be obtained from Dr. F. E Eckardt, Institut de Botanique, Montpellier, France

The Department of Physics and Astronomy of the Ohio State University is organizing a symposium on "Molecular Structure and Spectroscopy", which is to be held in the University during June 11–15 Further information can be obtained from Prof R A Oetjen, Department of Physics and Astronomy, Ohio State University 174 West 18th Avenue, Columbus 10, Ohio

Due to a change in date of the meeting of the International Pharmaceutical Federation, which will be held in Vienna during September 1–24, 1962, the third British Congress on the History of Medicine and Pharmacy will be held in London during September 27–28, 1962 The subject of the Congress will be "The History of British Hospitals" Further information can be obtained from Dr F N L Poynter, Wellcome Historical Medical Library, Euston Road, London, N W 1.

Erratum We regret to find that, in the article entitled "Action of Radiation on Synthesis of Deoxyribonucleic Acid in Normal and Tumour Strain Cells" (*Nature*, December 9, p 944), a line has been omitted and another duplicated In column 2, p 944, lines 15 and 16 should read " a decrease from the consequent depletion of the cell population However, as Painter and . " The present line 16 is a repetition of line 13

INTERPRETATION OF GEOPHYSICAL SURVEYS

RECENT improvements in geophysical instruments have not simplified interpretation of the data obtained by using them Indeed, their greater speed and higher accuracy increase the data to be analysed and foster attempts to interpret in finer detail Assessment of factors controlling the reasonable limits of interpretation was the main aim of a Geophysical Discussion of the Royal Astronomical Society, held in association with, and at the rooms of, the Geological Society on October 27 Introducing the four invited speakers, the chairman, Dr. W Bullerwell (Geological Survey), remarked that geophysical interpretation is usually tested by subsequent geological exploration, thus it was appropriate that discussion about interpretation should take place among geologists and benefit from their comment

In opening the discussion, Prof J M Bruckshaw (Imperial College of Science and Technology, London) reviewed the treatment of gravity and magnetic survey data, where interpretations aim to deduce sub-surface mass distributions accounting for observed surface fields Fundamental difficulties are ambiguity and lack of resolution Ambiguity reflects dependence of the gravity anomaly on several factors, including form, size, depth and density contrast When all these factors concerning a buried mass are known, it is possible to predict uniquely the effect on gravity at any point on the surface, but when interpreting a gravity profile recorded in a new area, the factors cannot be evaluated individually, and possible permutations allow many solutions consistent with the profile Only geological control can limit the inherent ambiguities Lack of resolution was illustrated by indicating that the interpreter cannot distinguish a point mass from a cube or sphere, since all produce similar gravity profiles Depth to centre, however, can be computed with reasonable accuracy and an estimate of size is obtainable from the maximum of the anomaly, though it depends in turn on an estimate of density contrast

Various numerical methods of analysis have been proposed, but Prof. Bruckshaw stressed that mathematical transformations cannot overcome limitations inherent in the original data, while subjective bias may be introduced in the process As a preliminary step, it is usual to isolate local anomalies thought to represent shallow geological structures by removing a regional background corresponding to smooth average variations Only rarely, however, can the regional be assigned geological significance, and the smoothed form assumed varies between different interpreters Attention has been given to the second vertical derivative of gravity following claims of higher resolution from a fourth-power relation with depth as compared with the normal square law, and correspondence between zero contours of second derivative and vertical density boundaries Unfortunately, precise evaluation of second derivatives requires continuous information whereas field observations are made at a series of discrete points One process of analysis depends on the subjective drawing of smooth curves through available points Alternatively, an interpolation formula may be applied, but developed formulæ give different solutions depending on size and form of primary grid Final stages depend on comparisons with model curves, and it is important to derive these using the same grid In reconnaissance of new areas, such methods are rarely warranted No amount of mathematical analysis can remedy insufficient geological control, and if only geophysical data are available, interpretation always remains ambiguous This was demonstrated recently in an example published by P E Jacobson, of the Creole Petroleum Co , showing differences between two independent interpretations of a magnetic survey in eastern Venezuela However, if good geological control is available and physical properties of the geological formations have been extensively studied, as in parts of England, some difficulties can be resolved and confident estimates advanced

Dr M H P Bott (University of Durham) agreed that gravity and magnetic data are ambiguous, but felt that useful conclusions can generally be salvaged from the sea of uncertainty by applying simplifying assumptions legitimately drawn from geological knowledge of similar environments The practical limit of interpretation is frequently determined by how far such reasonable assumptions permit assessment of background and isolation of the interesting anomaly from other disturbances Once the anomaly has been isolated, total mass can be accurately evaluated by areal integration of the gravity contours Russian work, recently developed further by Dr R A Smith, shows that if only a few conditions are satisfied a unique solution may be possible As an example, if an anomaly arises at a single interface across which the density contrast is constant and known, then the shape of the interface can be interpreted directly Usually the limiting conditions are not accurately known but can be expected to lie within a certain range, and this information permits use of an uncertainty principle through the inequality theorems of Bullard and Cooper, later extended by Bott and Smith Minimum and maximum values for depth to the source of the anomaly can be obtained Limiting depth estimates can be derived also from second and third horizontal derivatives of the gravity anomaly, through formulæ of Dr R A Smith relating maximum derivative and maximum depth The limiting case, corresponding to a symmetrical truncated wedge-shaped body with each edge contributing equally to the derivative, can be modified for the situation where one edge is distant from the point of observation, as for measurements at the margin of an extensive body Dr Bott showed how, using this method, he had analysed profiles across the margin of the Weardale anomaly for an estimate of depth to the top surface of the postulated Weardale granite, afterwards proved (at a depth of 1,281 ft) in a deep borehole at Rookhope, Co Durham Using maxima in second and third horizontal derivatives calculated from closely spaced observations by gravity meter and assuming density contrast of 0·2 gm /cm.^{3}, analysis had shown that the top of the postulated granite must lie at less than 3,500 ft beneath Weardale It is difficult to measure gradient maxima sufficiently accurately with a gravity meter, since an average over a base-line and not a point-value is observed. Dr. Bott believes that the torsion-balance, little used recently, might be used advantageously.

Similar methods often prove satisfactory for deciding between alternative geological possibilities, as in distinguishing gravity lows over sedimentary basins from those due to granites. In the former case the boundaries of the lighter sediments slope in towards the centre of the anomaly, whereas the granite margins slope out at depth. Different amplitude ratios between derivative maximum and minimum flanking the anomaly are detectable, and contrasting results from gravity traverses crossing the margins of the Cheshire Basin and the Dartmoor Granite were displayed. Finally, useful results can be derived from comparisons with models, since modern digital computers will check quite speedily whether a postulated geological structure would produce the profile observed or whether marked divergences necessitate a new hypothesis. Dr. Bott concluded by saying that, applied to analyse magnetic data, these methods encounter added difficulties due to variations in direction of permanent magnetization.

Mr. G. Tait (Iraq Petroleum Co.) contributed a paper outlining the geophysicist's views on interpretation of seismic surveys. He remarked that recent instrumental developments aim at producing a greatly simplified presentation of results in the hope that by transforming a series of impulses into a variable density profile the main features of the geological structure may be comprehended at a glance. No method of presentation, however, alters the fact that the behaviour of the seismic wave depends on elastic constants in the rock formations concerned and progress or difficulties in interpretation relate to whether and where variations in these constants occur. It is not always possible to predict from lithology, for seismic velocities in adjacent sandstones and limestones can be identical. Local velocity control is doubly important, because velocity discontinuities may not correlate directly with stratigraphic interfaces and because travel-time and velocity have equal weight in seismic determination of depth. Satisfactory velocity data can be obtained from well-shooting or by recording velocity logs in deep boreholes, but in virgin areas, where information must come from surface shots, available values may be inadequate. Accurate velocities can help when distinguishing between multiple reflexions and deep reflectors from curvatures in the moveout or by recognizing anomalous dips.

Several of these and other complications which the interpreter must disentangle were clearly demonstrated from oil exploration surveys by the Iraq Petroleum Co. One slide showed how tangential distortion against high-speed beds can simulate the indications of a natural structure. Importance of spread-length was apparent in the same example, since curvatures which could not be identified on a short spread became clearly visible at 12,000 ft. Records of a noise-spread indicated that alignments in background may confuse with weak reflexions. Profiles shot in the foothills of Iraq demonstrated both the problems and the importance of identifying multiple reflexions and diffraction cross-overs which may have amplitudes greater than those of the geologically significant events. Lower-amplitude contrast between events in a profile across the main producing oilfield of Iraq made it appear somewhat of an anticlimax, though containing important information. Mr. Tait's evidence made clear that, in dealing with seismic records, the interpreter must continuously bear in mind natural conditions under which the survey has been performed, velocity data available, instrument lay-out and complications which may have confused the records with multiple reflexions and diffraction effects.

Mr. A. N. Thomas (British Petroleum Co.) discussing geological factors in the planning and interpretation of seismic surveys, stressed the need for close co-ordination of geological and geophysical information. His first example, taken from the Po Valley, showed seismic reflexions on a phantom horizon to which geological information had been added, seismic evidence controlling the section as to fault zones and dips, while stratigraphical detail came from boreholes. Following examples came from south-west Iran, where initial interpretation difficulties with seismic refraction surveys were resolved when stratigraphical considerations correlated highest velocity with Middle Cretaceous Rudist limestone, not with the massive Asmari limestone 2,000–3,000 ft above. Refraction arcs in the Lali Plain, starting from positions determined by surface geological mapping, directed attention progressively southwards to prove concealed Asmari limestone structures. In other British Petroleum Co. surveys across Agha Jari and Lali, surface dip observations by geologists in an area of disharmonic folding gave valuable checks on geophysical evidence about elevation of a concealed crest. In exchange, structural interpretation of the surface geological maps was aided by the geophysical information. Evidence from Iran showed that, by producing regional isopach and facies maps, the geologist can illuminate seismic interpretation wherever such variations control velocity or ability to act as a refractor.

The bearing of velocity anomalies on seismic interpretation was next considered using an example by D. C. Skeels from a Devonian reef oilfield in West Canada. Reflexion times at boreholes to the Lower Cretaceous gave a velocity map used for controlling the seismic data, and the final seismic depth-map agreed with later sub-surface information more closely than an earlier map based on reflexion times only. In more complicated conditions accompanying Reef limestones in Western Alberta, structure contour maps and seismic isochron maps may show different relationships on successive horizons. Causes are sometimes difficult to assess, but it is clear that seismic evidence must be checked against drilling data at all available positions and levels. Difficulties in correlating reflexion pulses with specific geological formations were illustrated in results obtained near Calgary by Dr. T. C. Richards, in which corresponding seismic peaks and troughs could be seen to change stratigraphic horizon in different parts of the area. The sections also showed how ideas about the structure of the area changed as various stages of drilling, seismic survey and further drilling were completed. This analysis indicated the tendency of the seismic interpretation to smooth out complications and inability to locate with certainty the top of the Palæozoic, which is the most important lithological break.

Mr. Thomas concluded by saying that too much should not be expected from a seismic survey. It cannot eliminate the need for sound geological investigation; both lines of approach are necessary. The seismic method cannot give a complete picture of sub-surface conditions in areas of complicated structure, and geologists should realize the limitations imposed by the nature of the method.

Opening the meeting for general discussion, Dr Bullerwell recalled a remark of Prof Bruckshaw that potential theory has been known a very long time and that in many fields interpretation shows no fundamental advance over the past fifteen years Noting that during this period there has been increased use of systems like the airborne magnetometer for recording continuous profiles, he asked whether these removed some of the subjective elements mentioned Prof Bruckshaw replied that the subjective element remains in spite of such developments, due to the gaps between profiles and uncertainties in completing contours Though coverage is improved, it is never complete Dr W Domzalski felt that some views expressed had been unduly pessimistic Even though interpretations might prove quantitatively in error, qualitative results obtained following an imaginative approach often lead to new discoveries For reasonable success in interpretation it is also important to choose the geophysical method most suited to the geological problem Even where no control information to guide the interpretation is available, the possible results may justify geophysical work as the most economical means of preliminary reconnaissance

On the question of economics, Dr Bullerwell asked how soon during a survey should the results be tested by drilling Mr. N L Falcon said that satisfactory exploration implies a balanced programme combining geology, geophysics and drilling It is desirable to establish control, pursue the investigation comprehensively and not anticipate conclusions on the basis of too few results, but geophysical interpretation remains speculative until the key localities are drilled Dr T C Richards said that geophysical experiences in Canada emphasize the importance of early drilling control A quantitative approach is essential throughout and second-derivative gravity maps have proved unreliable for this Seismic methods must be introduced at a very early stage and have adequate geological and drilling control, especially in the Reef field, to overcome difficulties due to facies changes Otherwise, occurrences of Reef limestone in limestone or local velocity anomalies, as at Swan Hills, Alberta, would not be recognized

In closing the meeting the chairman remarked that personal experience often decides for one interpretation method rather than another Perhaps a real advance during the past fifteen years is represented by pooling of experience through free discussion and improved publication of geophysical results There is certainly a clear recognition of fundamental problems W BULLERWELL

TWELFTH INTERNATIONAL ASTRONAUTICAL CONGRESS

THE International Astronautical Congress is an annual meeting of the interplanetary societies of the world Societies take it in turn to act as host, and the recent meeting, during October 1–7, in Washington, was the first occasion that the meeting had been held on the American continent Next year's conference was fixed for Sofia, Bulgaria, it having been held in Stockholm last year and London in 1959 Of the 750 people registered, some 300 came from outside the United States The president of the International Astronautical Congress this year was Academician L I Sedov, of the U S S R , and for the forthcoming year Prof J Pérès, of France, was appointed the new president

The week following the Congress was the occasion of a meeting in New York by the American Rocket Society—"Spaceflight Report to the Nation"

There was perhaps a feeling that the International Astronautical Congress was of not quite such a high technical standard as previous ones, as many Americans seemed to consider the American Rocket Society meeting to be the more important of the two—a view not altogether shared by the European delegates

The opening ceremony on Monday was followed by a reception and buffet at the Bolling Air Force Base, where Dr Theodore von Kármán was very much in evidence among the delegates

The technical sessions started at 9 a m on October, 3 with three simultaneous sessions on space propulsion, astrodynamics and guidance, and the Fourth Colloquium on the Law of Outer Space (the Third Colloquium was held in Stockholm in 1960).

The session on space propulsion had two lectures from British delegates, the first by Mr A W T Mottram (Bristol Siddeley Engines, Ltd , Coventry) entitled "Propulsion of the Final Stage of a Satellite Launcher using Liquid Hydrogen as Fuel". Although this was the first lecture of the morning, it was well attended and there was a good discussion, although Americans in the audience were perhaps a little reluctant to describe some of their more recent failures in this field It must be remembered that liquid hydrogen has not yet been used successfully to place a satellite in orbit, and that it was announced during the course of the Congress that the *Centaur* rocket using liquid hydrogen as a fuel has been officially set back eight months

The other British paper, "An Examination of the Characteristics of Propulsion Systems for Inter-Orbital Transfer", by P A. E Stewart (Hawker Siddeley Aviation) was read in his absence by his colleague, Dr. W. F. Hilton. This described the more exotic forms of propulsion, including an omnidirectional method of focusing the Sun's rays for the direct heating of liquid hydrogen for use as a working substance.

Undoubtedly the most noteworthy paper of the morning was by another British delegate, Arthur C Clarke (Clarke-Wilson Associates, Colombo), under the title "The Social Impact of Communications Satellites" This was presented during the Space Law Colloquium and attracted widespread interest A future state of affairs where worldwide communications are as cheap and reliable as our present local communications was investigated. and its effects on social behaviour and trade were discussed Mr Clarke is, of course, well known for having started the idea of communications satellites by his article in October 1945 in *The Wireless World,* and indeed a reprint of this article was issued to all delegates

The Space Law Colloquium continued in the afternoon and there was a session on energy conversion and yet another simultaneous session on astrodynamics and guidance This last subject was treated as a round-table topic where a number of contributors made both prepared and unprepared comments, and included Prof Zdenek Kopal (University of Manchester)

On October 4 the round-table concept was continued with a discussion on exploration of the solar system by radar and radio astronomy, the Moon and Venus being considered in the morning and Jupiter and future experiments in the afternoon The radio reflexion experiments from the surface of the Moon appear to show fairly conclusively that much of the Moon is covered with a layer of dust having a thickness less than 1 cm The session on Jupiter was of interest on account of the detailed description of the radio emissions from the Great Red Spot The nature of these emissions is now fairly well established, but their cause appears to remain a mystery

The most significant communication at the Conference was given by the Russian delegation, who revealed that their cosmonaut Titov was space-sick throughout the duration of his 24-hr trip around the Earth, although this did not prevent him from sleeping in accordance with plans, or from carrying out routine duties which had been assigned to him

On October 5 the weather was fortunately much better, and the now traditional conference boat-trip was made down the River Potomac past the Naval Research Laboratory's radio telescope, on to Mount Vernon This informal occasion probably gives more opportunity for relaxed discussion between *ad hoc* groups of experts than is possible in the more formal atmosphere of the conference hall The Congress banquet was held in the evening and was addressed by Academician Sedov

On October 6 another British paper was presented, "Blue Streak as First Stage of a Satellite Launcher", by G K C Pardoe This described the present position of Britain's only large rocket, based on the design of the American *Atlas* rocket, which is the largest they have attempted to fire to date No particular reference was made to possible British or European payloads, although possible European collaboration in the future was mentioned Simultaneously, there was a round-table meeting on space communications, but most amazingly no reference was made to the use of radio, which is of course the obvious way for exploiting space for communications purposes The signals to be conveyed would be modulated on to beams of light emitted from optical masers This method gives a promise of tremendous band-width at some remote time in the future Use of ultra-violet light for communications was proposed by Mr J W Ogland, and one rather speculative paper suggested the use of γ-rays for communications If the vehicle is propelled by a nuclear rocket, the γ-rays are available as a by-product and some form of hinged lead sheet has to be contrived for signalling purposes This method is limited to use in space in the absence of any atmosphere and was shown to work at ranges up to 100,000 miles but the signalling frequency is limited to one cycle per second

Finally, on October 7, delegates were taken on a tour of the NASA Goddard Space Flight Center at Goddard Maryland

W F HILTON

THE VEHICLE DRIVER

A MEETING of the British Occupational Hygiene Society was held at the Traffic and Safety Division of the Road Research Laboratory during November 9–10 Six papers were read and discussed, for the first two, which dealt with bus drivers the chair was taken by Dr L G Norman, the president of the Society

Examination by Dr P Froggatt of data from Northern Ireland revealed that a curve of accident-rate plotted against age of driver had a broad minimum over the ages 45–55 years which was due to the beneficial results of experience being opposed by the decline in performance with advancing age The accident-rate of experienced drivers rose above 50 years of age

Mr C J Cornwall had examined the records of London bus drivers for the period 1957–59 for which the average rate was 1 37 accidents per driver-year on a mileage of about 14,000 Accidents were associated with traffic density The accident-rate fell with increasing experience more rapidly than it rose with age, the fall was marked up to 47 years and slower up to 70 Replacements of old drivers by younger men would increase the number of accidents

Dr R J Smeed, deputy director of the Road Research Laboratory, occupied the chair during a session which was opened by Mr W Gissane, who described the nature and cause of injuries resulting from motor-car accidents in a city Squadron-Leader D I Fryer told of the development of safety belts in aircraft, going back to Colonel Cody, who was the first man to fly a powered machine in England The design and testing of modern safety-belts were described in detail, and the value of rearwards-facing seats, adequately attached to the airframe, was emphasized

Road experience with safety belts was dealt with by Dr A J Moreland, who approached the problem of assessing accidents quantitatively by defining a damage index This number was evaluated from the damage to chassis, compartment and bodywork When divided by the weight of the motor-car it correlated well with the difference between the squares of the velocities of the objects coming into contact

The damage index divided by weight was used as a basis for comparing the frequency of casualties of various degrees Fatalities commenced at a figure near 15 and rose steadily, serious injuries rose from near the origin while slight injuries attained a maximum at about 25

Safety belts of good design, firmly attached, could prove beneficial, but there were suggestions that people drove faster when wearing them, and they did not check the forward velocity of the head on impact or protect it from objects flung forward from the rear The steering arrangements, likewise, might either promote injury or provide protection

The final paper was delivered by Mr I D Brown with Prof D D Reid in the chair Observations of police and other skilled drivers had been used in an attempt to relate driving performance to fatigue and distraction It seemed likely that there was spare capacity to draw on in case of need and that fatigue from general occupations before driving might be more serious than that induced by driving Tests of distraction by using tape recordings in which the subject was either interested, or not interested, were also carried out

The Road Research Laboratory demonstrated the telemetering of movements of the controls of a vehicle under way, of recording what happens in an accident and of experimental collisions The incorrect basis of early tests on motor-cycle crash-helmets was shown and specimens illustrated the protection afforded by helmets and the distribution of the injuries of motor-cyclists

The bulk of the meeting was concerned with the results of accidents and diminution of their effects on people, little attention was given to the prevention of motor-car accidents by methods within the control of the vehicle driver The papers and discussion will be published in the *Annals of Occupational Hygiene*

C. N DAVIES

MECHANISMS OF VIRUS INFECTIONS

A DISCUSSION meeting of the Royal Society, arranged by Prof Wilson Smith, on the subject of "Mechanisms of Virus Infections", was held on November 2 Owing to the large numbers attending, the meeting was transferred, by courtesy of Sir Lawrence Bragg, to the lecture hall of the Royal Institution The president of the Royal Society, Sir Howard Florey, took the chair

In his introduction, Prof Wilson Smith traced briefly the progress of virological knowledge from the early almost metaphysical questioning into the nature of viruses to the present wealth of information regarding their chemical constitution, structure and intracellular behaviour. He wondered, however, whether these particular aspects were not in danger of being over-emphasized at the expense of more traditional problems relating to virus disease The natural history of any virus disease is intimately related to the survival cycle of the virus concerned and, in the metazoon host, this cycle is necessarily complex The route of infection and primary lodgement, paths and mechanisms of spread through the host, replication and ultimate release must be considered at the various levels of the cell, the intact host and the host population It was with this in mind that the present programme had been built up around the concept of the survival cycle

Prof A W Downie (University of Liverpool) opened the discussion with a paper on "Mechanisms of Virus Entry and Spread within the Host (Animal Viruses)" Although a rapid survey of the various routes of infection known to occur was given to illustrate the extent of the subject, and problems associated with the question of neural spread in such diseases as poliomyelitis, herpes and pseudo-rabies were briefly discussed, the paper was centred on the pathogenesis of disease in the pox group of viruses Fenner's detailed work on ectromelia has laid a pattern which serves as a standard of reference for investigations with other members of this group, and a study of the highly lethal rabbit pox infection shows that it follows the same general course The main difference is that the stages of primary and secondary lodgement with final involvement of the skin, and of primary and secondary viræmia, are less well defined in rabbit pox than in mouse pox Despite thorough investigation of animals infected by the intra-nasal route, no primary lesion is identifiable although virus can be readily demonstrated in the tissues of the nasal passages and trachea The blood contains virus as early as the second day after infection and thereafter remains positive Clinical disease develops on the fourth day The finding that provoked the greatest interest and discussion was that animals do not become infectious by contact until the fifth or sixth day, at least 24 hr after the onset of clinical disease, a finding closely similar to that in human smallpox Following intranasal infection, virus may be isolated from the tissues of the upper respiratory tract in rabbit pox, and smallpox virus is presumed to invade by this route in the majority of human cases Why then is the patient not infective during the incubation period ? Dr C H Cadman (Scottish Horticultural Research Institute) dealt with the same subject in its relation to plant viruses and pointed out that there is an essential difference in the problems involved, since plant viruses are unable to effect entry into the tissues without external aid Trauma is an essential prerequisite to infection and is usually inflicted in Nature by insect vectors In the laboratory the necessary damage is achieved by rubbing the leaf, but the inefficiency of this procedure may be gauged from the fact that about 10^6 infective particles are necessary in order to produce a lesion. Further, since the plant does not possess conveniently isolated internal organs which can be segregated by dissection and to which the virus can track, spread of virus cannot be followed as in the animal host It is probable that virus spreads in the translocation stream, but if so the form of the virus at this time is not known Since certain viruses are apparently capable of dispensing permanently with their protein coat, it is possible that spread within the plant occurs in the form of ribonucleic acid The possible role of plasmodesmata and the problem presented by the fact that virus appears to spread through callus tissue cultures just as fast as through intact plant tissues were also discussed.

It was clear from both these papers, in their different contexts, that there is a long way to go before the problems of virus invasion of host tissues can be considered to be satisfactorily explained

"Intracellular Replication Mechanisms" were discussed by Prof M Stoker (University of Glasgow), who considered the subject from the point of view of the interrelationship of virus growth to cell growth The types of interrelationship exemplified by bacteriophage infection are (1) the extreme virulence found in the *T*-even phage where adsorption of the protein coat is sufficient to cause immediate death of the cell with breakdown of deoxyribonucleic acid; (2) intermediate virulence where the host cell is not immediately killed but cell division is prevented, and (3) the avirulent prophage relationship In the case of animal viruses, infection usually results in death of the cell, and the difficulties in demonstrating non-cytopathic infection of cells were discussed in detail For proof it is necessary to study isolates of single cells and demonstrate, first, release of virus and, second, cell division in that order

Prof Stoker concluded that there is as yet no satisfactory evidence for infections of this type with normally cytopathogenic viruses, but this has recently been acquired by Rubens with the Rous sarcoma virus so that this virus, which is not normally cytopathogenic, is the only one for which the formal criteria have been satisfied In the case of polyoma virus, either cytopathic effect or cell transformation may follow infection *in vitro*, but in the latter case, it has not proved possible to demonstrate virus in the transformed cells and these remain super-infectable with polyoma virus, albeit with difficulty. It must be concluded that persistence of the virus is not necessary for these cells to retain their character once transformation has occurred

Thus three types of non-cytopathogenic relationship appear to be possible In the case of the Rous virus both virus and cell multiply with the intermittent release of infective virus, in lysogenic bacteria both multiply together but virus is not released without induction and death of the cell occurring, and finally with polyoma the virus may induce a cell transformation without itself persisting

Dr A Isaacs (National Institute for Medical Research) considered "Interference Phenomena" as a further aspect of intracellular behaviour Many interference phenomena have now been shown to be mediated by interferon, and the properties of this substance were described It is a protein of molecular weight 63,000 which is produced by all vertebrate tissues so far tested All viruses examined have been found to provoke production of interferon and all are to some extent susceptible to its action In its protective action it possesses some specificity for the species in which it is produced It is non-toxic at viral inhibitory concentrations and does not inhibit cell division In infected cells it suppresses the production of viral nucleic acid, possibly through its inhibitory activity against oxidative processes providing the energy for synthesis As might be expected, therefore, it is found that viruses depending on oxidative processes for their replication are more strongly inhibited than those which do not have this dependence

The possible role of interferon in recovery from virus infections was discussed and some interesting speculations added on the possibility that the action of interferon in virus infections is incidental to its role in the normal cell. Dr Isaacs suggested that its function might in fact be that of excluding extraneous ribonucleic acid of whatever nature It is not formed during the first third of chick embryonic life when ribonucleic acid might be acting as a vital intermediary in the processes of morphogenesis, but thereafter is formed in increasing amounts in response to the appropriate stimulus It might be that its formation in later embryonic life is a mechanism for the protection of the tissues from unwanted morphogenetic stimuli This suggestion aroused some argument and it was pointed out that interferon does not exclude viral ribonucleic acid from the cell but apparently suppresses its formation.

The last paper on cellular relationships of virus was given by Prof G Belyavin (University College Hospital Medical School, London) on "Latency in Virus Infections" Prof Belyavin treated the subject from a purely theoretical point of view and demonstrated by means of a mathematical model that in any system where persistence of infection depends on a balance between the rate of replication of virus and the rate of replication of cells, an impossibly critical state would exist Such a system would inevitably lead either to the virus gaining the upper hand with eventual destruction of the whole cell population, or to cell multiplication outstripping virus multiplication with the elimination of the latter The assumptions on which the model was constructed did not include variation in the susceptibility of the cell population or the role taken by such substances as interferon The intervention of some such factors would be essential if a stable balance between virus and host cells were to be achieved

Epidemiological factors in relation to animal and plant virus diseases respectively were presented by Prof F. J Fenner (Australian National University) and Dr L Broadbent (Glasshouse Crops Research Institute) These two papers provided an interesting comparison of the problems in these two fields, the more so as Prof Fenner confined himself to a discussion of myxomatosis, a vector-transmitted disease A comparison of the Brazilian, Californian and Australian viruses in rabbits native to those regions suggests that, in the establishment of the disease in Nature, natural selection acts not on the virus as such but on the factor of transmissibility Thus the three types of virus are capable of infecting the Californian rabbit *S buchmani*, but whereas the attenuated Australian strain fails to give detectable virus in the skin lesions and the South American strain gives a titre of only 10^4/gm, the Californian virus achieves a titre of 10^8/gm and only the last is found to be transmissible by mosquitoes In Australia since 1952, the original virus, which killed close on 100 per cent of infected animals, has been largely replaced by less virulent variants The fall in mortality has not, however, been extreme or progressive, and the dominant strains have for some years remained in the 'grade III' category in which mortality is about 90 per cent; but since the infected rabbits live longer, the virus is available for transmission for a period of about 15 days as against 5 days with the highly virulent strains However, the increase in the numbers of genetically resistant rabbits may ultimately favour the more virulent virus.

The highly complex interplay of factors governing the epidemiology of myxomatosis was reflected in the field of plant viruses Dr Broadbent dealt with the epidemiology of plant virus diseases in wider terms, but the main interest in his paper lay not in the artificial spread of viruses by human agency or even by contact, but in the intricacies governing its transmission by vectors While the nature and static habits of plants eliminate the complications arising from behaviour patterns, they equally introduce factors specific to plant epidemiology Thus, while it is easy to understand that the size of plants and their position relative to each other and to sources of infection external to the crop must influence spread of infection, the results of the microclimate within the crop are more subtle. If plants are closely spaced, the transfer of vectors will be facilitated, but this may be offset by the development of temperature and humidity conditions within the crop which are not tolerable to them The vector will then be confined to the new shoots which may contain little virus Similarly, plants are in general more susceptible to infection when young, so that climatic influences on the time of greatest prevalence of the vector may determine whether or not infection is widespread irrespective of whether the vector is particularly numerous Despite these factors, which have no real counterpart in the animal virus field, there are more similarities than differences apparent in the epidemiology of plant and animal viruses and Dr Broadbent's paper served to illustrate some of these For example, some viruses, such as potato virus *Y*, can be picked up in a few seconds feeding and immediately spread to uninfected hosts These viruses do not persist in the vector for more than a few hours Others, such as potato leaf roll, require prolonged feeding to infect the vector, cannot be transmitted before the lapse of a latent period, and thereafter persist in the vector for many weeks. This contrast is very similar to that between myxomatosis and yellow fever

Finally, the damage which virus infection inflicts on the host was discussed by Dr J C N Westwood (Microbiological Research Establishment, Porton) in relation to the intact host and by Dr Alison Newton (University of Cambridge) at the cellular level Dr Westwood discussed "Virus Toxicity" and its possible role in the production of disease and ultimately death in fatal virus infections The term 'virus toxicity' was originally coined to cover the production, by massive doses of virus, of lesions or death in susceptible or non-susceptible animals without the occurrence of multiplication of the virus The toxic factor has always been inseparably associated with the virus particle itself Since virus disease is normally initiated by small doses of virus and accompanied by multiplication, it has seemed unlikely that 'toxicity' could be involved, and the latter has always been regarded as a freak laboratory phenomenon unassociated with production of disease It was suggested that this concept might be wrong Experiments were described which showed that the toxicity of rabbit pox virus is associated with a partial cycle of replication, as is the toxicity of other viruses in which such partial cycles have been sought Death in rabbit pox is preceded by the abrupt onset of shock, with some evidence of increased capillary permeability, and the clinical picture can best be explained by the presence of some toxic factor in the blood During the four days preceding death in acutely fatal infections, the virus concentration in the blood may rise to as high as 10^6 infective units/ml and similar concentrations occur in fatal smallpox and herpes infections in man The cytotoxic effect of such virus concentrations on the capillary endothelium could be the cause of the increase in capillary permeability and so precipitate the animal into shock Since no toxic factor other than the virus has ever been demonstrated to result from cell-virus interaction, the possible role of direct virus toxicity in disease must be seriously considered

Damage to the intact host depends on the damage produced at the cellular level, and Dr Newton considered the "Biochemical Aspects of Virus Infectivity" in relation to modification of cellular metabolism The main modifications are found first in the production of new virus and second in changes in the nucleic acid and protein synthesis Viruses which multiply in the cytoplasm make less use of the cell metabolism—for example, vaccinia virus does not affect the metabolism of deoxyribonucleic acid—and such viruses probably produce their damaging effects by competition for metabolites Those which multiply in the nucleus produce a more profound effect, since they must not only compete for metabolites but must also overcome the normal control processes of the cell Thus pre-labelling of the cellular deoxyribonucleic acid with tracer thymidine shows that herpes virus causes a rapid breakdown of cell deoxyribonucleic acid probably due to increased deoxyribonuclease activity similar to the increased ribonuclease activity stimulated by poliovirus It also stimulates synthesis of deoxyribonucleic acid These changes must damage the control mechanisms of the cell, and interfere with synthetic activities in the host In some viruses, such as encephalomyocarditis, viral ribonucleic acid is elaborated in the nucleus and protein in the cytoplasm It is probable that in these instances cytoplasmic protein metabolism is governed by viral ribonucleic acid which migrates from the nucleus The production of cell damage does not depend on completion of the multiplication cycle of the virus, for example, with poliovirus, herpes and rabbit pox viruses, inhibition of infective virus production does not prevent cell destruction It was suggested that cell damage results from the release of lysozomes, disruption of the controlling mechanism of the cell, competition for metabolites and disturbance of metabolic balance

The meeting was successful in provoking lively discussion and considerable disagreement on nearly every point raised by the main speakers Dr Sanders, in summing up, congratulated the participants on avoiding all reference to infective nucleic acids, and he also stressed the gulf which still exists between academic virology and virology as applied to disease

J C. N WESTWOOD

COTTON-GROWING IN NIGERIA

AGRICULTURAL progress relies on the pure sciences, on engineering and on economics These cannot come into play until major practical problems have been resolved into their scientific components Resolution is one of the functions of husbandry investigation Recognition of the nature and importance of this function has come only within the past three decades In this same period the other function of husbandry investigation has also been developed, namely, improvement of accepted practices in raising crops and livestock Cultivations, drainage, sowing, grazing, housing of stock and other day-to-day matters were not looked on, formerly, as problems amenable to study they were matters for the farmer's experience and tradition Modern husbandry investigation seeks to improve all working practices by studying their underlying purposes and interpreting their effects

That physical environment is primarily important for agriculture is now an axiom, though some costly flouting of it is yet fresh in memory It is largely through husbandry investigation that scientific attention has been brought to its present intensity in such matters as rainfall characteristics, solar radiation, air temperature, shade, wind and moisture-holding capacity of soils

Some highly successful husbandry investigations on cotton have recently been reported* from the Ministry of Agriculture Research Station at Samaru, Northern Nigeria

Past work at the Station and in its area of interest had revealed a discouraging situation Local yields were 150–200 lb of seed cotton an acre Late sowing partly accounted for this, but earlier sowing in experimental plots gave only 240 lb Assisted by fertilizers and by early planting the maximum yield was 600 lb. At these yields insecticidal protection did not pay Yield in a bad year was only half of that in a good one and in any small field good and bad patches markedly influenced growth and yield An improved variety of cotton, bred at the Station,

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* Lawes, D A, *Emp J Exp Agric*, 29, 116 (1961)

Table 1 ELECTROPHORETIC ANALYSIS OF BRONCHIAL MUCUS

Patient	Diagnosis	O	A_1	Streak	A_2	B	C	D	E	F
1 ♂	Rhinorrhœa	28 8	—	6 4	—	35 3	4 1	9 7	15 9	0
2 ♀	Aspergillosis	7 4	10 4*	—	14 6	31 0	?	14 2	21 7	1 3
3 ♂	Hæmoptysis	8 3	8 1†	4 8	—	27 2	—	21 0	20 0	10 3
4 ♂	Hæmoptysis	15 1	—	7 4	—	31 8	—	38 9 (mainly E)		6 7
5 ♂	Pneumonia	8 8	5 8	7 2	—	28 6	—	21 5	18 4	9 5
6 ♂	Pneumonia	20 1	—	6 9	—	24 5	5 2	14 6	22 4	6 3
7 ♂	Bronchial asthma	12 5	—	4 1	—	19 1	—	52 6 (mainly E)		11 4
8 ♀	Pulmonary alveolar proteinosis	12 8	—	—	—	22 5	—	32 6	20 2	12 6
9 ♂	Chronic bronchitis (L U L collapse)	14 3	—	15 2	—	9 6	—	53 (mainly E)		7 3
10 ♂	Chronic bronchitis	11 0	—	8 6	—	20 0	—	25 4	28	7 0
11 ♀	Chronic bronchitis	8 0	—	8 3	—	27 6	—	27 8	20 8	7 0
12 ♂	Chronic bronchitis	10 4	—	13 1	—	34 1	—	? 17 1	18 1	7 2
13 ♂	Chronic bronchitis	24 6	—	11 4	—	26 3	5 0	→ 13 4	16 5	2 3
14 ♂	Chronic bronchitis	9 6	—	3 6	—	18 6	—	54 8 (mainly E)		13 3
15 ♂	Chronic bronchitis	34 3	—	13 3	—	9 9	—	21 9	7 9	11 9
16 ♂	Chronic bronchitis (and emphysema)	16 1	—	12 4	—	14 8	—	43 7 (mainly D)		12 1
17 ♂	Lung carcinoma and chronic bronchitis	19 3	—	16 8	—	15 4	12 4	26 7 (mainly D)		7 9
18 ♂	Lung carcinoma	17 3	17 5 ?	4 7	—	25 1	—	17 5	14 7	3 3
19 ♂	Lung carcinoma	16 2	— ←	26	—	18 4		14	18 7	6 7
20 ♂	Lung carcinoma	9 8	10 8	10 1	—	27 8	—	21 2	13 3	6 0
21 ♂	Lung carcinoma	9 4	13 7	13 1	—	21 9	5 4	21 3	13 5	1 5
22 ♂	Lung carcinoma	6 5	9 7	5 3	—	32 2	5 4	16 1	21 6	3 1
23 ♂	Lung carcinoma	24 7	—	11 4	—	16 3	6 3	17 4	21 4	2 3
24 ♂	Lung carcinoma	7 4	—	11 6	—	15 4	39 2 (mainly D)		6 4	20 1
25 ♂	Lung carcinoma	17 4	—	18 4	—	14 2	6 2	22 1	10 0	11 8
26 ♂	Lung carcinoma	14 4	—	7 4	—	28 4	10 6	23 3	13 2	2 7

* Mobility, 0 09

† Mobility, 0 19

(M_F) A_1, 0 14 ± 0 04, A_2, 0·37, B, 0 57 ± 0 04, C, 0 67 ± 0 04, D, 0 79 ± 0 04; E, 0 88 ± 0 04; and F, 1 (9–10 cm in 16 hr) All components moved towards the negative electrode in the same direction as the endosmotic flow when the mucus was applied near (at 5 cm distance) the Whatman No 3 paper bridge (5 × 6 cm.) immersed in the anode vessel of an Evans Electroselenium apparatus Strips were dried immediately with hot air and fixed in absolute ethanol Components were revealed by dyeing (15 min) with 0 5 per cent bromophenol blue in ethanol saturated with mercuric chloride, then washed with several changes of 2 per cent acetic acid[2] They were estimated with an *EEL* scanner after clearing the strips with liquid paraffin in vacuum (3–4 hr) For completeness the fractions absorbed at the origin O and others only discernible as a streak between A_1 and A_2 were also scanned and expressed as a percentage of the total applied Components B and E had similar mobilities to serum albumin and γ_1-globulin, respectively In samples of chronic bronchitis sputum and mucus only components B, D and E reacted with antisera to human serum proteins The results obtained with various conditions are shown in Table 1 The bronchial mucus samples used were uncontaminated with saliva and obtained at bronchoscopy

Components B, D and E appear in all mucus samples and, except in rare instances, are the three major components Component F also appears to be a common constituent but can fall to very low levels Only in the case of rhinorrhœa was it completely absent Chronic bronchitis mucus samples present the simple patterns B, D and E and a generally high level of F Invasion of the lung by carcinoma produces the additional components A_1 and/or C Of the other cases, that of aspergillosis exhibits component A_2 uniquely and has an A_1 component of abnormally low mobility Both these could be the metabolic products of the invading mould Pulmonary alveolar proteinosis mucus contains an abnormally high content of component D

A similar examination of sputum samples, of which the bronchial mucus would constitute a high proportion, showed the same overall picture The cancer sputum samples (from seven patients) again showed additional components (usually C) not present in chronic bronchitis sputum and F was very low (1–3 per cent) Examination of sputa from one chronic bronchitic was commenced immediately after he had a 14-day course of tetracycline The following day he started a 7-day course of chloromycetin and throughout the period under review was having bronchodilators, breathing exercises and vigorous physiotherapy Following this treatment his general condition improved and his sputum gradually decreased from 2½ oz to ½ oz daily Over a period of three weeks analyses showed a remarkable decrease of component F from 17 2 to 4 3 per cent The neuraminic acid content of the sputum varied slightly over the same period from 1 23 to 1 39 per cent Strips stained with the periodic acid–fuchsin sulphite reagent[4] showed positive reactions only with components B, D and E Another chronic bronchitic with a carcinoma in the left hilar area maintained a high F content (9 5–21 5 per cent) throughout a three-week period of treatment with penicillin and achromycin

An alternative method of analysis of the bronchial mucus samples involved the use of the immunological reaction in agar gel diffusion cells[5] with an antiserum raised against human serum proteins Although no anti-F was present, it sufficed to distinguish the mucus of chronic bronchitis (3 lines) from that of patients suffering from bronchial carcinoma (4 lines), hæmoptysis (8 lines) and aspergillosis (at least 10 lines) Further development of this approach with additional antisera raised against component F and against selected whole mucus samples promises to afford a useful addition to methods of analysing bronchial mucus

This work was supported by grants from the Medical Research Council and the British Empire Cancer Campaign

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orcinol[7-9] or anthrone[10] method used. Detection of free sugar spots on paper chromatograms of acid hydrolysates of the protein (3 *N* hydrochloric acid up to 21 hr , 100° C) has not been achieved, possibly because of the low carbohydrate content, strong protein–carbohydrate bonds and/or sugar destruction The major sugar was, however, identified as galactose by a spectrophotometric method[7] and the nature of the spectrum as well as ratios between extinctions at various wave-lengths[7] suggested the presence of lesser amounts of glucose Similar criteria made probable the essential absence of keto-hexose, mannose and pentose The nature of the spectrum from the carbazole reaction[11] tended to eliminate hexuronic acid as a major constituent, while the presence or absence of hexosamine bound in the preponderant glycoprotein has not been definitely established Both hexuronic acid and hexosamine may be present in the scant orthochromatic material or sections stained with toluidine blue Sialic acid was essentially absent as judged by the observation that the tryptophan–perchloric acid test[12] yielded only a light colour, the intensity of which did not greatly exceed that obtained from an equal amount of beef serum albumin The amino-acids of the protein moiety have been examined by 2-dimensional chromatography of acid hydrolysates (6 *N* hydrochloric acid, 24 hr , 100° C) of the lipid-free material followed by ninhydrin development and visual comparison with spots given by an equal amount of bovine serum albumin of known amino-acid composition[13] which was hydrolysed in a manner identical with that used for the unknown protein Several comparisons were made for each amino-acid and the average results were accepted as a reasonable basis for calculating approximate amino-acid composition It was assumed that, for most amino-acids, errors associated with destruction or other variables were approximately the same for both preparations Perhaps of most immediate interest here is the fact that the protein is characterized by unusually large amounts of serine This substance was estimated to comprise approximately 15 per cent of the total amino-acid residue content on a weight basis The glutamic acid content was also found high as compared with many proteins (about 20 per cent) and aspartic acid low (about 5 per cent) Cystine was quite low, but quantitative estimates are not justified because of large variability in the destruction of this amino-acid as a function of the carbohydrate content of the hydrolysis medium[14] The levels of amino-acids did not vary significantly between batches obtained from normal and dystrophic animals It is not certain whether there are other quantitative differences in the composition of the two types of calculi

The mouse calculus material exhibits a chemical make-up which is markedly different from the organic matrix of crystallized human[15,16] or bovine[17] calculi and from a large number of mammalian proteins for which recorded data have been examined The composition is similar in a general way to that of the Bence Jones protein with regard to the contents of phospholipid, cholesterol and carbohydrate[18] as well as the high serine-level[19] but deviates radically in the contents of other amino-acids The significance of the influence of sex and of muscular dystrophy on the incidence of calculus excretion remains to be determined, however, beyond these considerations, it seems possible that the strain 129 mice may be a useful source of experimental animals for the study of calculus formation

This work was aided by a grant from the Muscular Dystrophy Association of America I wish to thank Dr Kay Fink for valuable suggestions concerning the preparation of the manuscript and Mrs Shirley Strachan for expert technical assistance

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MUCOPROTEINS OF BRONCHIAL MUCUS

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WARFVINGE[1] found that analyses of tracheo-bronchial secretions by electrophoresis on Whatman No 1 paper in barbiturate buffer *p*H 8 6, revealed at the most three mobile mucoproteins, the bulk of the material being absorbed at the origin

We have found that electrophoretic separation (16 hr) on cellulose acetate foil (5 × 25 cm) in citrate buffer (*p*H 5 1, μ 0 05) at 175–180 V and 0 6 m amp /cm revealed as many as seven well-defined components having electrophoretic mobilities

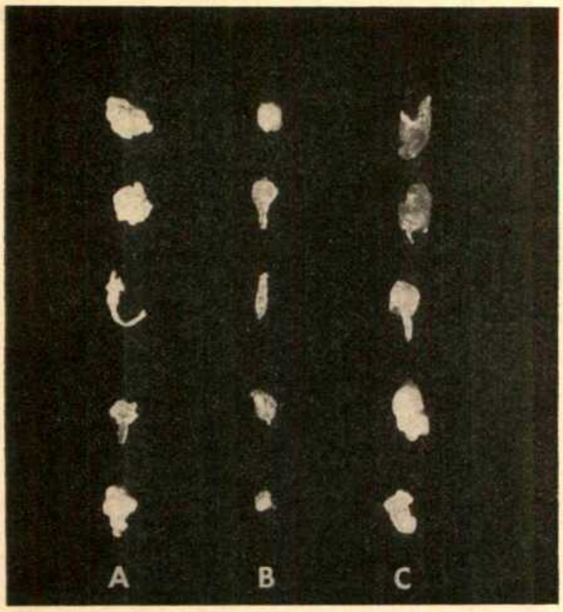

Fig. 1. Urinary calculi excreted by strain 129 mice. *A*, from homozygous normal mice; *B*, from 3–5 month-old male dystrophic mice; *C*, from male dystrophic mice in a more advanced stage of the disease. (× *c*. 2)

and staining techniques. Photomicrographs of portions of sections stained with various reagents are shown in Fig. 2. An outer wall was usually apparent and was defined best with hæmatoxylin and eosin stains (Fig. 2*A*). The portion of the wall shown is much wider than was generally seen but serves to illustrate certain small areas which stain blue with hæmatoxylin and appear dark in Fig. 2*A*. Since these areas did not react positively to Feulgen stain, they may represent minute zones of calcification rather than deoxyribonucleoprotein. The remainder and by far the major portion of the concretion showed only the light pink colour of the non-specific eosin. A strongly positive periodic acid–Schiff reaction (Fig. 2*B*) was shown throughout the calculi, indicating the presence of significant amounts of carbohydrate. Toluidine blue stain (Fig. 2*C*) showed thin sparse concentric rings of orthochromatic material only near the periphery while the remainder of the section reacted negatively to this stain suggesting that acid mucopolysaccharides are not essential for the formation of the calculi. Von Kossa stain for insoluble phosphates, carbonates and soaps gave a negative reaction and a sparse positive response was obtained to sudan IV at room temperature, suggesting small amounts of low-melting lipid. No evidence of a nidus with a separate identity was seen.

Water-insoluble protein comprised about 75 per cent of the original wet weight of the calculi and water-soluble solids about 8 per cent. To prepare the protein for chemical analysis, the calculi were ground in a mortar with distilled water and the insoluble residue was centrifuged, washed twice and dried to constant weight. Despite the fact that the availability of material has been quite limited, the use of sensitive micro-techniques has permitted qualitative identifications and quantitative estimates of its major constituents, and some of the more important results are reported briefly here. The complex protein exhibited an unexpectedly high nitrogen content (16·9 per cent in one batch assayed by digestion and Nesslerization[2]) and contained 1·5–2 per cent easily extractable lipid (four extractions of 30–45 min. each with 3 : 1 ethanol–ether at about 75° C.). About 20 per cent of the lipid was phospholipid as determined by conversion of inorganic phosphorus levels (methods of Fiske and Subbarow[3]) in lipid digests to 'average' phospholipid values according to Gortner[4]. Cholesterol, assayed by the method of Searcy and Bergquist[5], comprised approximately 10 per cent of the lipid. Both free and esterified forms were present but in varying ratio. Amide nitrogen, analysed in *N* hydrochloric acid hydrolysates (4 hr., 100° C.) by the Conway diffusion method[6], constituted about 1·3 per cent of the lipid-free protein. Carbohydrate was present to the extent of a few per cent although the values have varied with the

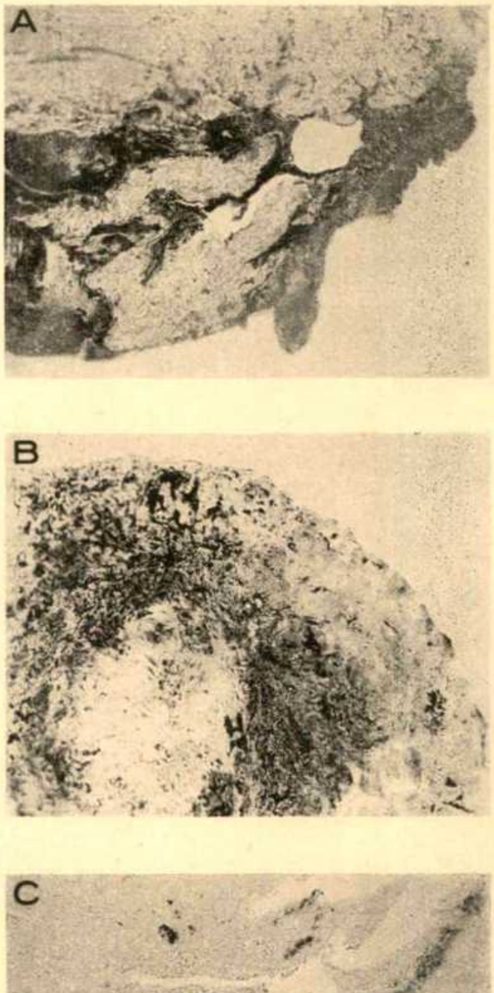

Fig. 2. Photomicrographs of outer portions of stained sections from urinary calculi. *A*, stained with hæmatoxylin and eosin; *B*, with periodic acid–Schiff reagent; *C*, with toluidine blue. (× *c*. 66)

by Medawar[18]). This theory postulates that all immunologically immature cells are susceptible to induction of tolerance, regardless of whether they are in the embryo or adult. The exposure of adult animals to antigenic stimuli should therefore set in motion two processes: sensitization of mature cells and induction of tolerance in immature cells. Tolerance would normally be masked by the immune response, but if the sensitized cells were to be removed by some means (as for example by prolonged contact with the antigens) the lymphoid cell population of adult animals might be changed progressively from a predominantly sensitized to a predominantly tolerant one[19]. Although nothing is at present known of the specificity or the mechanism of the striking effect described by Shapiro *et al.*[17] (for example, is it due to central or efferent inhibition?) their experiments are open to this interpretation. That smaller genetic differences can be overcome by a much less-formidable antigen dose suggests the possibility that the number of sensitized cells involved in such a situation is considerably smaller.

Our own results also could be accommodated by the stem-cell theory, the neonatal or very young mouse being vastly more amenable to induction of tolerance across the *H*-2 barrier than the 'adolescent' or adult mouse precisely because its population of lymphoid cells is still predominantly immature. The occurrence of increasing numbers of immunologically mature cells in developing animals would therefore be sufficient to account for the age-dependence of induction of tolerance. The experimental evidence of Howard and Michie[9] certainly suggests that even the neonatal mouse may already have some immunologically mature cells.

This alternative interpretation, according to which the difference between unresponsiveness in adults and tolerance in neonatal animals would be one of strategy rather than of basic mechanism, is of course highly speculative. Simonsen's[20] observation that adult lymphoid cell populations may, in time, develop tolerance of the host's tissue antigens while retaining the ability to respond to the tissue antigens of other mouse strains suggests that it may not be well founded; but much depends on the elucidation of the mechanism which underlies this observation.

We wish to thank Prof. P. B. Medawar for his interest in this work and for his much-valued criticisms.

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EXCRETION OF UNCRYSTALLIZED URINARY CALCULI COMPOSED OF GLYCOLIPO-PROTEIN BY NORMAL AND MUSCULAR DYSTROPHIC MICE

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STRAIN 129 mice, susceptible to a hereditary myopathy[1], excrete urinary calculi examples of which are reproduced in Fig. 1. These bodies have been located *in situ*, floating freely in the urinary bladder, and are probably vesical calculi. They may appear either hyaline or opaque and sometimes exhibit bizarre forms. The largest calculi, the maximum length of which was 3·5 mm. (Fig. 1*C*), were obtained from two dystrophic male mice which were in a more advanced stage of the disease than any of the other animals tested, with the hind legs extended and non-functional. Calculus excretion by one dystrophic and one heterozygous normal animal began at about three months of age.

The rate at which the calculi are excreted is a function of sex and of the presence or absence of muscular dystrophy (Table 1). The results shown are for representative animals; however, all dystrophic males studied excreted calculi at a greater rate than any of the normal males, and normal females have never yet been observed to pass a calculus. Dystrophic females occasionally yielded tiny amorphous hyaline bodies.

Sections were prepared for histochemical examination by formalin fixation of the concretions followed by conventional paraffin block mounting, slicing

Table 1. RATES OF EXCRETION OF URINARY CALCULI BY STRAIN 129 MICE

Type of mouse	Sex	Age* (months)	Collection period (days)	Rate (calculi/ 10 days)
Normal (Homozygous)	M	5	61	1
Normal (Homozygous)	M	3	36	0
Normal (Homozygous)	F	3	36	0
Normal (Heterozygous)	M	5	36	4
Normal (Heterozygous)	F	5	29	0
Dystrophic	M	3	63	6
Dystrophic	M	—	61	13
Dystrophic	M	—	18	8
Dystrophic	F	5	36	0

* Age at which collection was begun.

—, age unknown but the mouse was in an advanced stage of dystrophy and died during the collection period.

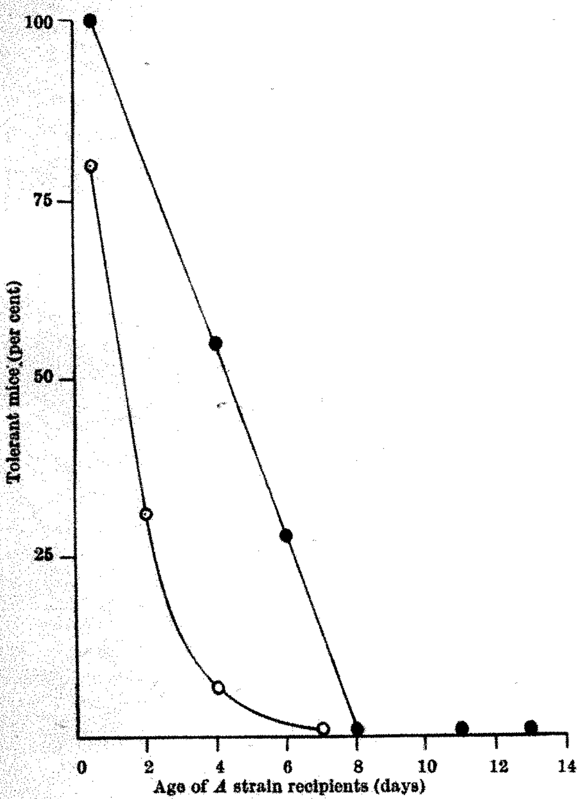

Fig. 2. Age-dependence of tolerance induction in *A* strain mice. Criterion of tolerance: skin graft survival at 5 × control median survival time (= 63 days). ○——○, 4–10 × 10^6 parental strain (*CBA*) cells per mouse. ●——●, 5 × 10^6 F_1 hybrid cells (*CBA/A*) per gm. body-weight

of these mice gave a weak second-set response, that is, they behaved as if the allogeneic (= homologous in the old terminology; see Gorer, Loutit, and Micklem[3]) cells had sensitized them.

The 11- and 12-day-old mice in the experiments described so far were injected with doses of cells which, scaled down according to body-weight, would have been more than adequate to bring about tolerance in neonatal mice. In order to investigate still further the relationship between tolerance and immunological paralysis groups of 12-day-old mice were injected with up to 10 times the weight-adjusted dose of *CBA/A* hybrid spleen cells (max. 3 × 10^8). The results are summarized in Table 1. Of the 26 mice not one was made tolerant by these massive stimuli and the mean survival-time of the grafts actually declined slightly with increasing dose, suggesting that larger numbers of cells sensitized more efficiently. (As the sensitivity elicited by allogeneic lymphoid cells declines relatively rapidly[4,5] a greater expression of sensitivity than that encountered by us could scarcely have been expected.) Hence, confrontation of these mice with a single cellular stimulus, however large, did not lead to the induction of tolerance.

Table 1

Exp. No.	Cell dose	No. of mice	No. of surviving grafts			Approx. MST (days)
			8 days	10 days	12 days	
6	WAD*	19	18	8	5	9½
8	3 × WAD	12	6	1	0	8
10	6 × WAD	2	2	0	–	8
15	6 × WAD	7	3	0	–	
17	4 × WAD i/v 6 × WAD i/p†	5	2	1	1‡	

* WAD, weight-adjusted dose = 5 × 10^6 cells per gm. body-weight = approx. 30 × 10^6 cells per mouse.

† I/p injection carried out on the following day.

‡ Graft destroyed by the thirteenth day.

It may be argued that the shift of the proportion of mice made tolerant in the first post-natal week is due to the fact that in Billingham and Brent's experiments parental strain cells rather than hybrid cells were used and that, in their experiments, the number of tolerant mice was therefore reduced by deaths from runt disease. Such an explanation is unconvincing, for a repetition of their experiments, using a constant number of *CBA/A* hybrid cells instead of *CBA* cells, is giving almost identical results. (This experiment is still in progress.) It is much more likely to represent a true shift produced by the higher antigen dose, an explanation which obliges us to revise our concept of the "neutral" period[6]. Such a period would appear to exist, if at all, only in a relative sense; it evidently depends not only on the degree of genetic affinity between cell donor and recipient[7,8] but also on the number of cells injected. In other words, immunological 'neutrality' for one antigen dose does not necessarily apply to another which is either larger or smaller—a conclusion which is in harmony with the observation that even new-born mice may be capable of immunological responses provided that they are stimulated by rather small doses of allogeneic cells[9]. That tolerance induction during the 'neutral' period should depend on antigen dose is perhaps not too surprising in view of the fact that induction of tolerance in new-born mice is also dependent on dose[1,9,10].

The point which stands out clearly from our results is that induction of tolerance in very young mice is not simply a scaled-down version of the kind of immunological unresponsiveness which can be brought about with soluble proteins in adult animals (for comprehensive reviews, see Chase and Smith[11,12]). Experiments somewhat similar in design but concerned with the induction of tolerance of bovine serum albumin in rabbits have been described by Smith[13], who found that with weight-adjusted doses tolerance could be elicited at birth or 7 and 14 days later, but not when injection was delayed until 21, 28 or 40 days after birth. (See also the work of Dresser[14] with mice.) Smith concluded that "... the induction of tolerance during the neonatal period depends upon special conditions which are unique to this period of life ...". Our own results are not at all at variance with this conclusion.

Nevertheless, it is possible to interpret our results by stipulating that the difference between the neonatal or very young mouse and the adult mouse is quantitative rather than qualitative. Such an alternative interpretation is prompted by findings reported from several other laboratories. (1) Tolerance of male isogenic grafts can be brought about in adult females by a single intravenous inoculation of approx. 20 × 10^6 male spleen cells[15]. (2) If donor and recipient differ only at the *H*–3 histocompatibility locus, adult mice can be sensitized or rendered unresponsive to skin grafts depending on the number of lymphoid cells injected[16] (1–5 × 10^6 cells sensitized, whereas 2 × 10^8 produced unresponsiveness). (3) Adult mice can be made unresponsive, even when donor and recipient strains differ at the *H*–2 locus, if they are injected intravenously and over a prolonged period of time with large numbers of spleen cells[17] (1·5 × 10^9 in 7 weeks).

These observations could be accounted for by the stem-cell theory of tolerance induction (see discussion taneously prevented (effect mentioned in 3), and the net result of efferent bundle activation is to gate out auditory input to the brain.

This work was supported by the U.S. Air Force (*AF* 61(052)120) and by the National Institute of Neurological Disease and Blindness, U.S. Public Health Service (*B*2482).

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CELLULAR DOSE AND AGE OF HOST IN THE INDUCTION OF TOLERANCE

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THERE has so far been no systematic study of the relationship between antigen dose and age of host in the induction of tolerance of tissue homografts. In previous experiments[1] conducted with mouse strains differing at the *H*-2 histo-incompatibility locus a constant number of adult spleen cells (4–10 × 10^6) were injected into groups of mice of various ages and the decline of tolerance induction with increasing age was studied. This decline occurred in the first few days of life and was found to be very rapid: only 13 per cent of *A* strain mice injected on the 4th postnatal day with *CBA* spleen cells showed any significant degree of tolerance when skin-grafted 6 weeks later. This kind of approach is open to the objection[2] that the antigen dose does not keep pace with the increase in weight of the young recipients; our latest experiments, of which a preliminary account is given here, were designed to re-investigate the age-dependence of induction of tolerance with this objection in mind.

The mouse strains were identical with those previously used except that *CBA*/*A* hybrid cell donors were preferred to *CBA* donors in order to avoid the complications of graft-against-host reactions[1]. Spleen cells from hybrid donors were injected into 6 groups of *A* strain mice ranging in age from about 12 hr. to 13 days after birth. Each group comprised 16–20 individuals, and all injections were made intravenously. In the youngest groups the sigmoid sinus or the orbital branch of the anterior facial vein were used[1]; in the older groups, one of the lateral tail veins. The dose of cells was 5 × 10^6/gm. of body-weight (weight-adjusted dose) and the cell suspensions were prepared according to the method described previously.

All mice were grafted with female *CBA*/*A* hybrid skin 55 days after injection. The grafts were inspected 8 days later, that is, at a time when a first-set response can still be clearly distinguished from a second-set response, and they were observed for 100 days. The median survival time of control grafts (*CBA*/*A* hybrid skin on normal adult *A* strain mice) being 12½ days, the grafts were classified according to whether they survived for at least twice the mean survival time (25 days; Fig. 1) or 5 times the mean survival time (63 days; Fig. 2).

The results are set out in Figs. 1 and 2 alongside the original (*constant dose*) data of Billingham and Brent. They show very clearly that, in the first week after birth, the injection of a weight-adjusted dose gave rise to a consistently higher proportion of tolerant animals. For example, in the 4-day-old group the proportion of tolerant mice rose from 13 to 85 per cent, and even in the 6-day-old group it remained at a relatively high level (45 per cent compared with 0 per cent) (Fig. 1). Similar increases become evident when the data are plotted according to our more stringent criterion of tolerance (Fig. 2). Without doubt the use of a weight-adjusted dose added about 4 days to the period in which tolerance could be induced in this particular strain combination.

The second point emerging from these results is that the injection of a standard dose into 12- or 13-day-old mice did not lead to induction of tolerance in a single mouse; on the contrary, a small proportion

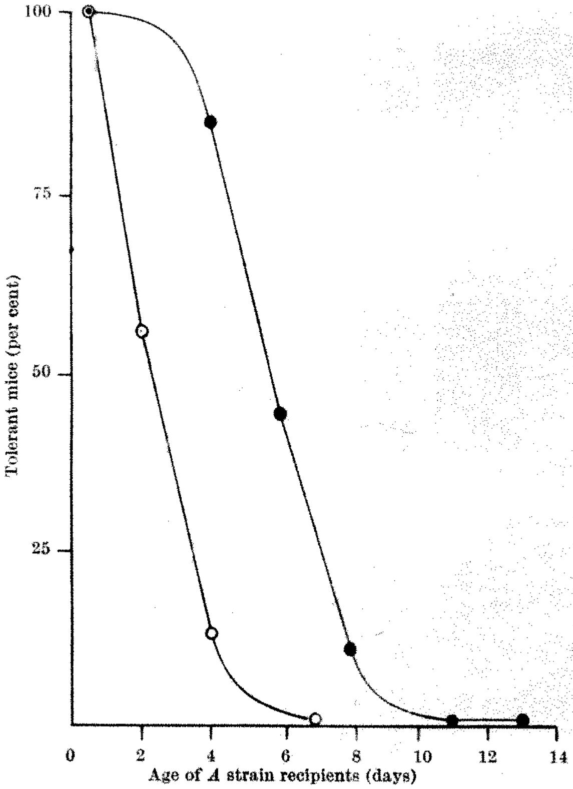

Fig. 1. Age-dependence of tolerance induction in *A* strain mice. Criterion of tolerance: skin graft survival at 2 × control median survival time (= 25 days). ○——○, 4–10 × 10^6 parental strain (*CBA*) cells per mouse. ●——●, 5 × 10^6 F_1 hybrid cells (*CBA*/*A*) per gm. body-weight

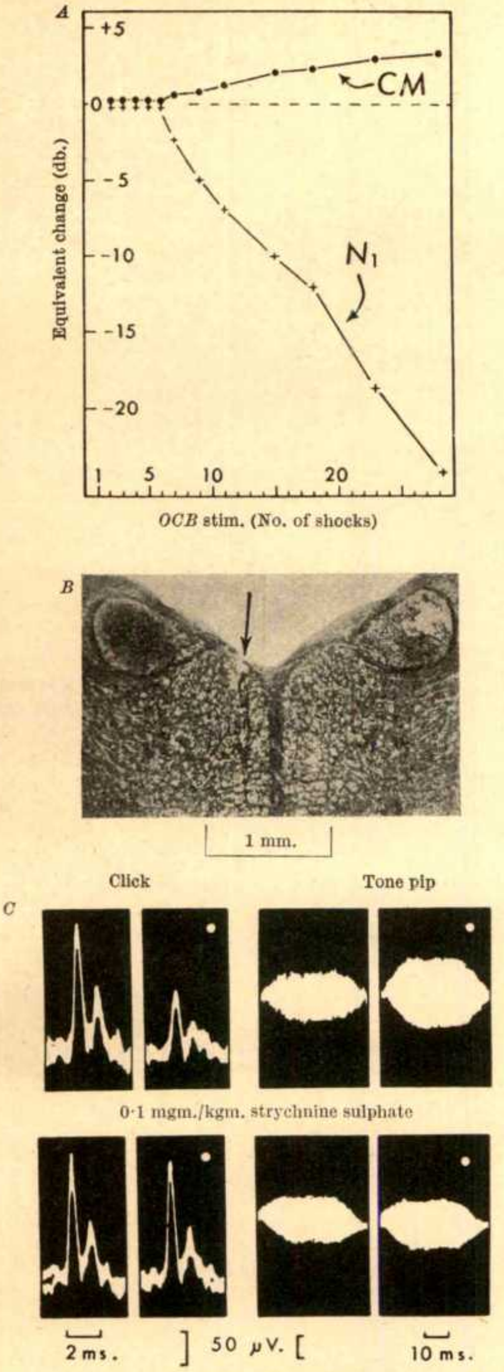

Fig. 1. Cat No. *A*295. Middle ear muscles cut. Clicks or 3 kc./s. tone pips, 30 db. above acoustic threshold, were delivered to the ear 10 msec. after delivery of a train of electric pulses at 300/sec. to the crossed olivo-cochlear bundle (*OCB*). *A*, amplitude changes in cochlear microphonic (*CM*) and neural (N_1) responses to sound as a function of the number of shocks in the preceding train delivered to *OCB*. *B*, histological control: section (modified Bielchowsky) with prussian blue mark of tip of stimulating electrode in *OCB*, between the two facial genua. *C*, oscillograms (several sweeps superimposed) showing suppression of N_1 spike and potentiation of *CM*, before and after strychnine. In the frames marked with a white dot, the sound was preceded by *OCB* stimulation

(*c*) *Neuropharmacology*. The intravenous injection of 0·05–0·15 mgm./kgm. strychnine sulphate which abolishes the N_1 inhibition[5] also eliminates the *CM* potentiation (Fig. 1*C*). The related alkaloid brucine produces the same antagonism to efferent bundle effects. No changes have been observed with metrazol, picrotoxin, ammonium chloride, eserine or dihydro-β-erythroidine.

For quantitative assessment, the changes in response voltage induced by bundle stimulation have been evaluated in relation to the intensity function of responses to sound alone. In many experiments the maximal suppression of N_1 was strikingly powerful, being equivalent to a 24-decibels attenuation of the sound energy. By contrast the maximal *CM* potentiation only amounted to a 3 db. increase (Fig. 1*A*). The two effects are thus of a quite different size, but both have to be considered when discussing the mode of action of the efferent axons on the inner ear and the following implications seem warranted.

(1) Since *CM* is generated in the hair cells of the organ of Corti[6,7] and is a 'receptor' potential[7], the recorded *CM* potentiation implies that the efferent axons must go as far peripherally as the hair-cell membrane. This is a useful cue for electron microscope studies.

(2) In view of the similar neuropharmacological properties, the efferent axons should manufacture and release the same inhibitory chemical transmitter as the axons responsible for post-synaptic inhibition in the cat's spinal cord[8]. Both inhibition of N_1 and potentiation of *CM* are ascribable to this transmitter (Fig. 1*C*).

(3) The olivo-cochlear suppression of N_1 being an obvious case of post-synaptic inhibition would depend on stabilization and possibly hyperpolarization of the terminal membrane of the afferent auditory axon since the latter represents the post-synaptic component in the excitatory afferent synapse between hair cell and auditory axon (Fig. 2).

(4) In order to account for the *CM* potentiation, we further suggest that part of the inhibitory transmitter is released on to the (pre-synaptic) membrane of the hair cell the resting potential of which would be increased by the usual selective change in ionic permeability. The *CM* receptor potential would thereby be potentiated since it is generated as a modulation of the hair cell resting potential (plus endolymphatic potential) by the acoustic transducer effect[6,7]. This would be the first evidence for a lack of restriction of transmitter effect to the post-synaptic membrane. Such a phenomenon might only occur in special and/or primitive synaptic organizations.

(5) The potentiation of the receptor potential is a paradoxical effect since the activation of the auditory axon by sound via the hair cell transducer is simul-

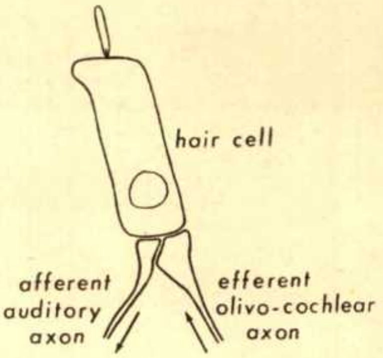

Fig. 2. Tentative diagram of synaptic organization in the organ of Corti, as suggested by the physiological evidence discussed. Electron microscope data would appear compatible with the occurrence of two types of axon terminals at the base of the hair cell, but the efferent endings have not yet been precisely identified

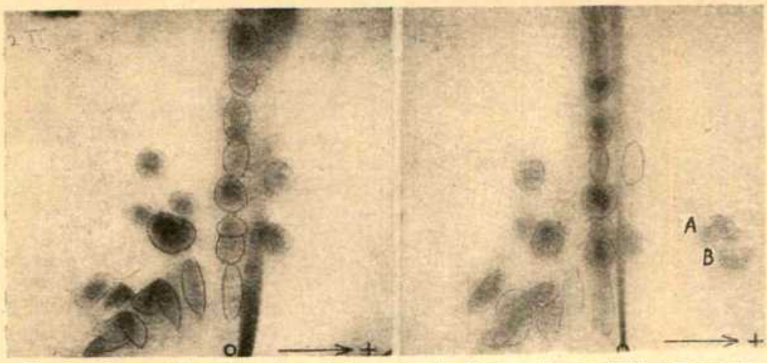

Fig. 3. Fingerprints of trypsin and chymotrypsin digests of hæmoglobin components separated on carboxymethyl cellulose columns described in Fig. 1. Paper electrophoresis of fraction II (left) and III (right) of the 'experimental' components applied at the origin (*O*) was carried out in the horizontal direction (cathode on the left), in pyridine/glacial acetic acid/water (10 : 0·4 : 90), *p*H 6·4 buffer, followed by paper chromatography in the vertical direction (ascending) using butanol/acetic acid/water (3 : 1 : 1) as solvent. Two peptides are demonstrable in the abnormal hæmoglobin component (*A* and *B*). No similar peptides were present in the other fractions

produced by nucleoproteins entering the cell and successfully competing or interfering with normal information transfer within the cell. (In unpublished experiments carried out in this laboratory, using similar culture conditions described here, influx of phosphorus-32-labelled DNA into rabbit marrow cells has been demonstrated[12].) It is unlikely that the abnormal hæmoglobins are due to artefacts such as denaturation or altered pigment formation since a distinctive peptide pattern has been observed in two experiments where fingerprint characteristics have been investigated. Experiments investigating the exact nature of the agent producing the change as well as structural considerations of the abnormal hæmoglobin are in progress.

In summary, abnormal hæmoglobin formation can be induced in human megaloblastic marrow cells exposed to nucleoproteins derived from sickle-cell anæmia. The data are not sufficient to identify with certainty either the product formed or the agent producing these changes. The electrophoretic behaviour of the abnormal hæmoglobin and its elution from carboxymethyl cellulose closely resembles sickle hæmoglobin, but the peptide analysis is distinctly different.

This investigation was supported by a research grant (*C*–4944) from the National Cancer Institute, National Institutes of Health, United States Public Health Service. Portions of this article were presented at the seventy-fourth annual meeting of the Association of American Physicians, May 3, 1961, and are published in the *Transactions* of the Association. We thank Drs. John Spizizen, Jack Pensky and Melvin Schoenberg for valuable advice and support.

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MODE OF ACTION OF THE EFFERENT OLIVO-COCHLEAR BUNDLE ON THE INNER EAR

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THE Rasmussen olivo-cochlear bundle[1] represents the most peripheral part of a polysynaptic centrifugal system presumably responsible for control of acoustic input[1,2]. It originates in cells of the superior olive complex and terminates close to the organ of Corti in the inner ear[1]. Direct faradization of the bundle is known to depress acoustic evoked responses[3] and the results to be presented are concerned with the peripheral mechanism of this gating effect.

In experiments on cats (pentobarbitone, chloralose or high spinal section; middle ear muscles stapedius and tensor tympani cauterized; 'Flaxedil' infusion) the auditory nerve compound action potential (N_1) evoked by clicks and the cochlear microphonic potential (*CM*) evoked by tone pips have been recorded through an electrode placed on the round window. Trains of electrical pulses were applied to the crossed olivo-cochlear bundle through bipolar stereotaxic electrodes (Fig. 1*B*) at known intervals before delivery of the testing sound (click or tone pip) to the ear. As illustrated in the upper row of records of Fig. 1*C*, effective stimulation of the efferent bundle produces two effects, namely, inhibition of the N_1 spike[3] and potentiation of *CM* (ref. 4). Our observations suggest that both effects are genuine and that they are simultaneously produced by a chemical transmitter released from the olivo-cochlear axon terminals.

(*a*) *Parameters*. The two effects disclose proportional quantitative changes when the parameters of the preceding bundle stimulation are systematically modified. In the graph of Fig. 1*A*, trains of 1–28 pulses at 300/sec. were delivered to the bundle 10 msec. before the testing sound: changes in N_1 and *CM* appear after the train of 6 shocks and increase in parallel with the number of shocks.

(*b*) *Dissipation*. When the testing sounds are applied at increasing intervals after a standard train, of say, 20 pulses at 400/sec., N_1 suppression and *CM* potentiation appear maximal at the end of the bundle stimulation and dissipate exponentially with a time constant of about 0·15 sec.

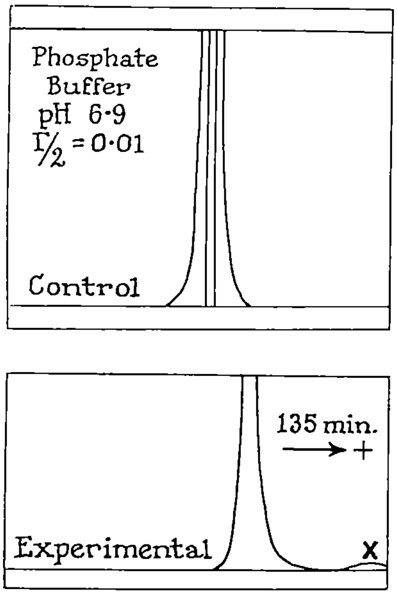

Fig. 1. Tiselius electrophoretic pattern of hæmoglobin derived from megaloblastic marrow incubated with and without sickle cell nucleoproteins. A single peak was observed in the control whereas an additional component (*x*) appeared in the experimental hæmoglobins. (Phosphate buffer, *p*H 6·9, ionic strength 0·1)

To the experimental flask was added 2 mgm. of sickle-cell nucleoproteins dissolved in 2 ml. of 0·9 per cent sodium chloride. The control incubation mixture was identical except that an equivalent volume of saline was substituted for the nucleoproteins. Incubation was carried out for 24 hr. at 37° C. with gentle agitation and the reaction terminated by the addition of deoxyribonuclease and 0·01 *M* magnesium sulphate. The cells were sedimented by centrifugation at low speed and washed 4–6 times, sedimenting each time from about 4 volumes of 0·9 per cent sodium chloride. The cells were lysed by freezing and thawing and the cell ghosts were removed by centrifugation at 30,000*g* for 30 min. The resultant hæmoglobin solutions were dialysed for 3 days against 0·001 *M* phosphate buffer, *p*H 7·2, with three changes of buffer followed by high-speed centrifugation. Altered hæmoglobin formation was detected by various electrophoretic procedures, ion-exchange chromatography and peptide analysis. An unexpected and consistent finding was that the radioactivity present in the hæmoglobin derived from marrow incubated with the nucleoproteins was 2–4 times that present in the control samples. Thus, in the experiment reported in this article, 0·8 per cent of the iron-59 added was incorporated in the control as compared with 2·35 per cent incorporated in the experimental.

The electrophoretic mobility of the hæmoglobins was first compared on either agar-gel or starch-gel and by moving boundary electrophoresis. Abnormal components were demonstrable in the experimental hæmoglobin solutions as compared with the control samples in five of nine experiments. One such component demonstrable by Tiselius electrophoresis is indicated in Fig. 1. Elution of an abnormal component from a carboxymethyl cellulose column[10] was achieved by establishing a *p*H gradient with 0·01 *M* phosphate buffer. This abnormal hæmoglobin component (III, Fig. 2) closely resembled sickle-cell hæmoglobin in its elution behaviour. No difference has been observed between control and experimental carboxymethyl cellulose elution patterns of hæmoglobin when megaloblastic marrow was incubated with DNA derived from normal marrow.

The abnormal component was concentrated by evaporation of the eluates, dialysed in the same manner as described, and a peptide analysis performed. Since there was no comparable peak in the control, the peptide composition of the abnormal component (III, Fig. 2) was compared with the peptide composition of hæmoglobins derived from peaks I and II in both the control and experimental samples. Peptide fingerprints of the hæmoglobins were carried out by the procedure of Ingram[4] using the modified digestion method of Anfinsen *et al.*[11]. Samples were run in pairs under identical conditions using the same quantity of hæmoglobin. Two peptides which are not demonstrable in any other fraction were present in the fingerprint of the abnormal hæmoglobin (*A* and *B*, Fig. 3). This peptide fingerprint does not, however, resemble that derived from sickle-cell hæmoglobin where the abnormal peptide is found in juxtaposition to peptide No. 4. Neither does it resemble the peptide pattern of fœtal hæmoglobin when compared with fingerprints of cord blood obtained by the same technique. An identical abnormal peptide pattern was obtained in another experiment of this type using similar conditions.

The possibility therefore exists that the megaloblasts can be induced to form a new hæmoglobin as the result of altered peptide synthesis and that therefore a phenotypic alteration or mutant has been produced. The electrophoretic behaviour of the abnormal component and its elution from carboxymethyl cellulose columns closely resemble those of sickle-cell hæmoglobin but the peptide analysis is distinctly different. It may be postulated that such a mutant could be

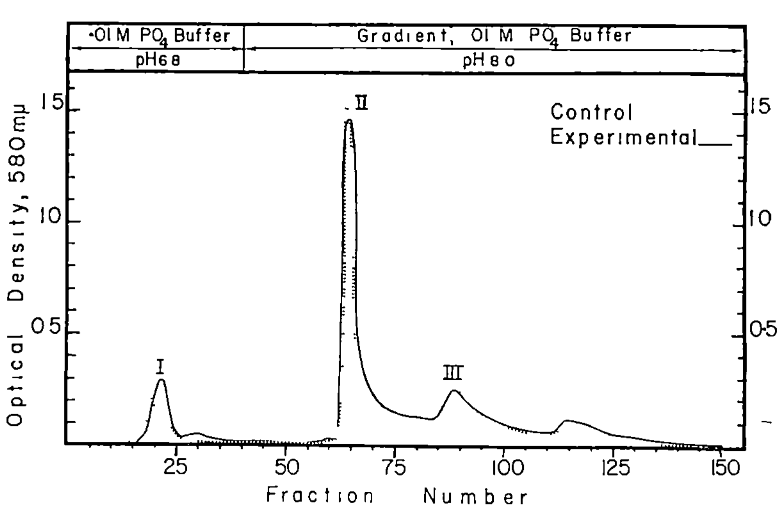

Fig. 2. Separation of hæmoglobin components of megaloblastic marrow incubated with and without sickle-cell nucleoproteins. Approximately 150 mgm. hæmoglobin described in Fig. 1 were introduced on to a carboxymethyl cellulose column (52 × 1·3 cm.) and the hæmoglobins separated by elution with 0·01 *M* phosphate buffer (*p*H 6·8–8·0). 4·0-ml. aliquots were collected at flow-rates of 20 ml. per hour. Hæmoglobin concentration was measured at 580 mμ. An abnormal component (III) was demonstrable in the 'experimental' as compared with the control

organs *in vitro*[3] but to be largely confined to glomerular basement membranes *in vivo*[10].

Since anti-rat and anti-dog placenta sera have been shown to be nephrotoxic, and nephrotoxic sera are always localized on the glomerular basement membranes *in vivo* and *in vitro*, it would appear reasonable to speculate that anti-human placenta sera which are localized on human glomerular basement membrane *in vitro* might also contain glomerular-damaging antibodies among the localizing antibodies. Certainly absence of localization on the human glomerular basement membrane by anti-human placenta sera would be substantial evidence against glomerular-damaging antibodies being present.

Recently, Dixon[11] has shown that repeated intravenous injections of foreign protein produces glomerular damage in rabbits by means of antigen–antibody complexes. These complexes become localized in and damage glomeruli without any immunological specificity for kidney antigen. This alternative method of damaging glomeruli would have to be considered in the etiology of toxæmias of pregnancy because placental antigens might continually leak into the maternal circulation and form antigen–antibody complexes which become localized in glomeruli for non-immunological reasons and damage them.

Although the basement membranes in many organs may contain antigens in common with the maternal human glomerular basement membrane, the placenta is unique during pregnancy. The placenta is the only organ in the body which has the capacity to vary its antigenic structures by paternal contribution to its composition. This variation may be sufficient to cause some of the antigens to appear 'foreign' to the mother yet sufficiently related to cause the antibodies produced to cross-react with closely related antigens in the human glomerular basement membrane and produce glomerular damage. Since the human placenta may be considered a highly vascular structure, rich in antigens closely related to human glomerular basement membrane, it is possible that such antigens, slightly altered, could enter the maternal body and act as an antigenic stimulus. In such cases the maternal human glomerular basement membrane might act as one of the most important and vulnerable target organs.

These preliminary experiments will be reported in greater detail elsewhere[12].

This work was supported (in part) by grant *H*-4785 from the National Institutes of Health, U.S. Public Health Service, the Chicago Heart Association and the Schering Corporation. I would like to express my gratitude to Dr. M. Edward Davis, Department of Obstetrics and Gynecology, and Dr. Edward Henderson, Schering Corporation, for their interest and support in this work, and Dr. F. H. Fitch, Department of Pathology, for the use of facilities.

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ABNORMAL GLOBIN FORMATION IN HUMAN MEGALOBLASTS

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THE following work was undertaken to determine whether altered production of globin could be induced in human megaloblasts by exposing them *in vitro* to nucleoproteins derived from sickle-cell anæmia. More than thirty biochemically distinct characteristics have been successfully introduced into bacteria by exposing them to deoxyribonucleic acid (DNA) derived from homologous species[1]. Similar work on mammalian cells has been hampered by the obvious difficulties of using such cells as compared with bacteria and also by the difficulty of demonstrating that a successful transfer of genetic information has occurred. The abnormal hæmoglobins, however, provide an approach to such investigations. They are, at present, the only well-investigated system in human biochemical genetics where it can be shown that mutations produce demonstrable alteration in a protein molecule. Thus, Ingram has shown that sickle-cell hæmoglobin differs from normal hæmoglobin by the substitution of valine for glutamic acid in a specific peptide of the β-polypeptide chain[2–4]. Abnormal amino-acid sequences have now been shown for a number of genetic hæmoglobin diseases[5–8] and could therefore serve to indicate successful transfer of genetic information.

Megaloblasts were chosen because they are arrested at a primitive stage and might be susceptible to the influence of genetic material during maturation. Sickle-cell anæmia was chosen as a source of the nucleoproteins because it is the most readily available source of the genetic hæmoglobin diseases.

Marrow was aspirated from patients with sickle-cell anæmia, the DNA extracted with 2 *M* sodium chloride, deproteinized with deoxycholate and isolated by precipitation with 95 per cent ethanol. The final product was a highly viscous material containing 5–10 per cent ribonucleic acid and a similar amount of protein in addition to the DNA. No further attempt was made to remove these substances because of their potential contribution to transforming activity. This material was dialysed against 0.1 *M* sodium chloride and 0.05 *M* sodium citrate in the cold for 24 hr. and incubated with megaloblastic bone marrow. Each incubation mixture contained 10 ml. of sternal marrow aspirated from a patient with megaloblastic anæmia and 10 ml. of *S*-15 tissue culture fluid[9] which was supplemented by 5 mgm. heparin, 0.50 mgm. folinic acid, 0.10 mgm. vitamin B_{12}, 20,000 units penicillin and 0.12 µc. (80 µgm.) of ferrous citrate (iron-59)

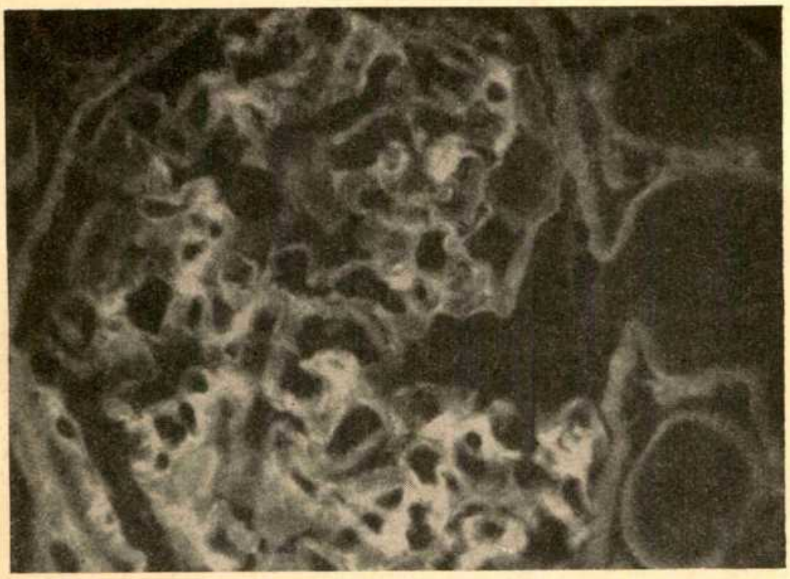

Fig. 1. Fluorescent photomicrograph of a human kidney stained *in vitro* with sheep anti-human placenta fluorescein conjugates. The basement membranes of glomeruli, Bowman's capsule and tubules show specific apple-green fluorescence which appears white in the photograph. Note the tubule opening into Bowman's space. Cellular structures do not stain. Identical results were obtained with sheep anti-human glomerular basement membrane fluorescein conjugates. (× *c.* 525)

rabbit anti-rat glomeruli serum stained connective tissue structures in various rat organs and was nephrotoxic for rats. Evidently nephrotoxic antibodies are part of the localizing antibodies. Since direct bioassay is not possible in man, the demonstration of heterologous antibodies to human placenta which collect in human glomeruli would prove that human placenta and glomeruli share closely related antigens. Such localizing antibodies might be nephrotoxic.

Since little is known about the relationship of antigens in human placenta and kidney, this investigation was undertaken to determine if human placental antigens were present in human kidney and where such antigens were located, using the immunofluorescent technique of Coons[4].

Human glomerular basement membrane was prepared as previously described[5]. The membrane was suspended in isotonic saline to a concentration of about 80 mgm. per ml. A 1 : 1 water-in-oil emulsion was made with Freund's adjuvant consisting of 85 ml. 'Bayol *F*', 15 ml. 'Arlacel *A*' and 200 mgm. *Mycobacterium butyricum* (Difco).

A human placenta was obtained shortly after birth, freed from membranes and washed grossly free of blood. Minced placenta and isotonic saline were mixed in a Waring blender for several minutes and then incorporated into Freund's adjuvant. Additional saline was added until the final concentration of placenta in the emulsion was about 1 gm./6 ml.

About 6 c.c. of the human glomerular basement membrane emulsion and 30 c.c. of the placental emulsion were injected into individual sheep by intramuscular, intradermal and subcutaneous routes every 2 weeks. Each sheep was bled to death one week after the fifth injection. The sera were removed and stored in the deep freeze (− 15° C.) until needed.

The globulin fractions were separated from the sera by half-saturation with ammonium sulphate or the cold alcohol fractionation method of Deutsch[6]. The γ-globulin was conjugated to fluorescein isothiocyanate according to Riggs's method[7].

Human kidney tissues were obtained soon after autopsy or from surgical specimens. Human placentas were obtained immediately after birth. Portions of tissue were frozen in isopentane cooled by a mixture of dry ice and acetone, and sections 4 microns thick were cut in a cryostat. The fluorescein - conjugated antisera were passed through a 'Dowex' resin column and then absorbed with 150 mgm. of acetone-dried mouse liver powder per c.c. of conjugated serum. In addition, 1 part of antiserum was absorbed with 2 parts of oxylated whole human blood.

The absorbed antisera were then used directly to stain the tissue sections and careful controls were used as described by Coons[4]. The slides were examined with a Leitz microscope fluorescence unit and permanent photographic records were made.

Fluorescein-labelled sheep antibodies to human glomerular basement membrane or human placenta stained identical connective tissue structures in the kidney. These structures may be defined as the basement membranes of glomeruli, tubules, Bowman's capsules and intertubular capillaries. Certain extracellular structures in the media and adventitia of arteries were also stained. The stained structures in the media of arteries might correspond to the basement membranes of smooth muscle cells according to Gersh[8]. The structures in the adventitia of arteries might represent irregularly condensed ground substance.

Fluorescein-labelled sheep antibodies to human placenta and human glomerular basement membrane stained the placenta in similar manner. Specific staining was diffusely present throughout the entire connective tissue core of the villus with heavy con-concentration in blood vessel walls and in the subsyncytial cell layer.

Cellular structures in the kidney and placenta did not show specific staining. None of the control sections showed the specific apple-green fluorescence. An interesting incidental finding was the observation that autofluorescent internal and external laminæ were not seen in placental blood vessels encountered even though some were of large size.

The results of these preliminary experiments indicate clearly that human placenta and human glomerular basement membrane share a closely related antigen or group of antigens. Further, that the placenta contains an antigen or group of antigens which is present in various connective tissue structures of the kidney.

The chemical nature of the antigen (or antigens) is unknown. The areas that stain most intensely in the placenta and kidney with fluorescein-conjugates are also periodic acid–Schiff positive. Mature collagen is reported not to stain in rats[3] and man[9], using anti-glomeruli fluorescent conjugates. It would seem unlikely that collagen fibrils are the antigen involved in the present investigation.

In general, nephrotoxic sera have been shown to stain many connective tissue structures of many

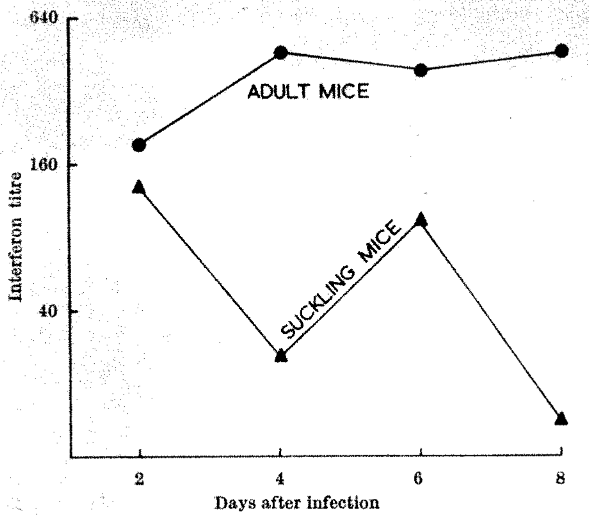

Fig. 3. Interferon titres in lung extracts of adult and suckling mice at various times after infection. Pools of three lungs tested at day indicated, mean titres of three experiments. Titres shown as the reciprocal of the dilution of lung extract causing 50 per cent reduction in the virus plaque counts

The lungs taken at each time-interval were pooled and extracts prepared. The amount of saline used for the extracts was adjusted according to the weight of the lungs. The material was tested for the amount of virus present (ID_{50} titrated in chick embryos) and interferon content (plaque reduction test in mouse embryo fibroblast monolayers infected with approx. 100 plaque-forming units of encephalomyocarditis virus[5]). Four such experiments were carried out, and the combined results are presented in Figs. 2 and 3. As can be seen from the graphs, the virus titres were higher in suckling mice, and interferon content lower than in the infected adults. No significant differences in interferon content were seen at the early stage of infection but differences became evident towards the end of the infectious process—during recovery.

As was indicated earlier, the speed of virus elimination as an indicator of the recovery process in adult mice might have been influenced by locally produced antibody. In the lungs of the infected suckling mice no antibodies were found, and there is suggestive evidence from the experiments described here that the recovery process was influenced by the amount of interferon produced. The lower content of interferon in the lungs of the infected suckling mice could be responsible for both the higher virus titres during the course of infection and the longer persistence of virus.

Differences in the course of both naturally and experimentally induced viral infections in young as compared with adult animals have been repeatedly described in the past and with few exceptions[10] they indicated that younger animals were more susceptible to infection with many different viruses[11-22].

Acquired resistance of growing animals to certain neurotropic viruses in the absence of humoral antibodies or previous exposure to these viruses was described as early as in 1936 by P. K. Olitsky *et al.*[11]. The factors which might have influenced the refractory state of adult animals were considered obscure.

Very young mice showed a higher susceptibility to rabies virus infection than adult mice, but only when fixed virus was used[19]. In this connexion it is worth mentioning a recent finding that attenuated virus is a better producer of interferon than virulent virus[23].

It has been found that during maturation of the embryo there is a steady increase in the sensitivity of the cells to interferon and in their ability to produce interferon[6]. The present results suggest that this process may continue during post-embryonic life and that this may be a factor governing the response to virus infection in young animals.

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CLOSELY RELATED ANTIGENS IN HUMAN PLACENTA AND KIDNEY

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ONE of the many theories of the etiology of toxæmias of pregnancy maintains that maternal antibodies formed against placental antigens may damage maternal glomeruli. In order to support this hypothesis, it would be desirable to demonstrate that human placenta and human glomerular basement membrane contain closely related antigens; and secondly, that the placenta has a mechanism by which these closely related placental antigens might vary sufficiently to 'appear' foreign to the mother so as to provide an antigenic stimulus. The antibodies formed against this antigenic stimulus could be expected to collect in the human glomerular basement membrane and initiate glomerular damage. This hypothesis is based on the premise that in order for antibodies to damage tissues, they would first have to be present in the affected tissues. Such antibodies could properly be called autoantibodies and this mechanism an autoimmune mechanism.

Seegal reported that the rat[1] and dog[2] placenta and kidney contain nephrotoxic antigens in common. Cruickshank and Hill[3] reported fluorescein-labelled

It seems that the feeding of the meal part of each of these diets in the form of a gruel enabled a large proportion of it, if not all, to by-pass the rumen. This was associated with markedly better utilization of dietary protein, presumably brought about mainly by a more efficient use of dietary energy

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INFLUENCE OF AGE OF MICE ON THE RECOVERY FROM EXPERIMENTAL SENDAI VIRUS INFECTION

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THE view is now prevalent that Sendai virus[1] (*Myxovirus parainfluenzae* 1)[2] persists in laboratory mice mainly in the Far East Attempts to produce a persistent infection in mice, carried out by C H Andrewes and me[3], were unsuccessful Intranasal infection occasionally caused lethal pneumonia, but among animals which recovered it was observed that adult mice eliminated the virus much earlier than the suckling ones. This indicated that the recovery process in very young mice might be handicapped

In order to study this further 4 weeks old mice were inoculated intranasally with 0 05 ml and suckling mice infected during the 1st day of life with 0 01 ml of allantoic fluid containing 100 EID_{50}/ml of Sendai virus Five adult mice along with 5 sucklings were killed 4 and 6 days and daily from 8 to 15 days after infection The presence of virus was checked by sub-inoculation of the lung extracts from individual mice into 11-day-old chick embryos The results of this experiment are shown in Table 1 In adult mice, virus was no longer recovered after the 8th day of infection, whereas in suckling mice virus was detectable up to 14 days, the titres being high up to the 9th day after infection (ID_{50} tests in pieces of chick chorioallantoic membrane[4]) In the same experiment sera of adult mice were tested at various times after infection for the presence of hæmagglutination inhibition antibodies No antibody was found Lung extracts of infected adult mice contained small amounts of viral antibody as measured by the hæmagglutination inhibition test from the 8th day after infection (Fig 1) No antibodies were found in lung extracts of the suckling mice up to the 15th day after infection. While one cannot exclude the possibility that local hæmagglutination inhibition antibodies might have influenced the elimination of virus in adult mice, it is difficult to support this explanation in the case of suckling mice which eliminated virus by the 15th day after infection despite the complete lack of any detectable hæmagglutination inhibition antibody in their lungs

Table 1 PERSISTENCE OF VIRUS IN ADULT AND SUCKLING MICE AT VARIOUS TIMES AFTER INFECTION
Numerator number of mouse lungs yielding virus. Denominator number of mouse lungs tested

Mice	Days after infection									
	4	6	8	9	10	11	12	13	14	15
Adult	5/5	5/5	4/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Suckling	5/5	5/5	5/5	5/5	5/5	3/5	1/5	4/5	1/5	0/5

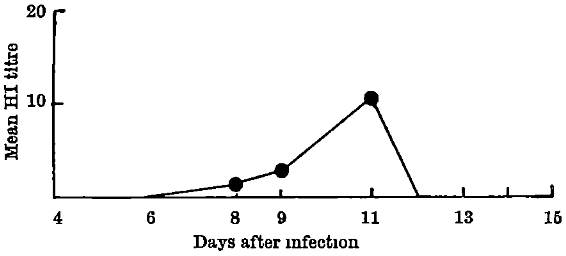

Fig. 1 Hæmagglutination inhibition (HI) antibodies in lung extracts of adult mice at various times after infection Mean titres of five lungs individually tested

There are reports which indicate that an important role in recovery from primary viral infections may be played by interferon[5-8] In this connexion it is of interest that recovery from viral infection can occur in the absence of any antibody formation, for example, in the chick embryo[7], or in the presence of only traces of antibody, for example, in patients with hypogammaglobulinæmia[9]

Experiments were therefore set up to compare interferon production in experimentally infected adult and suckling mice Three animals of each group were killed 2, 4, 6 and 8 days after infection

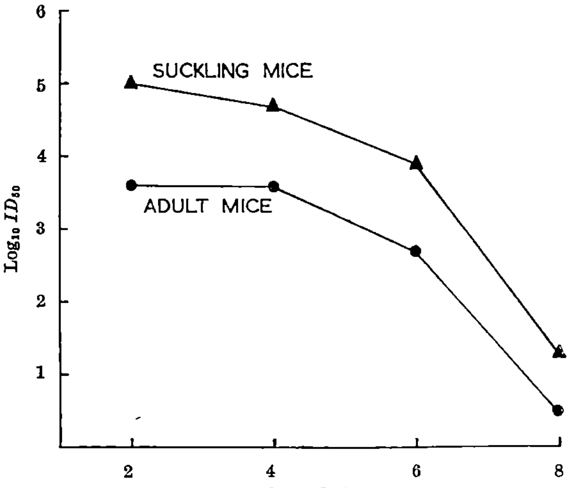

Fig 2 Virus titres in lung extracts of adult and suckling mice at various times after infection Pools of three lungs tested at day indicated, mean titres of four experiments

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Table 1 PERCENTAGE COMPOSITION OF THE DIETS

	Diet *X*	Diet *Y*
Ingredients		
Hay (crude protein, 6 99 per cent of dry matter, crude fibre, 35 5 per cent of dry matter)	25	25
Spray-dried, separated-milk powder	20	—
Fatted milk powder*	—	40
Rolled barley	30	7
Barley meal	10	10
White fishmeal	15	18

* The fatted milk powder was prepared by the homogenization of crude red palm oil with liquid separated-milk, followed by spray drying in a commercial plant to produce a powder containing 50 per cent of fat

utilization of those carbohydrates which are capable of being well assimilated by monogastric digestion It seems likely that even when calves are receiving hay, and behaving as functional ruminants, the feeding of the concentrate part of their diet as a gruel enables this food to bypass the rumen If the carbohydrates are then more efficiently utilized than when fed dry this should increase the available energy of the diet, and thereby improve nitrogen retention[2] It was therefore decided to examine the comparative effects of wet and dry feeding on the nitrogen balance of calves

Four ruminating Ayrshire bull calves, approximately 14 weeks old and housed in metabolism crates, were used for the investigation Two diets were made up as shown in Table 1, the hay being kept separate from the other components Chlortetracycline (17 mgm per lb of diet) and supplements of trace minerals and vitamins A, D_3, E and the B complex were included Each diet was allocated to two calves In all the balance periods (periods 7, 8 and 9) the daily allowance of meal for each calf (1,050 gm) was given in two equal feeds, at 9 a m and 5 p m The daily allowance of chopped hay (350 gm) was given immediately after the morning feed For the first and last parts of the experiment (periods 7 and 9), the 525 gm of meal for each feed was mixed with two litres of warm water and bucket-fed immediately During period 8, the meal was fed dry, followed by two litres of warm water per calf Each balance period of eight days was preceded by a preliminary period of six days The collection and analysis of fæces and urine were carried out as previously described[3], except that fæcal nitrogen determinations were made on composite wet samples at the end of each period

The effects of dry and wet feeding on the mode of absorption of the carbohydrates were studied at the end of periods 8 and 9 by measuring the changes in reducing sugars of the blood following a normal meal, and also one containing in addition 200 gm glucose The reducing sugars were determined by King's method[4] on samples of blood taken at 0, 1½, 3, 6 and 8 hr after feeding

It is evident from the results in Table 2 that the retention of nitrogen by each calf was markedly lower with dry feeding than with wet feeding Since dry feeding was associated with a somewhat greater digestibility of the crude protein, the differences in nitrogen retention were due to differences in the extent to which absorbed nitrogen was retained It should also be noted that the efficiency of nitrogen retention was much higher on diet *Y* (the high-fat diet) than on diet *X*, with each method of feeding, suggesting that lack of available dietary energy was at all times an important factor in limiting the retention of nitrogen

From the changes in reducing sugars of the blood 1½ hr after feeding the respective meals without added glucose (Table 2), it is evident that all four calves showed appreciable rises following wet feeding compared with much smaller changes after dry feeding This difference in response to wet and dry feeding supports the view that wet feeding enables a considerable proportion of the food to pass directly into the abomasum, whereas dry feeding is associated with entry of the food into the rumen, with subsequent fermentation Lactose has previously been shown to be capable of producing an appreciable rise in reducing sugars of the blood[5], and since the single feed of each diet would supply an estimated 70 gm of lactose, some of the response must have come from this source

Further evidence is provided by the results obtained when 200 gm of glucose were added to the food Calves 1, 2 and 3 showed little or no response to the added glucose with dry feeding, but very marked responses with wet feeding In the case of calf 4, although there was an appreciable rise in blood glucose following dry feeding, this was greatly increased with wet feeding

It is of interest to note the greater rises in blood sugar on the high-fat diet since this has also been observed in studies of human nutrition[6]

Table 2 COMPOSITION AND DIGESTIBILITY OF DIETS, NITROGEN BALANCE, AND BLOOD REDUCING SUGARS

Composition of diets (dry matter basis)	Diet *X*						Diet *Y*					
Crude protein (per cent)	25 7						24 3					
Ether-extractable material (per cent)	2 49						24 0					
	Calf 1			Calf 2			Calf 3			Calf 4		
Period No	7	8	9	7	8	9	7	8	9	7	8	9
Mean live-weight of calf* (lb)	158	167	176	149	161	167	160	179	185	159	174	188
Method of feeding meal	Wet	Dry	Wet	Wet	Dry	Wet	Wet	Dry	Wet	Wet	Dry	Wet
Apparent digestibility of diets												
Dry matter (per cent)	73 0	79 1	77 2	75 6	81 7	78 9	78 6	77 3	75 0	77 6	79 3	79 2
Crude protein (per cent)	75 8	83 0	79 9	82 5	85 5	81 7	81 6	82 7	80 9	80 3	84 0	80 2
Nitrogen balance												
Intake (gm /day)	51 7	51 7	51 7	51 7	51 7	51 7	50 0	50 0	50 0	50 0	50 0	50 0
Retention (gm /day)	13 5	6 65	10 1	14 9	10 2	13 4	23 7	14 4	21 3	25 5	17 2	24 3
Retention (per cent of intake)	26 1	12 9	19 5	28 8	19 7	25 9	47 4	28 8	42 6	51 0	34 4	48 6
Blood reducing sugars												
Change at 1½ hr after feeding (mgm./100 ml)												
Meal only		+2	+27		+11	+21		−4	+44		+7	+53
Meal + 200 gm glucose		−12	+38		−2	+30		+6	+85		+44	+143

* Mean of initial and final weight for each period

occur and the lizard's tail habitually regenerates rather a makeshift for the original structure. This may adequately serve its biological purpose, however, and the whole variety in degree of convergence is consistent with a natural selection basis.

It must also be remembered that the power of regeneration is as ancient as that of ontogenesis itself, that asexual reproduction and other morphogenetic processes grade into that of regenesis[2], and that ontogenesis and regenesis have evolved together, step by step, throughout their long history. It would perhaps be surprising to find anything but quite close convergence between them. It also seems probable that in general the same genes control both. In arthropods abnormalities such as the regeneration of a leg in place of an antenna are paralleled by similar abnormalities of ontogenesis, and these are known to be determined in typical Mendelian fashion. Regeneration probably can be regarded as just a special case of what Wigglesworth[3] has called 'developmental polymorphism', the insect embryo produces a first set of legs and other organs, and then a new, imaginal set is produced at metamorphosis. There are also other pieces of evidence that ontogenesis and regenesis are components of a common mechanism[2], and we can scarcely regard the convergence between them as special evidence in favour of final causation. This does not affect the possibility that they, and all biological phenomena, show more general evidence of the operation of final causes, but most biologists believe that natural selection could have produced the apparent purposefulness in the many interesting cases discussed by E. S. Russell and others. Among these, internal homœostatic mechanisms do figure, of course, if not as frequently as external anatomical features.

Final causation as usually understood is perhaps a problem purely for the philosopher, since it is scarcely amenable to the scientific approach. At the same time biologists must be as much concerned as philosophers in the problem of reconciling their respective approaches to the acquisition of knowledge. This problem stems largely from the differences between the axiomatic premises of the two approaches and, so long as these remain, it is not reasonable to expect any ultimate integration of their views. By axiomatic law final causation, as usually understood, is ruled out of the scientific court and no amount of subsequent pleading can bring it in again. It may be a reality, but it cannot be tested experimentally.

Some biologists might regard natural selection as somewhat in the position of a final cause[2] in the sense that it has the appearance of acting in the manner of a *deus ex machina* on the fundamental processes governed by efficient causes. It has the virtue of being a cause which can be investigated by observation and experiment. For this very reason it is not a final cause in the usual sense but may prove to be all we need to seek. It has been pointed out[4] that the conception of natural selection is just another way of saying that at every time and place only the viable systems survive: few would object to the view that the final cause of life is its viability. Survival of the viable, and extinction of all other, systems may be generalized into the principle that what actually occurs in each spatio-temporal context is the most probable event in that context. The viable automatically behave as though directed towards the goal of survival. In the behaviour of the higher animals this has become very purposeful in appearance. The anticipatory character of ethological responses and of ontogenesis certainly appears purposive to an observer but it should be remembered that these activities are largely recapitulatory of past experiences, of the individual and the species respectively, and are not purposive aims at entirely novel objectives.

The 'most probable' conception of final causation, originating entirely from within a biological outlook, could ultimately provide a coherent and comprehensive account of evolution which might meet the requirements of philosophers. At the same time it discourages the idea that there is necessarily a sharp psychophysical dualism in the phenomenon of life. Spilsbury believes that this dualism also corresponds to one between subjective and objective experience, but it is perhaps debatable if our subjective experience is anything beyond an objective study of ourselves.

Dr. Spilsbury raises a number of minor issues which many biologists might wish to take up, but it would be wrong to obscure his main thesis by pursuing them here. There are also one or two puzzling passages, such as the apparent contradiction between the two properties demanded of any prospective change in the genetic control of regeneration, and some unguarded phrases such as "organic causation", "expedite future survival" and " 'inner' and 'outer' aspects of behaviour".

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COMPARATIVE EFFECTS OF WET AND DRY FEEDING ON THE UTILIZATION OF PROTEIN BY CALVES

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It is generally accepted that when liquid milk is fed to young calves it by-passes the rumen and reticulum, by means of the œsophageal groove[1], and enters directly into the abomasum. This permits the food to undergo monogastric digestion. The recent trend towards the early weaning of calves by a system involving dry feeding of concentrate meals is associated with the early development of a functional rumen. However, fermentation in the rumen is a relatively inefficient process, which penalizes the line between development and behaviour is a fine one If behaviour is not fully describable in spectator-language, the same may be true for development (This does not imply that developmental processes may be conscious We learn from self-consciousness that some vital functions are directed at future ends It does not follow that all future-directed processes are conscious Consciousness may reveal one facet of organic functioning without constituting its essence) This possibility cannot be excluded, so long as the problem of development, as at present conceived—how the 'genetic code' is 'made flesh' and conditions the psycho-physical attributes of the adult—remains unsolved In principle, the attempt to define ontogenetic precursors or rudiments of mind seems not less justified that the corresponding attempt in phylogeny (Not on the ground that ontogeny repeats phylogeny, but on the ground that we should seek to connect later states of a system with earlier ones) A conceptual bridge is yet to be built between the human zygote, considered as a biochemical system, and the purposiveness of the adult man, if the latter is to be explained partly as a consequence of the former. It is plausible to look for this bridge in the intervening processes of development, and especially in the interpretation of the 'anticipatory'[4] appearance of development, the pre-functional elaboration of parts destined to become an integrated system of functional organs in the adult. No theory of development can be deemed satisfying which does not offer an explanation of this progressive realization of functions, just as no theory of the neuro-physiology of behaviour is satisfying which does not explain how the progressive realization of a future end-state is possible (In neither case can selection theory provide a substitute for an analysis of the concurrent causes of this central feature of development and behaviour

That there has been selection for mechanisms which expedite future survival does not tell us what these mechanisms are. Disregard of this led to the nineteenth-century error of substituting speculations about phylogenies for analysis of ontogenies Development theory has been retarded first by being viewed exclusively through Darwinian spectacles, and more recently through Mendelian ones. Mendelism abstracted from the forward thrust of developmental processes, and concentrated on correlating starting-points with end-points, parental phenotypes with filial phenotypes Necessarily, the concept of the gene arrived at by these methods is inadequate as a basis for development theory This abstractive tendency of genetics is re-inforced by modern concentration on the genetics of micro-organisms, with their relatively simple ontogenies It is possible in the future that development theory will influence conceptions of genetics and evolution as deeply as it has hitherto come under their influence) The temporal organization of vital processes, which makes meaningless any analysis of the organism-at-an-instant, presents similar patterns and problems for all the life sciences Information theory has made familiar the notion that organisms store up information from the past, both in their brains and their germ-cells Possibly a similar generalization will prove fruitful with respect to the forward direction of organic processes, which often proceed as though organisms possessed information about the future, and may produce an adaptive end-result even in circumstances different from those encountered in the (phylogenetic) past—as when a normal adult develops from the fusion of two amphibian eggs Unless some generalized concept can be made to cover all instances of future-directed functioning, it is hard to see how the reference to the future involved in conscious anticipation can be made intelligible in relation to its ontogenetic and phylogenetic history, and one possible ideal of theoretical biology will have to be abandoned

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Dr Spilsbury renders a general service in bridging the gulf between philosophy and biology, and a special service to biology in focusing attention on regeneration as a critical test case of the ability of the Darwinian theory to explain the mode of evolution. His suggestion that the genetic determination of regenesis (the process of regeneration) may be independent of that of normal ontogenesis also must interest biologists Can we be sure that natural selection is a factor powerful enough to establish perfect convergence between the paths of these two morphogenetic processes, and also to maintain the perfection of the convergence throughout a long evolutionary series of changes in the final, adult form ? As Spilsbury has further pointed out in correspondence, selection in favour of regenerative ability seems to be much weaker than that in favour of normal ontogenesis since, while the latter is inevitably demanded, the former is only possibly evoked in each individual life Normal development is a *sine qua non* for propagation by the individual whereas often regeneration is called for only late in the reproductive phase of life Consequently even if natural selection provides an adequate explanation of the evolution of normal ontogenesis, it may not be adequate to explain the perfect convergence by regenesis. By the same token, however, if it should prove a sufficient explanation of the latter then we need have little doubt that it can explain the evolution of ontogenesis and of biological adaptations in general

An exact mathematical approach, by Fisher and Haldane in particular, has emphasized how small an advantage may have selective value for the individual, and survival value for the species Successful regeneration, even if called for in only a small percentage of individuals, provided it enables them to make a further small contribution to the size of the next generation may make just the difference between an assured survival and an inevitable extinction for the species. This is true even on the assumption that regenerative power is equal in all the individuals of a species, but there is also likely to be selection differentially in favour of individuals combining a high regenerative power with other advantageous characteristics Moreover, regeneration is not so rare in the field[1] as implied above and this is further supported by a recent census on populations of three species of *Planaria* Between 25 and 50 per cent of the individuals of the different species were in process of regeneration at that moment, so that each individual is likely to be challenged several times during its life. Selection in favour of regenerative ability therefore is stronger than appears at first sight

The perfection of the convergence between ontogenesis and regenesis should not be overstressed It is usually extremely good, but mistakes sometimes

EVOLUTION OF REGENERATION, AND ITS POSSIBLE BEARING ON PHILOSOPHY

NEEDHAM[1] has recently criticized the view of Morgan and others that the distribution of regenerative capacities is not closely correlated with their adaptive value This criticism, if accepted, can be used to answer one objection to the Neo-Darwinian interpretation of regeneration and its evolution Other problems remain Regeneration, when adaptive, is a secondary adaptation; it is the adaptation of an adaptive embryonic sequence to the special conditions under which regeneration occurs, the relation between the primary and secondary adaptation being one of likeness in the final developmental product, and often of unlikeness in the processes of development. A spectacular instance is the regeneration of the urodele lens In cases such as this, it may fairly be assumed that regenerative processes are adaptive only if they restore a close copy of the part lost This imposes a stringent condition for the selection of regenerative mutants, if the capacity for regeneration has evolved in this way.

In the present state of ignorance about the genetic basis of regeneration, the following alternative assumptions appear open to orthodox evolutionists The first alternative assumes that different or partly different groups of genes are active in regeneration and embryonic development, each set embodying different instructions for, for example, lens-making On the other assumption, the same genes are involved in both cases, causing different developmental processes in different circumstances

The first alternative implies a highly improbable convergence in the evolution of embryonic and regenerative development, with close correspondence, at different evolutionary stages, between the end-products of independently varying gene assemblies Such an ontogenetic convergence, maintained throughout an evolutionary series, would be less comprehensible, on the assumption of random genetic change, than convergent evolution between different species, where the resemblances are less exact

On the alternative assumption, the evolution of regeneration may be ascribed to selection for genetic changes which facilitate double-track development by the same genetic determinants Such genetic changes are required (1) to leave unchanged former developmental tracks, at least with respect to their end-product, (2) to initiate new tracks, the usefulness of which depends on their leading to the same end-state as the old tracks The same paradox arises here as on the assumption of independent genetic controls for embryonic and regenerative development the paradox of ascribing the replication of identical structures, through different developmental processes, throughout an evolutionary sequence, to random genetic changes which have been selected It is legitimate to doubt whether so exact a coincidence of results could have come about in this way There seems to be no evidence for the occurrence of equifinal regenerative mutations, having the developmental effects outlined above

Equifinality or end-invariance, attained through variable precurrent events, is a pervasive property of life and the products of life[2], from the cellular to the social and technological grades of organization. Its elucidation would probably be of philosophical significance, as offering the lure of the long-awaited solution of the mind/body problem, and of a general concept of organic causation connecting final with efficient causes The split between these may be linked with the dualism of spectator and participant Activities which, to a spectator, appear determined by past causes, to their participants, appear aimed at future ends While in atomic physics it has become necessary to emphasize the impact of the observer on the observed, in evolutionary biology it is necessary to note the impact, mediated by a common descent, of the processes studied on their students' ways of thinking about them Awareness of this impact implies an almost existentialist sense of personal involvement in evolutionary processes, out of which has somehow surprisingly sprung the human capacity for concern over evolution, past and future. What may be called a detached spectator's view of evolution, the *post factum* assessment of evolution as determined by efficient causes, makes a coherent and comprehensive theory of evolution impossible, so long as the relation between these efficient causes and the development of the capacity for future-directed action remains a mystery. (Darwin's famous personification of natural selection, as the never off-duty scrutineer of variation, vividly reflects the view that the course of evolution has been determined by factors in principle observable by a sufficiently acute and unremitting onlooker. Another symptom of the spectator's point of view, in Darwin and some modern writers on evolution, is the disproportionate emphasis on external adaptations to specific features of the environment, and the comparative neglect of the integrative and regulative capacities of the organism in development and self-maintenance)

All historical studies of past life are liable to be conducted from a spectator's point of view, including the study of human history One reason for this is that the working back from present to past, characteristic of all historical inference, reverses the natural order of events, and obstructs their evaluation from past to future, from the participants' point of view

From our present grasp of the mechanisms of evolution, we can no more derive the evolution of purpose than from our present understanding of cerebral mechanisms we can derive either the 'inner' or 'outer' aspects of behaviour Consequently, claims that Neo-Darwinism provides a coherent synthesis of all the known relevant facts are baseless The interest and challenge of equifinality lie in its hint that we do not have to resign ourselves to this incoherence, and that the struggle for a unitary conception of unconscious organic processes and mental processes, within an evolutionary framework, may not be vain Possibly these processes are not merely analogous, with respect to equifinality, but even homologous, in the sense of having a common evolutionary origin There seems no compelling reason why the search for homologues of mind should be restricted to the field of behaviour As Sinnott[3] and others have pointed out, the dividing both the earliest and the established pattern of the disease, the patient with a skin blemish can watch, if he wishes, the growth of a curable cutaneous cancer Carcinoma of the lung is different from both, it produces symptoms (that is, something of which the patient complains) as a by-product of its naturally hidden position and will produce them when quite small should it simultaneously involve a blood vessel and a small peripheral bronchus, when blood-stained sputum may be expectorated, but commonly is measured in centimetres and produces the first symptoms due to remote spread The size of a tumour, its duration or its clinical accessibility or visibility are not in themselves important in prognosis The overriding factor is our knowledge based on experience of the natural history of each type of tumour For example, the prognosis in the case of squamous cell carcinoma depends on which organ it primarily involves, and extrapolation from one species to another must be done with extreme caution Nevertheless, any basic studies of that neglected field—animal pathology—are most welcome A complete understanding of tumour behaviour must include such knowledge, hence the work of Silver and Cater on the treatment of spontaneous tumours in animals by radiotherapy or by chemotherapeutic agents offers a little hope that research workers have not forgotten the value of comparative investigations

Prof Bullough's group at Birkbeck College continue work on the diurnal mitotic rhythm of normal mouse epidermis and conclude that it is due to the hour-by-hour variations in the ability of prepared cells to enter division and not to hour-by-hour variations in the numbers of cells preparing to divide, that the ability of cells to enter division depends on variations in the secretion-rate of adrenalin, and that these variations in turn depend on a degree of stress felt by the animal, stress being higher when a mouse is awake than when it is asleep On this fascinating work one could build, with little imagination, a hypothesis accounting for human cancer as, in part, a psychological disorder (This has been attempted in the past with no convincing success, but a careful psychosomatic approach to human cancer is worth while)

With the chemotherapeutic agents at present available, one of the practical problems is that of delivering a dose of a drug which will result in the death of tumour cells, but which is not lethal to the patient, drugs with a greater specificity towards tumours are being developed while, at the same time, techniques using existing drugs aim at better localization Where the tumour is in a limb, this becomes a fairly simple matter of perfusion, where the growth is widely disseminated, the urgent problem is to preserve a bone marrow which can regenerate after severe depression or even total death of bone marrow following administration of chemotherapeutic agents Techniques vary from using living fœtal marrow from a fœtal tissue bank or the storage of the patient's own aspirated marrow for later re-injection, to an ingenious technique reported by Starr in New South Wales, where the patient's aspirated marrow is injected into a drill-hole in the tibia after a tourniquet is applied, only to be released after an otherwise lethal intravenous dose of cytotoxic drug has become fixed or eliminated, in 1–3 hr

At Makerere College, Uganda, animal tumours are investigated by exfoliative cytology, many tumour cells being studied to greater advantage when flattened on glass slides than in paraffin sections In the case of the transmissible (venereal) tumour of the dog it has been found that the round cells infiltrating the lesions are plasma cells, frequently binucleate with a 'mirror image' pattern In enzootic hæmaturia of cattle (a disease characterized by progression of papilloma of the bladder to carcinoma) investigations have confirmed the findings of some Turkish pathologists, and support a viral origin of the condition These cytological observations show that the bovine urinary bladder has secretory function Well-known virus diseases of animals—canine distemper and infectious pneumonitis of cats—are now being investigated by this rapid method of study

As for many years past, the report provides plenty of evidence of the widespread use of physical techniques involving radiation and radioisotopes, this year there is evidence of the rapid extension of diagnostic and research uses of the latter in remote parts of the world But there is little which is sufficiently novel to require mention. Hospital physicists, perennially suffering from erratic radiation background, will be interested to know that, at the Royal Cancer Hospital, an attempt is being made to build a low-background room on a small outlay of money. Chalk in paper bags, 2 ft thick, is being used for this, the inner surface of the room being lined with ½-in aged lead. Another development which looks like being of practical importance is taking place at the Middlesex Hospital, where the radiation-induced destruction of the fluorescence of quinine sulphate is being investigated as a method of measuring γ- and X-ray dosage The results suggest that doses of 10 rads or even less can be estimated with good accuracy; it will be interesting to hear further of this work.

Among radiotherapeutic developments note should be taken of the Royal Cancer Hospital's use of solid sources which have been made radioactive by neutron bombardment These include a Souttar's tube, for treatment of the œsophagus, made of silver-plated nickel wire containing a core of gold, and two laryngectomy tube applicators of copper-nickel alloy, giving, after activation, a dose-rate of the order of 200 rads/hr The Department of Radiotherapeutics at Cambridge has continued its efforts, noted here last year, to use the selective uptake of tritiated 'Synkavit' in cancer as a therapeutic measure Information is given on methods of administration, dosage, and means for increasing selective concentration Information is also given on the degree of selective uptake in various types of tumour-tissue as compared with bone-marrow Investigations, subject to the usual limitations, have been made on patients and the results are so far inconclusive On the other hand, parallel investigations on animals which are also reported look promising A similar line of investigation is reported from the Sheffield Regional Radiotherapy Centre, where the selective uptake of radioactive zinc-labelled hæmatoporphyrin has been investigated with encouraging results, however, no details are given

The Leeds centre is making a new approach to the study of bone mineralization Attempts are being made to relate the transmission through bone of radiations of markedly different quality to the quantity of calcified bone lying in the path of the beam Thulium-170 and cæsium-137 have been used in this work which, if successful, will make it possible to study bone mineralization by *in vivo* methods

D G ARNOTT
I HIEGER
A L LEVENE

the problem seems to have been entirely overlooked by the legislators There is, therefore, a renewed interest in compounds of the flavone and isoflavone series described later in this report"

Salaman and co-workers find that new-born mice are highly sensitive to carcinogens, after one year, of 26 mice of the '101' strain which had received 30μ of dimethylbenzanthracene neo-natally, 9 developed leukæmia, 25 lung adenoma, 7 skin papilloma and 16 papillomas of the forestomach

Cunningham and colleagues at the Royal College of Surgeons have been studying cytologically the process of liver cancer induction by dimethylaminoazobenzene using a technique of section cutting which permits the life-like preservation of cellular structures in that mitochondria, free lipid, lipid-protein bonds and dehydrogenases are retained intact, the tissue being sectioned at − 25° after cooling to − 70°

Orr has been continuing his work on the chemical induction of mammary cancer in the mouse, such tumours did not develop in female mice of the *C*57*B*1 strain after painting the skin with dimethylbenzanthracene (DMBA)—a standard technique—but if the females were made pseudo-pregnant by being caged with vasectomized males and then painted with DMBA, 21 out of 29 mice developed breast tumours due to the added stimulus from progesterone in pseudo-pregnancy

Orr and Mandl find that senile virgin rats are very liable to spontaneous mammary tumours, of 69 such females aged between 600 and 1,200 days, 34 (49 per cent) showed macroscopic fibroadenomata, while a parallel series of 46 female rats used for breeding had an incidence of only 4 per cent mammary tumours "It is tentatively suggested in the light of these observations that continuous secretion of œstrogen by virgin animals, uninterrupted by phases of progesterone secretion during pregnancy, stimulates the anterior pituitary The œstrogen-stimulated pituitary secretes appreciable quantities of luteotrophin even before it becomes enlarged, at the same time output of other gonadotrophins decreases The secretion of luteotrophin would account for both the mammary hyperplasia and the presence of excessively large corpora lutea Absence of corpora lutea could be explained by insufficiency of LH with failure of rupture of Graafian follicles"

Baldwin, at Nottingham, finds that a commercially available motor-engine oil additive is highly carcinogenic for mouse skin, it consists of a mixture of compounds which include lead naphthenate, carbon tetrachloride, 1,1,1,-trichloroethane and a petroleum oil base, the indications are that the active factor is present in the oil base

King, Hughes and Louis at Melbourne, in interesting work on the fluorescein conjugate staining of tissues, state "The studies of the last four years have been continued and extended In addition to serum globulins, serum albumin has been conjugated with fluorescein and rhodamine The differential avidity of the dye for serum albumin as opposed to globulins has been confirmed In addition, sera from species other than those previously examined have been investigated with similar results, normal tissues of all species studied stained but malignant tissues failed to stain

"Tissues grown in tissue culture show the staining and fluorescence characteristic of normal tissues for the first three days By the fourth day staining becomes patchy and subsequently no staining can be obtained, that is, after the fourth day the culture shows the same staining characteristics as malignant tissue This conforms with observations made on re-introduction of such tissues into the original animal or one of the same species, when they grow in the same manner as true neoplasms"

In Melbourne also, Trautner and Coats have been using an ingenious technique in the study of species immunity to cancer, mouse ascites carcinoma cells were suspended in nutrient fluid in 'Perspex' chambers (0 1 ml) fitted with non-reactive 'Millipore' filters of pore size 0 6–0 3μ "Attempts to transplant the tumour directly from the mouse to either the rat or the guinea pig were uniformly unsuccessful " When the chambers were inserted intraperitoneally in rats or guinea pigs the growth of the mouse cancer cells was at least as prolific as in mice "It was found that growth of the tumour in the rats and guinea pigs was as good as or better than it had been in mice, and the day-by-day growth characteristics were studied It would appear that the tumour-host hostility evident on direct inoculation was dependent on cell-to-cell contact between tumour cells and the host "

Studies in geographical pathology come from the University College of North Wales ("Cancer Incidence in Relation to Trace Elements in Soil and Plants"), Makerere College, Uganda ("Cancer in African Children") and the University of Bristol

Statistical data from the Bristol Tumour Registry show that under the age of 40 the English incidence of bone sarcoma is similar to Norwegian figures After that age the tumour is much more common in south-west England, due largely but not entirely to the frequency of Paget's disease of bone in the English population, but not in the Norwegian population Except in those subject to Paget's disease the tumour has a monomodal age incidence curve, the English incidence showing a typical bimodal curve

Paget's disease (osteitis deformans), probably the commonest non-traumatic disease of bone seen in an orthopædic department, was described in some detail in 1876 by Sir James Paget It has been described in animals, too, and is an etiological feature of canine osteogenic sarcoma, where it is much more common in some breeds than in others The author of this study is clearly aware of the need to combine a knowledge of geographical pathology with genetics, comparative pathology and basic clinical observation in the elucidation of the most fundamental aspect of any disease —its natural history

A report on a clinico-pathological investigation of cancer of the cervix uteri concludes that (1) beyond a certain dosage (irradiation) range the physical modalities of irradiation do not significantly alter the cure-rate for cancer of the cervix, (2) the clinical extent of the disease and such quantitative biological characters as Broder's grade and size of tumour foci have a significant influence on the five-year survival-rate, (3) among the qualitative biological characters the histological type of the tumour and its ability to respond initially with an increase in differentiation profoundly affect the cure-rate, (4) systemic factors, of yet unknown character, can control the growth of persisting cancers for long periods

The same, or similar, methods of treatment, unmodified in essentials for perhaps 20 years or more, are in use in hospitals throughout Britain, and naturally their reports read much the same At present the clinician subscribes to earlier diagnosis as well as better surgery, irradiation or chemotherapy as one of the principal means of improving results Not in all cases is this meaningful, the patient with an enlarged lymph node due to lymphosarcoma has

Of particular scientific interest is the large programme of work now in progress on the quantitative aspects of fire in structures, both real and idealized. A particular object of this is to establish scaling laws for rate of burning, size of flames and the spread of fire by heat transmission from them, and the disposal of smoke by ventilation.

The statistical inquiries, as always, are interesting, and this year include reference to the incidence of fire in post-war houses, fires caused electrically, and aircraft crash incidents. Tests have been carried out on the relative effectiveness of audible warning devices in traffic, from which the traditional fire bell emerges rather badly. Preliminary experiments on the use of a gas turbine as an inert gas generator for extinguishing fires in large building spaces have given encouraging results. Fire-resistance testing of building materials, work on the prevention or mitigation of gas and dust explosions, and a number of other projects have been continued.

J. H. BURGOYNE

PROGRESS OF CANCER RESEARCH

THE annual report of the British Empire Cancer Campaign continues to expand year by year; the thirty-eighth edition* is a massive book of nearly 800 pages, the scientists working either wholly or partly under the ægis of the Campaign number nearly 1,500, and a million pounds was spent on research in 1960.

Some idea of the trends of cancer research, as described in the report, can be suggested by breaking down, into subject-matter and class, the total of papers published in 1960 by these investigators and comparing the resulting catalogue with that for 1950; the papers will, of course, represent the work more or less completed before 1960 rather than the present progress, but the main lines will undoubtedly remain substantially the same (Table 1). The table shows that in the ten years the trend has been to increase the volume of work on biochemical themes at the expense of carcinogenesis and radiobiology, and that the immunological and statistical approach is attracting more research.

Only a little of the large amount of research work detailed in the report can be referred to here. One of the most interesting investigations continues to be on the Gross leukæmia-inducing virus and the related polyoma virus. Miller confirms that removal of the thymus in *C3Hf/GS* mice at 3–4 months after they have been neo-natally inoculated with the Gross virus prevents the development of lymphocytic leukæmia which otherwise appears in 100 per cent of such inoculated mice. Grafting isogenic thymus into the thymectomized mice restores the growth potentiality of leukæmia. *C3H* mice which had been made tolerant to tissue from *Ak* mice at birth were inoculated with normal *Ak* thymus and became leukæmic; transplantation tests showed that the lymphocytic neoplasm arose from host cells and not from the inoculated *Ak* cells, thus demonstrating that a non-cellular factor from *Ak* thymus is a necessary component in the origin of this lymphocytic leukæmia.

C3H mice injected at birth with the Gross virus were later given a lethal dose of radiation and protected with normal isogenic marrow from untreated mice; most of the injected mice developed leukæmia, and tests with protecting cells carrying a marker chromosome indicated that the leukæmogenic virus is not only radio-resistant but is also resistant to any immune attack that might have been exerted by the protective marrow cells, and therefore there is not much hope of treating leukæmias of viral origin by total-body irradiation and marrow replacement.

Ambrose and colleagues, in experiments on normal and malignant cells grown *in vitro*, conclude that "The characteristic properties of the tumour cells so far suggested in the present studies are therefore as follows: (1) the tumour cell membranes show reduced mutual adhesiveness as compared with normal cells; (2) several lipases and phospholipases are more toxic for tumour cells than for normal cells and the tumour cells are also more sensitive to lipid in the medium, suggesting that more exposed lipid is present on the tumour cell membrane; (3) the structure of the tumour cell involves a less basic protein than the normal cell, suggesting a more acidic micro-climate at the cell surface, in agreement with the electrophoretic studies indicating an increased negative surface charge".

The possible cancer risks attached to the use of œstrogen implantation in cattle and poultry is the subject of a report by Sir Charles Dodds. "There has been a new interest in the alleged carcinogenic action of œstrogens during the past year. The widespread use of stilboestrol and hexoestrol in agriculture has generally been approved by governments. Stilboestrol implantation in poultry for the purpose of caponisation has become almost universal and it is also estimated that a very large percentage of meat produced in the North American continent has been produced under the influence of stilboestrol, hexoestrol or some similar substance. There has been a change recently in the administrative outlook in the North American continent on the use of these substances and at the present time, in America, their use has been banned by what is known as the Delaney Amendment to an Act in the F.D.A. This whole question is being looked into and we have been particularly interested in attempts to assess the intake of œstrogens in a normal diet. This aspect of

Table 1. PAPERS PUBLISHED ON CANCER RESEARCH

Classification	In 1960	Total in 1960 (per cent)	In 1950	Total in 1950 (per cent)
Radiobiology, radiotherapeutics, physics, tracer studies	187	21	79	33
Biochemistry of DNA, RNA, proteins, enzymes, carbohydrate metabolism	177	20	33	14
Clinico-pathology	105	11.9	20*	8.3
Cytology, tissue culture, electronmicroscopy, cytochemistry	87	9.9	19	7.9
Carcinogenesis, carcinogens, biology of tumours	81	9.2	38	16.0
Cancer chemotherapy, cytotoxins	70	7.9	15	6.2
Hormones, steroids	54	6.1	12	5
Immunity, immuno-therapy, transplantation	50	5.8	4	1.7
Mutation, cytogenetics	25	2.8	5	2.1
Statistics, geographical pathology	26	2.8	1	0.45
Viruses	21	2.4	6	2.5

* Report of Clinical Cancer Research Committee.

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* British Empire Cancer Campaign. Thirty-eighth Annual Report covering the year 1960. Part 1. The Chairman's Statement and the Accounts. Pp. xxxviii. Part 2. The Scientific Report of the Researches undertaken by the Central Organization and its Autonomous Councils in the United Kingdom and by some of its Affiliated Organizations Overseas. Pp. xxxix–lviii+792. (London: British Empire Cancer Campaign, 1961.)

two similar receivers, the front one of which is exposed to the radiation. The target consists of a massive copper disk 4.5 cm. in diameter and 0.5 cm. thick to ensure temperature uniformity. Thermocouples are attached at a number of points in order to average the rise in temperature. The electrical heating element is cemented to the front disk on the side exposed to the radiation. The cycle of operations and the practice carried out in the Laboratory for the calibration of working-standard thermopiles against the Guild radiometer with a radiation source, consisting of a 1,000-W. tungsten lamp operated at a colour temperature of 2,850° K., together with water filter and heat-absorbing glass to absorb radiation of a wave-length longer than 1μ, are explained in detail.

The second booklet, No. 24 (ref. 2), is concerned with photometric standards and the unit of light. The development of light sources from the early nineteenth century and the evolution of the present primary standard, with the internationally accepted unit of luminous intensity, the candela, are discussed, followed by a detailed description of the procedures carried out at the National Physical Laboratory with regard to the arrangement of the primary standard, and calibration of secondary standards of luminous intensity and luminous flux. Finally, the present international situation with regard to intercomparisons of photometric scales is reviewed, and a table is given showing the relative magnitudes of the candela (2,042° K.) as found in the most recent determinations (up to 1957) at various national laboratories. The monograph can be recommended as a comprehensive and instructive statement of photometric standards and light sources, and should be of value to all concerned with illumination. S. Weintroub

[1] Department of Scientific and Industrial Research. National Physical Laboratory. Notes on Applied Science, No. 23: *Radiometric Standards and Measurements*. E. J. Gillham. Pp. iv+23. (London: H.M.S.O., 1961.) 1s. 9d. net.

[2] Department of Scientific and Industrial Research. National Physical Laboratory. Notes on Applied Science No. 24: *Photometric Standards and the Unit of Light*. J. S. Preston. Pp. iv+32+4 plates. (London: H.M.S.O., 1961.) 3s. net.

GEOLOGY OF BROWN CLEE HILL AND THE ADJACENT AREA

*The Old Red Sandstone of Brown Clee Hill and the Adjacent Area**, recently issued by the British Museum (Natural History), is an important paper as it deals with a classic area in which is displayed the most complete sequence of Old Red deposits of the Anglo-Welsh development. In the first quarter of the century Wickham King established a general stratigraphical succession for that development, which he based to a large extent on his findings in the Brown Clee district, but his supporting palæontological evidence was too meagre and scattered to provide really convincing fossil zones. At that time too many of the forms had been inadequately described or named from fragments. King's work, however, generated a stimulus that has led to a revolution in our knowledge of the British Old Red ostracoderm faunas. This has been the result of systematic collecting and accurate description. The latter we owe chiefly to the senior author, Dr. E. I. White, and Part 2 of the present paper is his latest contribution to the subject. While our knowledge of the fossils has been gradually accumulating, the palæontologist has had, to a great extent, to rely on King's stratigraphical conclusions, which were not always based on reliable mapping.

In the present case the two junior authors have presented an excellent map based on accurate 6-in. survey and elaborate collecting, which have enabled them to determine the range of the fossils with considerable accuracy. For the Downtonian Series, they have taken advantage of much recent palæontological work, and the zones for this series can now be regarded as well established, and their evidence as merely confirming conclusions reached previously in this and other areas. But for the desperately barren Dittonian and Clee Series, their painstaking field-work has supplied Dr. White with material, by which broad zones can now be defined with some exactness.

Part 1, devoted to the stratigraphy, includes interesting discussions concerning the geographical conditions governing sedimentation, the ecology of the fossiliferous 'pockets', the so-called *Psammosteus* Limestone (which has never yielded a fossil, let alone a *Psammosteus*), and foreign pebbles in the conglomerates, but its main significance is the correlation of the fossils with the stratal succession, and the determination of the range of each important species. A valuable feature is a list of more than a hundred localities with the fauna discovered at each.

Part 2 is descriptive of a number of new species and varieties illustrated by 60 text figures and 16 plates. Of particular value are the new forms discovered by the authors and others in the Clee Series and in the unconformable upper Old Red beds (the Farlow Sandstone Series). L. J. Wills

* Bulletin of the British Museum (Natural History). Geology Vol. 5, No. 7: *The Old Red Sandstone of Brown Clee Hill and the Adjacent Area*. 1: Stratigraphy. By Harold William Ball and David Lawrence Dineley. 2: Palæontology. By Errol Ivor White. Pp. 175–310+plates 33–48. (London: British Museum (Natural History), 1961.) 70s.

FIRE RESEARCH 1960

The recently issued annual report of the Joint Fire Research Organization* records the bringing into use, during 1960, of new buildings providing 30,000 sq. ft. of permanent floor area for the continued prosecution of fire research (see *Nature*, 189, 105, 1961).

Among the many investigations described, that which focused attention on the behaviour in draughts of oil heaters of the drip-feed radiant type has doubtless aroused the greatest public interest. The relatively new and unique wind-tunnel facilities of the Fire Research Station permitted an expeditious study of this problem, followed, within the year under review, by the publication of a report and a new British Standard and, finally, by the introduction of appropriate legislation.

* Department of Scientific and Industrial Research and Fire Offices' Committee Joint Fire Research Organization. Fire Research, 1960: *Report of the Fire Research Board with the Report of the Director of Fire Research*. Pp. viii+67+12 plates. (London: H.M.S.O., 1961.) 5s. 6d. net.

showed but a poor increase in yield (its merits were made clear in later trials under improved conditions). A belief had therefore grown up that the attainable yield of rain-grown cotton in the area was decidedly lower than that for other African territories.

Rainfall (total and effective) appeared to be the dominating factor for yield, and experiments were made during 1957–59 to assess its influence. From meteorological records and observations, 40 in. was deduced as the expected (1 : 1 chance) annual rainfall, 27·4 in. of which would come after July 1, and under local conditions a crop of cotton would need about 25 in. of rain in the growing season if growth was not to be limited by soil moisture (July 1 is recommended as the sowing date for cotton, as the earliest which allows food crops to be planted first). What happens to rain after reaching the soil and how control of this influences yield were the primary issues in the field experiments.

Surface 'capping' through heavy rain, to which the local soils are specially prone, was shown to be largely controllable by timely hoeing and by mulching, entry of rain into the soil being thus greatly increased. The land is ridged for cotton and other crops, and it was found that here, as elsewhere, water conservation could be improved by 'tying' the ridges with small bunds at intervals along the furrows. In dry years this considerably increased yield but depressed it in wet years. These effects were clearly interpreted by soil–moisture profiles.

The effects of varying degrees of these controls having thus been measured, field trials were arranged, having in mind steps which could be fitted into peasant practice. A light mulching, in the furrow bottoms only, proved effective, and by slitting the furrow bottom and working a small amount of mulch into the slit the effect was enhanced. Tying alternate ridges only worked well, and avoided yield depression through standing water in wet years. Groundnut shell, dead grass and chopped *Sorghum* stalks made good mulches, the shell being best. By preparing the land for sowing at the onset of the rains, instead of just before sowing, which is local practice, cotton yields were raised by 15 to 20 per cent in two trials. Increased penetration and retention of rain had evidently been secured. Good response to fertilizers was obtained where tied ridges and mulch were also used.

The practical inference was clear—fertilizers, insecticidal protection, improved varieties and other betterments are repaid only when water conservation is amply ensured and this is practicable at peasant husbandry level. To drive the lesson home, cotton was grown on a half-acre plot for four years running in which rainfall was 55, 35, 42 and 43 in. Yields were all of the order of 2,000 lb. of seed cotton an acre, which is good for rain-grown cotton anywhere and excellent in comparison with any other part of Africa. Moreover, picking was ended by Christmas whereas it dawdled on for another 2–3 months on other plots.

This successful husbandry investigation is of more than local and immediate value. It is part of the widespread studies of rainfall, soil moisture and crop water needs carried out by the Empire Cotton Growing Corporation. The basic work is done at its Central Research Station, Namulonge, Uganda. All this work deals not with cotton alone but also in conjunction with the customary food crops of cotton growers. Agriculture in general is thus benefited in cotton-producing countries. Moreover, the practical problems centring on rainfall are becoming so defined and resolved as to enable their deeper, physiological study to be effectively opened up. Plant breeding, also, gains a clearer sense of direction.

F. L. ENGLEDOW

RADIOMETRIC AND PHOTOMETRIC STANDARDS

TWO recent publications in the series *Notes on Applied Science* of the National Physical Laboratory, Teddington, illustrate some of the activities of the Light Division of the Laboratory.

No. 23 in the series[1], *Radiometric Standards and Measurements*, by E. J. Gillham, deals with the methods used at the Laboratory for the measurement of ultra-violet, visible and infra-red radiation. The contents consist of three main sections devoted to the measurement of total radiation, the measurement of monochromatic radiation, and the absolute measurement of radiation, respectively. Total radiation is usually more intense than monochromatic radiation and therefore can be measured more easily, but the total energy content of a beam of radiation is often of less practical significance. An instrument for measuring total radiation must be equally sensitive to radiation at all wave-lengths, and the only means of converting radiation into another form of energy with predictable efficiency over the whole wave-length range is by absorption at a blackened surface, that is, to use a thermal detector. Fortunately, blacks with an absorption factor of 95 per cent or more over the whole spectral range of interest are available, and consequently thermal detectors with an error due to selectivity of not more than 2 or 3 per cent can be obtained. The type of thermal detector commonly used is the thermopile, and in the booklet the Moll thermopile, which has stable characteristics and which forms a very useful working standard for measuring radiation or for calibration of other radiation detectors, is described in some detail. A set of six Moll thermopiles is maintained at the National Physical Laboratory for calibration purposes. The methods used for the intercomparison of thermopiles and for the accurate measurement of the selectivity of a thermopile using a Callender radio-balance and three different sources of radiation are discussed.

To illustrate the problems encountered in the measurement of monochromatic radiation a description is given of a routine test undertaken at the Laboratory to determine the special sensitivity of a photoemissive cell, in the 0·25–0·7μ wave-length range. The optical arrangements and apparatus required for the measurement of the spectral sensitivity, and also the absolute sensitivity of the photocell, are given. For the absolute measurement of radiation a thermal detector in which the target is provided with a resistance element or other means for supplying a measurable amount of electrical power is recommended. At the National Physical Laboratory the absolute radiometer used is the Guild drift radiometer. It is of the compensated type with

ULTRASTRUCTURAL ALTERATIONS DURING THE CRABTREE EFFECT IN ROUS SARCOMA CELLS

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ELECTRON microscopic studies show that glucose selectively alters the microstructures of Rous sarcoma cells and that the cellular alterations observed could account for suppression of respiration[1] (Crabtree effect). The mechanism of glucose suppression of respiration of tumour cells has generally been approached through biochemical investigations[2]. Work in this laboratory indicates that glucose utilization *per se* does not depress uptake of oxygen. It was reported that increasing the concentration of glucose in the medium containing normal tissue will increase glucose utilization to a rate comparable with that of tumour tissue without suppressing respiration[3,4]. This preliminary report shows that when Rous sarcoma virus-induced tumours of the chorioallantoic membrane are excised from embryonated chicken eggs and incubated *in vitro* in the presence of glucose the cells undergo microstructural changes. Degeneration of mitochondria is observed. This effect of glucose on mitochondria of Rous sarcoma cells is similar to that reported by Merker *et al.* for Ehrlich ascites tumour cells[5]. Changes in the nucleus and endoplasmic reticulum of Rous sarcoma cells were also observed. These changes were not observed when Rous sarcoma was incubated in medium without glucose or in normal chorioallantoic membrane incubated in the presence or absence of glucose.

Standard lot *CT* 842 of Rous sarcoma virus was obtained from Dr. W. Ray Bryan and used for inducing tumours of the chorioallantoic membrane as previously reported[3,4]. Rous sarcoma was dissected free of non-tumorous tissue and sliced with a Stadie–Riggs hand microtome. Tissue slices were incubated under aseptic conditions in a Warburg flask in a final volume of 2 ml. with modified Hanks's balanced salt solution in the absence of added bicarbonate, and without phenol red indicator. The *p*H was adjusted to 7·6 with dibasic phosphate (*M*/15). The glucose concentration used was 5·5 µmoles/ml. Control flasks were without glucose.

After 12 hr. incubation at 37° C. tissues incubated with and without glucose were removed from the flask and immediately fixed in 1 per cent buffered osmium tetroxide[6] for 1 hr. at room temperature. The fixed tissue was embedded according to standard procedures in a 4:1 mixture of butyl and methyl methacrylates. Polymerization was carried out overnight at room temperature under an ultra-violet lamp. Sections were made with a Porter–Blum microtome equipped with glass knives.

Oxygen uptake was determined manometrically on tissue slices of tumour and chorioallantoic membrane. Results are reported on the average hourly rate of oxygen uptake during the 12th to the 16th hr. of in-

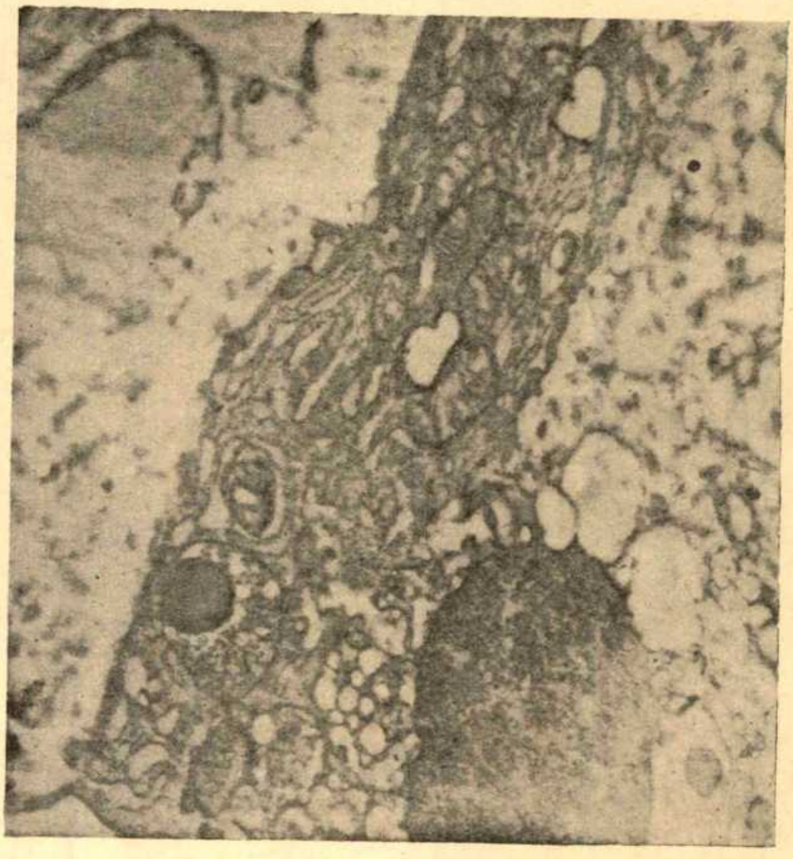

Fig. 1. Electron micrograph showing detail of a cross-section of a Rous sarcoma virus-induced tumour of the chorioallantoic membrane. Slices of tumour tissue were incubated in modified Hanks's balanced salt solution without glucose for 12 hr. at 37° C. The tissue was fixed in 1 per cent buffered osmium tetroxide and embedded in a 4:1 mixture of butyl and methyl methacrylates. The flattened surface cell shows mitochondria and cristæ and a well-delineated endoplasmic reticulum. Many vacuoles are present in the lower mesodermal cell and its nucleus shows a homogeneous composition. (× *c.* 16,500)

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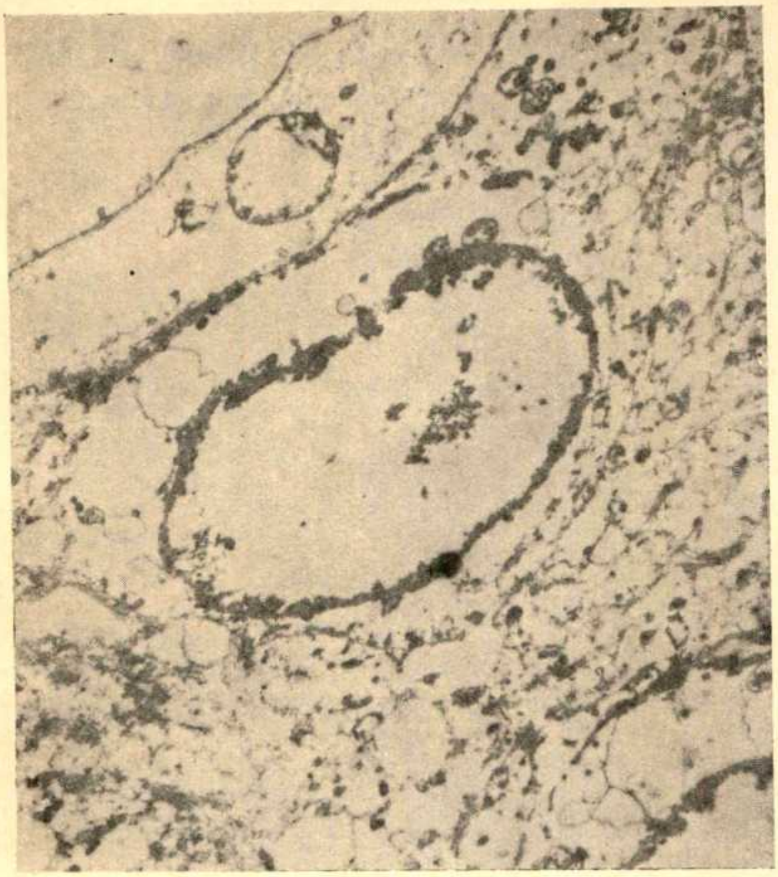

Fig. 2. Electron micrograph showing a cross-section of Rous sarcoma virus-induced tumour of the chorioallantoic membrane. Slices of tumour tissue were incubated in a modified Hanks's balanced salt solution with 5·5 μmoles/ml. of glucose for 12 hr. at 37° C. The tissue was fixed in 1 per cent buffered osmium tetroxide and embedded in a 4:1 mixture of butyl and methyl methacrylates. The flattened surface cell and mesodermal cell give the appearance of being 'leached out'. The degeneration of microstructures is evidenced by marginal clumping along the nuclear membrane, no well-defined endoplasmic reticulum, and no well-defined cristæ in what appears to be the remains of mitochondria. (× *c*. 11,000)

cubation to show the physiological state of the tissues at the time samples were taken for electron microscopic studies. Results given in Table 1 were determined on tissue slices from the same tumour viewed by electron microscopy (Figs. 1 and 2). Respiration results on chorioallantoic membrane are similarly reported for sections from the same membranes viewed by electron microscopy.

Slices of Rous sarcoma which had been incubated 12 hr. in Hanks's balanced salt solution were examined by electron microscopy (RCA-*EMU*-2). Well-delineated microstructures were observed (Fig. 1). The cells selected show a cross-section of the tumour with regard to the surface of the chorioallantoic membrane. It is not known if the flattened surface cell is ectodermal or endodermal, but distinct mitochondria with cristæ are observable as well as a defined endoplasmic reticulum. The nucleus of the mesodermal cell has a homogeneous composition, but the cytoplasm shows greater vacuolation. Representative sections of other mesodermal cells show well-formed mitochondria even though the increased numbers of vacuoles in mesodermal cells distort the endoplasmic reticulum. A comparable section (Fig. 2) shows a cross-section of tumour slices which had been incubated for 12 hr. with glucose. The flattened surface cell as well as the mesodermal cell give a 'leached out' appearance. There is no defined endoplasmic reticulum, and the only evidence of mitochondria may be cross-sections seen in the mesodermal cell giving a degenerate appearance. The nuclei show marginal clumping and the 'leached out' appearance seen in the cytoplasm. More than 200 sections of tumour tissue that had been incubated in glucose were made and examined. No well-formed mitochondria were seen. The 'leached out' appearance of the cytoplasm and nucleus was present in all cells observed.

Table 1. AVERAGE HOURLY RATE OF RESPIRATION DURING 12–16 HR.

Tissue	Medium	Oxygen uptake (μl./mgm. dry tissue/hr.)
Rous tumour	BSS	5·2
	BSS + glucose 5·5 μmoles/ml.	1·8
Chorioallantoic membrane	BSS	6·3
	BSS + glucose 5·5 μmoles/ml.	6·1

Direct method of Warburg 2 ml. final volume of reaction chamber at 37° C. Tissue weight range, 3–5 mgm. dry weight. Chorioallantoic membrane was from a 16-day-old embryonated egg. BSS, modified Hanks's balanced salt solution.

Comparable sections of normal chorioallantoic membrane, that do not give a Crabtree effect when incubated with glucose, appeared normal when examined under the electron microscope. In chorioallantoic membrane mitochondria were seen with well-defined cristæ, nuclei appeared homogeneous in composition, and the endoplasmic reticular system was well delineated.

It seems reasonable to propose that the physiological effect of glucose on Rous sarcoma respiration (Crabtree effect) may be a secondary result of the effect of glucose on the functional structures of the cell rather than a direct biochemical competitive inhibition. Damage to the cristæ of mitochondria results in 'leaching out' of enzymes and co-factors and respiration is interrupted[7]. It is proposed that glucose selectively induces damage to the mitochondria of Rous sarcoma cells and this may be the lesion of the Crabtree effect.

This work was supported in part by research grants: *C*–4692, of the National Cancer Institute, U.S. Public Health Service, and *E*–212, of the American Cancer Society, Inc. Grateful acknowledgment is made to the Indiana Elks' Association for support of the electron microscope work of this study.

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EFFECTS OF 2-DIETHYLAMINOETHYL 2,2-DIPHENYLPENTANOATE (SKF 525A) ON INSECTICIDAL POTENCY

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2-DIETHYLAMINOETHYL 2,2-diphenylpentanoate (SKF 525*A*) is well known to prolong the actions of a variety of drugs in mammals[1], and we have therefore investigated the effects of the compound on the toxicity to insects of pyrethrins and other insecticides, comparing its effects with those of the pyrethrin synergist 'piperonyl butoxide' (PB)[2,3] SKF 525*A* has been administered to mammals in the form of its water-soluble hydrochloride, but when injected the hydrochloride proved inconveniently toxic to the test insects, and if applied externally could be expected to penetrate only slowly through the insect cuticle, hence we dosed the insects externally with SKF 525*A* in the form of the free base, which, like PB, was then non-toxic at the doses used

In a series of qualitative tests, lesser mealworm beetles, *Alphitobius laevigatus* (F), were dosed topically between the hind coxæ[4] with a solution of SKF 525*A* or PB, and 5–10 min. later with a solution of insecticide on the neck[5]. Separating the applications reduced the risk of chemical reaction between the basic SKF 525*A* and those insecticides that were acidic, before they had penetrated into the insect 7 µgm. of SKF 525*A* or PB were applied in 0 07 µl. of solution in refined (odourless) kerosene, and an appropriate dose of insecticide in 0 025 µl of solution in a heavier refined petroleum oil, Shell 'Risella 17'. Applications of insecticide without SKF 525*A* or PB were made comparably, with a dose of oil solvent on the other site Use of the two different oil solvents enabled the two techniques of dosage to be used in conjunction In additional qualitative tests, houseflies, *Musca domestica* L , were dosed topically[4] with pyrethrins or malathion, mixed with SKF 525*A* or PB, in solution in refined kerosene Effects were determined by percentage of beetles paralysed 4 days after dosage (approximating to final mortality) and percentage of flies dead 24 hr after dosage The results, shown in Table 1, indicate a joint-action spectrum for SKF 525*A* qualitatively similar to that for PB, reduction by PB of the insecticidal activity of malathion in flies has been noted previously[6]

Table 1 EFFECTS OF SKF 525*A* AND PB ON THE TOXICITIES OF CERTAIN INSECTICIDES

Insecticide	*A laevigatus*		*M domestica*	
	SFK 525*A*	PB	SKF 525*A*	PB
Pyrethrins	+	+	+	+
Allethrin	+	+		
Barthrin	+	+		
Malathion	(–)	–	–	–
2-*n*-Valeryl 1,3-indandione	+	(+)		
2-*sec*butyl 4,6-dinitrophenol	0	0		
DDT	0	0		
γ-BHC	0	0		
Dieldrin	0	0		

+, Considerable synergism, (+), slight synergism, 0, no effect, (–), slight antagonism, –, considerable antagonism

The synergistic effects of SKF 525*A* and PB on the pyrethrins were compared quantitatively The synergist was used in mixture with the pyrethrins, in solutions in refined kerosene The volume was 0 07 µl. per beetle, applied between the hind coxæ[4], and 0 05 and 0·10 µl per male and female fly respectively, applied dorsally on the thorax[4] Factors of synergism[3,7] were determined, each the ratio of the ED_{50} for pyrethrins in the absence of synergist to that in the presence of synergist at 10 times the dose of pyrethrins With SKF 525*A* the factor was 1·8 for the beetles and 2 5 for the flies; with PB the factor was 3 2 for the beetles and 15 for the flies SKF 525*A* was less synergistic than PB

Neither 2,2-diphenylpentanoic acid nor 2-diethylaminoethanol affected the toxicity of pyrethrins Other congeners of SKF 525*A* are being investigated

In mammals SKF 525*A* depresses the metabolism, especially oxidative metabolism, of numerous drugs, thereby increasing[1] or decreasing[8] their effectiveness according as the metabolic products are more or less active than the drugs themselves In the present work SKF 525*A* depressed the effect of malathion, which is oxidized in the cockroach, *Periplaneta americana*, to the more insecticidal malaoxon[9] O'Brien[10] mentions that SKF 525*A* failed to reduce the action on the cockroach and the housefly of certain organo-phosphorus insecticides with more insecticidal oxidation products, but does not state the conditions in his experiments In the present work SKF 525*A* was found to increase the effectiveness of pyrethrins, which, like related pyrethroids, are metabolized in insects to less insecticidal compounds not as yet identified, though chromatographic evidence[11] indicates that they could be oxidation products. The influence of PB on the toxicities was qualitatively similar to that of SKF 525*A* Thus the findings reported here are in general accord with other evidence[12] that PB and other 3,4-methylenedioxyphenyl compounds synergize pyrethrins in insects by depressing oxidative detoxification

In mammals SKF 525*A* depresses drug metabolism brought about by the liver microsomes[1], a scheme has been put forward[10] to account for the apparently low specificity of the enzyme systems involved The present results suggest that there may be in insects systems analogous to those inhibited by SKF 525*A* in the liver microsomes of mammals

We are indebted to the Smith Kline and French Research Institute, Welwyn Garden City, for a sample of SKF 525*A*

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BLUE-GREEN ALGAE

Growth

It is well known that many species of algae fail to thrive in simple inorganic media and may be cultured with greater ease in the presence of ill-defined organic materials such as soil extract[1,2]. These observations have promoted the suggestion that such algae may have requirements for growth factors, especially since many species are now known to be unable to synthesize certain vitamins essential to their metabolism[3]. In an earlier discussion of this question, however, Gerloff *et al.*[4] have directed attention to the fact that, in general, the addition of organic solutions of unknown composition has never been found to be absolutely essential for growth. They conclude that stimulation to growth under these conditions may be ascribed to the physiological condition of the algae rather than to the provision of some essential vitamin-like substance. Nevertheless, their statement that it is unlikely that any members of the Cyanophyta will be found to exhibit a requirement for vitamins has been shown to be in error in at least one instance by Pintner and Provasoli[5], who have discovered an absolute requirement for vitamin B_{12} by *Phormidium persicinum*. It is particularly interesting to note that several species of algae have been found not to thrive in the absence of other micro-organisms. In fact, Schramm[6] found himself unable to induce growth of zoospores and zygospores of *Vaucheria*, *Oedogonium* and *Spirogyra* in bacteria-free conditions. Among the Cyanophyta, Fogg[7] records the common observation that *Gloeotrichia natans* fails to thrive as well in pure as in impure culture. Findings such as these seem to support the assertion of Pringsheim[1] that "multiplication is often harder to achieve than isolation from bacteria and other unwanted micro-organisms". Similar experience and difficulties and the absence of more precise information led me to undertake the present work, the chief results of which are described here.

In isolating bacteria-free cultures of blue-green algae from rich soils of tropical northern Australia[8,9], observations were made with an unidentified species of *Nostoc* which agree closely with those for *Gloeotrichia natans* recorded by Fogg[7]. Growth of the alga in impure culture was clearly superior to that in pure culture. The stimulatory effect was found to be due to a species of the stalked bacterium *Caulobacter* which was prominent among the contaminants. When the two organisms were grown together in the light in a suitable nitrogen-free mineral medium, considerably greater amounts of nitrogen were fixed than by the alga alone under otherwise identical conditions. These results are shown in Table 1. It will be seen that, whereas 9·64 mgm nitrogen were fixed by the alga in pure culture, 17·43 mgm were fixed in the presence of the bacterium. In each case, a notable proportion of the nitrogen fixed was released into the culture solution. Separate tests showed that the *Caulobacter* is unable to fix nitrogen in pure culture. At the present time, there is no evidence to indicate that the stalk has any special function in the association comparable with the attachments shown by Houwink[10] to cells of *Bacillus*, although it has been found that the structure reaches maximal development in the presence of the alga and does not appear to be formed on media such as meat extract agar. It is worth noting that stalked organisms of the same genus have been found commonly in impure cultures of other blue-green algae from widely separate parts of Australia.

Table 1. NITROGEN FIXED (MGM.) BY *Nostoc* ALONE AND IN ASSOCIATION WITH *Caulobacter* (KJELDAHL ANALYSES)

	Nostoc	*Nostoc* with *Caulobacter*
Cells	6·14	12·46
Culture solution	3·50	4·97
Total	9·64	17·43

To obtain greater understanding of the relationship, the stages in the growth cycle of the alga were followed in Petri dish culture. It was observed that hormogonia, on formation, move away from the colony margin and then undergo a series of reciprocal movements along the path first followed, this activity culminating in a sudden arching of the originally straight filament. At this stage, motility is lost and the individual cells begin to enlarge and divide in such a manner that the developing trichome becomes more and more contorted. Terminal and intercalary heterocysts become apparent, the latter being formed at fairly regular intervals along the trichome. The alga has never been observed to produce akinetes. Continued growth results in the formation of a roughly spherical trichome mass 0·1–0·2 mm. or more in diameter, from the interior of which a second generation of hormogonia emerges.

If single migrating hormogonia are transferred to fresh medium, their movements commonly cease, trichome formation begins almost immediately and the cycle of growth continues normally for at least a while. Frequently, however, the hormogonia either lose their pigmentation and die without further development or continue their normal growth and die at some later stage, often before the emergence of the second generation of hormogonia. Even when colonies 20–30 mm. in diameter are produced from a single hormogone, they are invariably pale in colour and thinly formed, and made up in large part of colourless and apparently non-viable trichomes. The capacity of hormogonia to continue developing has been found to be closely associated with the light history of individual colonies. Failure of hormogonia to develop on transfer may be induced most readily by frequent sub-culture under conditions of continuous and relatively intense illumination.

When small numbers of nitrogen-starved *Caulobacter* cells are added to single transferred hormogonia, growth is always more rapid than in the absence of the bacteria and is never accompanied by the death of the alga. Colonies produced in this way are larger, thicker and more densely pigmented than pure cultures and do not contain the colourless trichomes so characteristic of the latter.

Initially, it was supposed that some essential organic nutrilite might have been lacking in the absence of the bacterium, although this seemed rather unlikely in view of the fact that at least reasonably normal development was often observed to occur in pure culture as already described. None the less, the possibility was investigated. It was found that the incorporation in the basal nitrogen-free mineral medium of materials such as yeast extract, casein hydrolysate as well as the individual vitamins thiamine, biotin and B_{12} and a range of single amino-acids were without effect. Without exception, single hormogonia transferred to the supplemented media failed to develop. Similar results were obtained when low concentrations of ammonia or nitrate ions were supplied. Even when growth took place in the presence of inorganic nitrogen, it was not superior to that in control plates.

Later, it was discovered that the addition of one or two drops of a 1 p.p.m solution of indole-3-acetic acid was capable of effecting the recovery of bacteria-free hormogonia degenerated to the colourless condition preceding death Accordingly, tests were conducted using *Avena* coleoptile sections in an attempt to detect growth-stimulating hormones in cultures of the alga and the bacterium It was found that a highly significant increase in the length of coleoptile sections occurred in the presence of *Caulobacter* cells compared with controls A similar response was recorded with sections in contact with a reasonably healthy pure culture of the alga grown for three weeks at only 12 ft.-candles and also with 18-hr cultures, associated with *Caulobacter*, from which hormogonia were beginning to emerge at about 100 ft.-candles. In pure cultures more than 4 weeks old and grown at relatively high light intensities between 500 and 600 ft -candles, the growth of coleoptile sections was significantly inhibited.

From these observations, there seems little doubt that, in some way, substances with hormone properties are necessary to enable the alga to achieve normal development Here, attention is directed to a recent investigation in which Lazaroff and Vishniac[11] have concluded that *Nostoc muscorum A*, too, is dependent on a 'morphogenetic substance' to complete its normal cycle of growth With *N muscorum*, however, the substance is never limiting under normal conditions of cultivation in the light Abnormal growth is exhibited only when the alga is incubated in the dark with a suitable source of carbon

The inhibiting influence of older cultures on the growth of coleoptile sections is of interest That the effect might have been caused by excess hormone seems improbable It is known that auxin and indole-3-acetic acid are inactivated by light. Granick[12] has directed attention to the particularly destructive effect of blue light on auxin. In this connexion, it is relevant to note that the warm-white fluorescent lights used in the present work emit strongly in this region and have been found harmful to several species of chlorophycean algae by Algeus[13] A more plausible explanation for the results is suggested by the separate observation by Algeus[2] that the oxidation products of indole-3-acetic acid are inhibitory to growth However, it should not be overlooked that Stewart *et al* [14] have found a precursor of auxin in radishes that exerts a similar effect

Whatever the nature of the inhibitory substance or substances, it is possibly correct to assume that normal development of the *Nostoc* in question is dependent on the balance existing from time to time between hormone and growth inhibitor. As a corollary, it would be interesting to investigate the possibility that the reciprocal movements of hormogonia on agar media constitute a response to gradient concentrations of these substances around the margins of colonies.

In view of the fact that auxins tend to be destroyed in the light, it seems unlikely that the beneficial influence of *Caulobacter* is due solely to the contribution of a growth hormone, although this, undoubtedly, is of some importance Other observations support this contention. In particular, the alga is invariably seen to be dark in colour, sometimes almost black, in the presence of *Caulobacter*, whereas pure cultures are commonly a pale green The dark coloration is caused by phycoerythrin which appears to exercise a protective function by absorbing those wave-lengths to which the hormone is susceptible Recently, Boney and Corner[15] have presented evidence to show that this pigment also has a protective function in certain red algae The mechanism by which the bacterium stimulates phycoerythrin production by the alga is not known Apparently light of short wave-lengths is also necessary, however, because the pigment is not produced under red light even with *Caulobacter* present

In this matter, a further observation appears to be especially significant It has been noted already that considerable enlargement of the trichome immediately precedes formation of hormogone It seems not unreasonable to assume that such development might well be favourable to the conservation or build-up of hormone by the alga If the beneficial influence of *Caulobacter* lay only in the provision of hormone, it would be expected that, in cultures containing the bacterium, trichome enlargement would be obviated to some extent In fact, this is found to be true, although the effect is observed to operate only in the early phases in the growth of colonies from single hormogonia Therefore, it must be concluded that direct stimulation is relatively short-lived and must be supplemented or replaced at some stage by hormone production by the alga with the protection afforded by abundant phycoerythrin

A remarkable feature of the early growth of colonies containing *Caulobacter* is that fresh hormogonia are produced not only after minimal trichome development but also without the formation of heterocysts The heterocysts appear to be associated with full trichome enlargement Furthermore, it has been observed that hormogonia originating from fully developed trichomes always emerge from the interior of these bodies, never from the periphery One is immediately reminded of a suggestion by Fritsch[16] that these 'enigmatic' structures might well be concerned with the production of substances controlling growth, cell division, and other developmental processes of blue-green algae It is believed that the present work provides concrete evidence in support of this hypothesis and could also explain the reason why difficulty is sometimes experienced in securing the growth of algae in pure culture J S BUNT

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Isolation of Bacteria-free Cultures from Hormogone-producing Blue-Green Algae

THE difficulties associated with obtaining bacteria-free cultures of blue-green algae are well known Various techniques, involving the use of toxic physical or chemical agents combined with patient and careful sub-culture, have been tested in attempts to eliminate contaminants. Of the many agents examined, few have proved to be successful. Allison and Morris[1], Allison *et al.*[2], Bortels,[3] Gerloff *et al.*[4], Fogg[5] and Venkataraman *et al.*[6] have prepared bacteria-free cultures by exposing algal material to ultra-violet light. Fogg[7] purified one species with the aid of chlorine water. De[8] depended on the selectivity of mineral media solidified with silica gel and on extensive sub-culturing to obtain pure cultures. Unfortunately, these techniques have not been found generally applicable[7,8], partly because a large degree of reliance must be placed on chance for success.

In these Laboratories, none of the techniques described in the literature proved satisfactory. A number of others, based on the assumption that an agent might be found to which the algae alone would be insusceptible, gave equally disappointing results. The possibility of using antibiotics has been examined[9], again without success.

In view of these results, it was felt that some technique aimed at the selection of newly formed and uncontaminated trichomes as suggested by Pringsheim[10] rather than the destruction of micro-organisms closely bound in the sheaths of older material might be more rewarding. Such an approach has, of course, already been used where final isolation has been from newly migrated hormogonia. However, the procedures used have seriously limited the chances of success. The method to be described was designed to minimize the operation of chance.

A small amount of inoculum containing a single species of alga is transferred to the surface of a suitable medium solidified with agar previously prepared as a deep, one-third filling a 6 in. × 1 in. culture tube. The material is incubated under appropriate lighting until fresh growth is just visible to the naked eye. Sufficient medium is then added carefully to form a plugging layer 3–5 mm. deep over the developing colony. As soon as this plug has set, 5–10 ml. of the same medium, but without agar, is added and the tube returned to the light room. With the algae under investigation, it was found possible and worth while to incorporate 60 μgm./ml. aureomycin in the plugging medium as an aid to final isolation. After some time, 5–11 days in my experience, young hormogonia and usually small numbers of motile bacteria are found to be present in the supernatant liquid medium although not visible to the naked eye. At this time, a series of small aliquots is transferred to Petri dishes and poured with the chosen medium. In these Laboratories, 10–20 0.1-ml. aliquots are used. The number of plates and the size of the aliquots are chosen to favour the development of isolated uncontaminated colonies. These are located as early as possible by regular microscopic inspection of the plates and immediately transferred to fresh medium. A fine hypodermic needle mounted in a suitable holder and a dissecting microscope have been found indispensable for this purpose. The purity of cultures is checked by normal cultural procedures and by direct microscopic inspection.

Up to the present, six bacteria-free cultures have been obtained, including the genera *Nostoc*, *Anabaena* and *Gloeotrichia*. It is of interest to note that the cultures of *Gloeotrichia* are being maintained on nitrogen-deficient media. Kjeldahl analyses suggest a capacity for nitrogen fixation.

Unfortunately, the technique described has not been found applicable to species which do not form hormogonia readily. However, recent investigations indicate that exposure of such organisms to suitable concentrations of indole-3-acetic acid at light intensities of about 12 ft.-candles may be generally useful for inducing formation of hormogone. In fact, using this technique recently, two further bacteria-free cultures have been obtained of algae that otherwise could not be induced to form hormogonia.

This and the preceding work have been supported by a University of Sydney research grant. Thanks are due to Miss R. Webb for technical assistance.

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Use of Antibiotics to purify Blue-Green Algae

THE use of antibiotics in media for tissue cultures suggests that such techniques may also be useful for freeing blue-green algae of contaminants. Our results with antibiotics have not been entirely successful, but it may serve some useful purpose to report them here to avoid possible waste of time by other investigators.

The following substances are inhibitory to algal growth: cetrimide (*n*-cetyl-trimethyl ammonium bromide), dithionyl, streptomycin, neomycin, bacitracin. Actidione, penicillin, chloramphenicol, tetracycline, sulphafurazole, erythromycin, nystatin, novobiocin, oleandomycin, polymyxin, furodantoin and fungostatin are not inhibitory. It is possible to eliminate fungal contaminants by cultivation of algae in the presence of actidione and fungostatin, but not possible to free the algae from bacteria by using singly any of the substances mentioned here.

The following combinations have been used without success: (i) tetracycline–chlorotetracycline, (ii) penicillin–erythromycin–aureomycin, (iii) polymyxin–penicillin–erythromycin–tetracycline. No success was obtained when the algal cultures were pre-treated with 0.002 per cent dithionyl.

It should be reported here that the bacterial contaminants are sensitive to antibiotics when tested in the absence of algae but cannot be eliminated when the algae are present. The algae seem to protect the bacteria against the antibiotics in some way. Also, some of the contaminants would not grow in ordinary meat infusion broth. Therefore, the absence of bacterial growth in broth should not be regarded as a sole criterion for purity of algal cultures.

We wish to acknowledge financial assistance provided by the Commonwealth Scientific and Industrial Research Organization Division of Land Research and Regional Survey and the Wheat Industry Research Committee of New South Wales.

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LETTERS TO THE EDITORS

PHYSICS

Vortex Shedding from the End of a Tube as a Result of Buoyancy Effects

ONE of the most interesting phenomena observed in fluid flow is that of vortex shedding, the best known example of which is the von Kármán vortex street behind a cylinder or similar obstacles normal to the flowing stream. For such two-dimensional systems, there is considerable information available. However, for axially symmetric systems, there appears to be little more than the recent results of Magarvey and Bishop[1] on spheres. Therefore, a study of vortex shedding in axially symmetric systems was initiated under Battelle sponsorship. In one system, vortices as shown in Fig. 1 were observed in alkaline water (containing amounts of sodium carbonate neutralized by up to 1·24 c.c. nitric acid/litre) discharging from a long vertical tube into a large plenum chamber. The chamber contained slightly acidic water (up to 7·65 c.c. nitric acid/litre). Reynolds numbers down to the experimental limit of above 200 were investigated. The flow pattern was revealed by phenolphthalein added to the inlet water supply.

The Strouhal number, $\nu D/V$, which characterizes in a dimensionless manner the vortex shedding frequency, varied from about 0·3 to 1·4 in the experiments. The Strouhal number was observed to increase with increasing acidity, with increasing alkalinity, and with decreasing Froude number. Reynolds number did not appear to be an important variable. It would appear that the buoyancy effects produced by the small release of heat at the reaction interface causes the vortex shedding in an otherwise stable flow. This supposition is supported by the observation of similar vortex shedding when a charged platinum ring at the tube exit was used to generate hydrogen bubbles, and when the density of the inflow was reduced slightly by use of a small addition of alcohol.

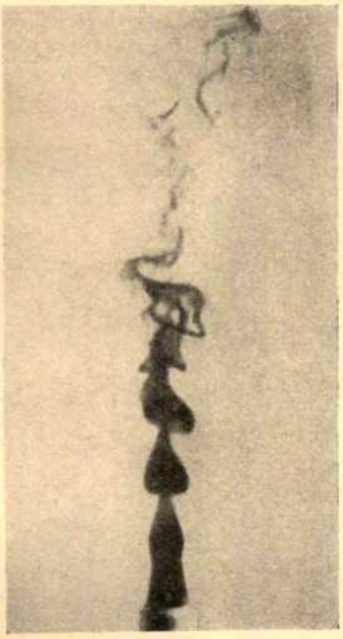

Fig. 1. Vortex formation at discharge of ½ in. diameter tube

One other observation should be reported. Tritton[2] observed, in an investigation of vortex shedding from rods, that at certain combinations of the independent parameters two alternative frequencies rather than one frequency could occur. Sato[3], in work on the instability of two-dimensional jets, made a similar observation. In my system, quite dissimilar to the previous two, we also observed that there were certain combinations of independent variables which led to two alternative frequencies, and, consequently, two alternative Strouhal numbers. In fact, the shifting from one frequency to the other, which often occurred in these instances, led to difficulties in obtaining accurate counts of frequency of vortex formation.

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Distribution of Pressure for Flow of Gas through Porous Media

THE permeability coefficient K for flow of gas through a porous medium is defined by the equation[1]:

$$G = -K\frac{\partial c}{\partial x} \qquad (1)$$

where G is the flowing flux in moles through unit cross-sectional area of the medium in unit time, and $\frac{\partial c}{\partial x}$ is the concentration gradient. When the concentration of the gas is expressed in terms of the pressure p, equation (1) becomes:

$$G = -\frac{K}{RT}\frac{\partial p}{\partial x} \qquad (2)$$

If K is a linear function of pressure:

$$K = a + bp \qquad (3)$$

where a and b are constants, equation (2) may be integrated using the boundary conditions: $p = p_i$ (inlet pressure) at $x = L$; $p = p_0$ (outlet pressure) at $x = 0$; $p = f(x)$ $0 < x < L$ for all time.

The solution is:

$$p^2 + \frac{2a}{b}p - \left(\frac{a}{b} + p_m\right)2\Delta p\frac{x}{L} - p_0^2 - \frac{2a}{b}p_0 = 0 \qquad (4)$$

where $\Delta p = p_i - p_0$ = pressure difference, $p_m = (p_i + p_0)/2$ = mean pressure, and L is the length of the medium. This is the general equation for a parabola.

We have measured the pressure at three points inside a split specimen for steady-state flow of nitrogen, carbon dioxide and freon-12 (CF_2Cl_2) through Acheson '*A*' and Morgan '*EY*9' graphites. The experimental pressure distributions approximate to

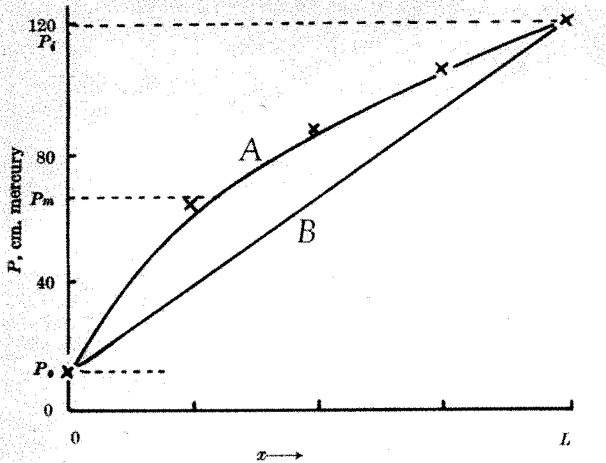

Fig. 1. Distribution of pressure through Acheson '*A*' graphite specimen. Flow gas, nitrogen. p_i = 120·6 cm. mercury, p_0 = 12·1 cm. mercury, L = 3·9 cm.; ×, experimental pressure. Full lines: *A*, parabola calculated from equation (4); *B*, chord

those predicted by equation (4) for $35 < \Delta p < 160$ cm. mercury and $20 < p_m < 120$ cm. mercury. A typical distribution is reproduced in Fig. 1. In practice, the experimental pressures diverge from the theoretical parabola by up to 12 per cent at the centre of the specimen, the experimental error being less than 3 per cent. This divergence may be attributed to three possible causes: (1) inhomogeneity in the graphite (the variation in K through specimens of the two graphites was 30 per cent and 55 per cent respectively); (2) non-linearity of the dependence on pressure of K (in one of the graphites $K = 0{\cdot}25 + 0{\cdot}065\,p - 7 \times 10^{-4}\,p^{1{\cdot}68}$), and (3) possible surface flow of adsorbed gas.

Carman[1] gives the integration of equation (2) as:

$$G = \frac{K}{RT} \cdot \frac{\Delta p}{L} \qquad (5)$$

which he states is valid when Δp is small or K is independent of pressure. Now it may be readily shown from equation (4) that the tangent to the theoretical parabola at $p = p_m$ is parallel to the chord from p_i to p_0, that is, at $p = p_m$, $\frac{\partial p}{\partial x} = \frac{\Delta p}{L}$. This means that (5) may be applied to calculate K at the mean pressure in a porous medium for any Δp provided that K is a linear function of pressure.

Biram[2], in a recent communication, has proposed that the constants a and b in equation (3) can be obtained by measuring the pressure at the mid-point and at both ends of a specimen. The equation he gives may be derived from equation (4). In practice, we find that his method may involve an error as high as 120 per cent due to the reasons mentioned earlier which cause deviations of the experimental pressures from the theoretical parabola.

We also take this opportunity to mention that we have observed minima in the variation of K with pressure for several gases in different graphites at low pressures.

We are indebted to Mr. A. McNab of the New Zealand Department of Scientific and Industrial Research for a helpful discussion. We acknowledge the support of the Australian Atomic Energy Commission in the award of a scholarship to one of us (G. J. F.), and the provision of graphite samples.

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The Reaction Silicon-29(*n*,α)Magnesium-26 at 14 MeV. Neutron Energy

A 400 Ω-cm. *n*-type silicon surface barrier detector was irradiated by neutrons of energy 14·03 ± 0·05 MeV. The experimental arrangement is identical with that described in an earlier communication[1] reporting results for (*n*,α) and (*n*,*p*) reactions in silicon-28 at the same neutron energy.

The reaction silicon-29(*n*,α)magnesium-26 has a *Q*-value of − 0·022 MeV., so that although its abundance in natural silicon is only 4·7 per cent it is possible to observe the reaction in competition with the more intense silicon-28 reactions, as the typical energy spectrum of Fig. 1 shows.

The results of Hinds and Middleton[2] for the energy-levels of magnesium-26 enable assignment of the two highest energy groups to the magnesium-26 ground-state and the excited state at 1·801 MeV. The ground-state and first four excited states of magnesium-25, populated by the silicon-28(*n*,α)magnesium-25 reaction, are also present and are labelled.

Dearnaley and Ferguson have examined the reaction silicon-29(*n*,α)magnesium-26 in the nuetron energy-range 5–8 MeV., and report a group which could correspond to transitions to a new first excited state in magnesium-26, located at 1·33 MeV. (ref. 3). Re-examination of the high-energy portion of our previously reported spectra did not yield evidence

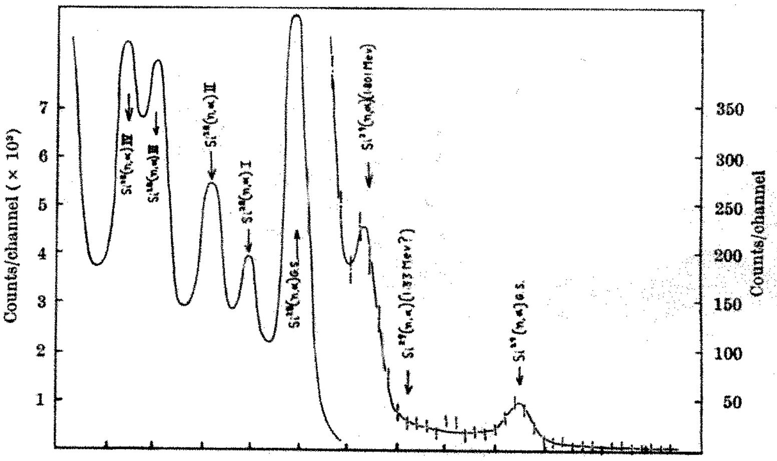

Fig. 1. Spectrum showing the reaction silicon-29(*n*,α)magnesium-26

for such a transition. Re-investigation of the relevant region with improved statistics was undertaken with the results given above, and fails to provide evidence for an alpha-particle group corresponding to the level postulated by Dearnaley and Ferguson.

The approximate cross-section ratio for the magnesium-26 ground-state and 1·801-MeV excited state groups, obtained in the present experiment, agrees with that obtained by Dearnaley and Ferguson at 7·6-MeV. neutron energy. In the absence of a selection rule inhibiting the transition, one would expect the 14 MeV (n,α) spectrum to exhibit a cross-section to the new magnesium-26 level similar to the ground-state cross-section. Fig. 1 indicates that this is not so, and if the level exists the cross-section for the transition to it is less than 0·1 mb.

Previous investigations of the energy-levels of magnesium-26, notably those of Hinds and Middleton, using the reactions magnesium-25(d,p)magnesium-26, aluminium-27(t,α)magnesium-26 and magnesium-24(t,p)magnesium-26, gave no indication of a level in magnesium-26 at 1·33 MeV, nor did the experiment of Glover and Weigold[4], investigating the reaction aluminium-27(n,d)magnesium-26.

Dearnaley and Ferguson have considered and investigated possible sources of contamination and we can offer no obvious explanation for the peak observed in their results. Additional experimental evidence is required to resolve the problem of whether or not the postulated level exists.

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GEOCHEMISTRY

Origin of High Tritium Content of Atmospheric Methane, Hydrogen and Stratospheric Water

RECENT work (ref. 1 and Suess, H. E., private communication) has shown that atmospheric methane has a fractional tritium content orders of magnitude higher than that of rain water and approaching that of atmospheric hydrogen and stratospheric water. The rate of increase with time of this tritium in methane makes it appear likely that it originates in the testing of nuclear devices. (The finding by Bishop *et al.*[1] that the specific activity of atmospheric methane appears to be lower in the troposphere than in the stratosphere or at the surface is, however, not obviously explained on the basis of origin in thermonuclear explosion, or for that matter by origin from any single source.) However, no satisfactory mechanism by which such tritium can be incorporated in methane has been advanced. The high temperature and oxidizing conditions supposed to exist in the bomb fireball seemed to rule it out as a source of tritiated methane[1]. Biological processes yielding methane of high specific activity are difficult to conceive; and indeed, sewage methane has little tritium content[1]. High atmosphere, radiation-induced mechanisms for exchange of methane with high specific activity atmospheric hydrogen can be formulated, but these mechanisms suggest that the methane is likely to be oxidized under the conditions required (Libby, W. F., private communication).

The thesis of this communication is that tritiated methane may after all be produced in the fireball, in a reducing atmosphere which I believe must exist at its centre. Thermonuclear weapons are presumably constructed primarily of reducing substances. Following detonation, the hot gas formed from bomb material will push back the surrounding atmosphere to form a reducing core within the fireball. On cooling to about 3,000° C this core will contract to a diameter approaching 100 metres, assuming some reasonable mass of the original weapon. During this stage the fireball is isothermal[2], and it is very unlikely that the large reducing core will mix with its oxidizing surroundings. On further cooling its contents will form molecular species. The tritium may be expected to combine largely as metal hydrides, molecular hydrogen, water, ammonia and hydrocarbons including methane. (The necessary carbon may be derived from conventional explosives and any other carbonaceous material contained in the device.)

At the end of the glowing, isothermal stage the reducing core, now hotter than its surroundings, will presumably tend to rise. During this convection, air will be entrained and the cooling, shrinking bubble of reducing gas may be expected to break up and mix. Insufficient data are openly available to speculate about how much combustion will occur during this process, but it would be surprising if such oxidation were complete.

If the high tritium content of atmospheric methane is due to this mechanism the question arises why this same methane does not also have a high carbon-14 activity[1]. It must be first pointed out that most of the carbon-14 from the $^{14}N(n,p)^{14}C$ reaction will be produced outside the reducing core and will therefore appear as $^{14}CO_2$. However, even if the amount of carbon-14 within the core were of the same magnitude as that of tritium, then atmospheric methane resulting from such events having an activity of 2×10^4 tritium units ($T\ H = 1·5 \times 10^{13}$) would have incremental carbon-14 activity amounting to only about 10 per cent of that of contemporary biosphere carbon.

The hypothesis of a reducing core in a nuclear explosion fireball also suggests that the high tritium content of atmospheric hydrogen[3] of the thermonuclear era arises directly, and is not dependent on, an exchange between molecular hydrogen and tritiated water. Most of the tritium in the fireball will probably be combined in molecular forms other than methane and hydrogen, particularly in metal hydrides. These, and hydrocarbons less stable than methane (for example, acetylene), will afterwards be completely oxidized in the atmosphere to yield water vapour of high specific activity found in the stratosphere[4].

The validity of the suggestion advanced here may be checked in several ways. The most direct involves sampling the fireball gases soon after they have cooled. Later tests on atmospheric gases may be based on the abnormally high ratio of deuterium to protium which may be presumed to exist in the fireball core

Thus there should be an unnaturally high probability that a molecule of atmospheric methane containing tritium contains a deuterium atom as well Such a measurement of the ratio CH_3T CH_2DT will require mass separation prior to the application of low-level counting techniques The ratio of HT DT in atmospheric hydrogen is much easier to determine since these isomers can be separated by gas chromatography[5] The answer here will depend not only on the origin of the tritiated hydrogen, but also on whether it can undergo exchange with atmospheric water

I am indebted to Prof W F Libby and to Dr E A Martell for stimulating discussions of the present proposal This work was supported by the U S Air Force and Atomic Energy Commission

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Formation of Pegmatitic Carbonatite in a Syenite–Marble Contact

Immediately north of the village of Karacayir, vilayet Sivas, Turkey, a small intrusion of rather coarse-grained syenite occurs in a banded marble formation of unknown age Here I had occasion to study in some detail a peculiar coarse-grained marble of pegmatitic appearance, containing biotite and apatite The banded marbles seem to be regionally metamorphosed, on which the thermal metamorphism caused by the intrusion is superimposed The part of the marble which protrudes into the syenite is changed into a carbonatite–pegmatite Otherwise the marbles retain their regular banding, which even seems to be accentuated when nearing the contact Within the aureole of thermal metamorphism small crystals of phlogopite and diopside develop in small amounts on the stratification planes as well as throughout the marble Near the contact small dikes of syenite–aplite and more-or-less crushed syenite–pegmatite cut through the marbles, the syenite itself contains locally muscovite

A non-metamorphic fossiliferous impure nummulitic limestone lies unconformably on top of both marble formation and syenite, thereby defining the age of the latter as pre-Eocene Intra-formational conglomerates are polymixt, containing quartz-pebbles, quartzites, quartzitic schists and acid intrusives

Next to calcite the pegmatitic carbonatite contains large (up to 1 in and more) biotite crystals, slender hexagonal prisms of green apatite, and locally small idiomorphic thorianite (ThO_2) crystals In one place a mass of large idiomorphic allanite crystals has been found, of platey habit, up to 1 in across Direct evidence that this carbonatite actually passed through a molten state is not available, as no carbonate-glass was found Wyllie and Tuttle[1] state that "Some carbonate liquids can be quenched to a glass by extremely rapid cooling, but in nature such rapid cooling is unlikely and evidence of melting in rocks will be difficult to find"

The total obliteration of the pre-intrusive structure of the marble, the idiomorphic and coarse-grained habit of its constituent minerals, its obvious permeation with magmatic fluids (addition of thorium, rare earths, phosphorus pentoxide, (Fe,Mg)O silica and water) indicate a pseudo-liquid condition of this carbonatite mass at the time of the syenite intrusion This condition is most probably caused by the presence of a liquid phase, a carbonate melt

According to the same authors[1] the partial melting of carbonates in the presence of water vapour starts at temperatures as low as 650° C , and at pressures reached at relatively high levels in Earth's crust This temperature is almost certainly exceeded during the intrusion of a syenitic magma In another publication Wyllie and Tuttle[2] state that· "Although calcite is generally the dominant mineral in carbonatites apatite and mica are constant accessories" The presence of radioactive and rare-earth minerals (thorianite and allanite) in the carbonatite mass shows its close affinity to 'normal' intrusive carbonatites It is believed, in the light of the results obtained by Wyllie and Tuttle[1,2], that pressure-temperature conditions in this contact provoked the (partial) liquefaction of the marble, which recrystallized on cooling as a pegmatitic mass of carbonatite composition The importance of this example lies in the fact that here a carbonatite, associated as usual with alkaline intrusives, is still indisputably recognizable as the direct continuation of a sedimentary marble Though I certainly do not claim definitely that carbonatites must be formed this way, it shows at least that they may

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METALLURGY

Embrittlement of Grain Boundaries by Equilibrium Segregation

Equilibrium segregation of impurities or solute elements has frequently been suggested as a cause of grain boundary embrittlement in metals and metallic compounds, but the evidence has been largely indirect Even if the existence of grain boundary segregation be granted in specific instances, the manner in which such segregation might give rise to embrittlement has remained wholely speculative It is the purpose of this communication to report that in a large number of cases where boundary segregation is established, suspected, or possible, a significant hardening of the grain boundary region relative to that of the interior of the grain is observed by microhardness measurements

A typical result is shown in Fig 1 for a high-purity iron sample treated by Ainslie *et al* [1] in a sulphur-bearing atmosphere and demonstrated by them with tracer techniques to contain sulphur absorbed in grain boundary regions This case appears to be the most firmly established instance of equilibrium grain boundary segregation on record Tests on a com-

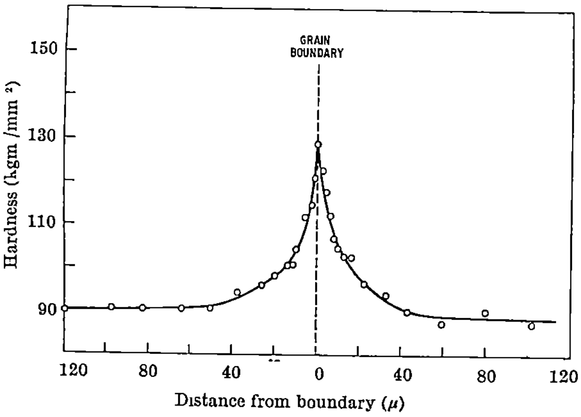

Fig 1

panion sample which had been subjected by Ainslie *et al* to identical heat treatment in sulphur-free atmosphere showed no boundary hardening whatsoever The intensity of the peak hardening in the sulphur-segregated iron (about 40 per cent), and the extent of the measurable effect (about 50μ either side of the boundary) are typical, but hardness differences of as much as 60 per cent and breadths as great as 100μ on each side have been observed in some cases with other systems Although effects of relative orientation of grains undoubtedly exist, they appear to be small since comparable hardening is observed in all boundaries of a randomly oriented polycrystalline sample

Table 1 lists some of the cases in which grain boundary hardening by equilibrium segregation has been observed in the present investigation Later publications will report these results in more detail

Table 1

Base material	Suspected segregant	Typical $\frac{\Delta H}{H}$ %	References suggesting segregation or reporting embrittlement by trace amounts of impurity
Iron	S	43	Ainslie *et al* (ref 1)
Iron	O	30	Low and Feustel (ref 2)
Aluminium bronze	Al	13	Perryman (ref 3)
Beryllides	O and/or N	50	Lewis (ref 4)
Silicides	O and/or N	33	Fitzer (ref 5)
Miscellaneous intermetallics, for example, AgMg, NiAl, Ni_3Al, CoCr-σ, Ni_3Ti, etc	frequently O and/or N	15–60	Westbrook and Wood (unpublished work)

Our experiments show certain features which appear to be common to the general phenomenology of the effect

Among these are

(1) Segregation is dependent on temperature In some instances, segregation, and therefore grain boundary hardening, disappears at high temperatures It is therefore possible, in favourable circumstances to eliminate segregation and improve toughness by quenching

(2) Segregation is frequently modified by the presence of another trace impurity, for example, segregation of oxygen does not occur in iron bearing small amounts of carbon but only in decarburized iron

(3) Grain boundary hardening by segregation in intermetallic compounds seems only to occur when there is a stoichiometric excess of a chemically active element

(4) Kinetics of segregation and desegregation are extremely rapid, suggesting lateral transport of segregant along some 'short circuiting' diffusion path

Previous suggestions for mechanisms of grain boundary embrittlement by segregation have involved internal stresses, changes in melting point, and changes in surface energy While all are conceptually plausible, no such mechanism has had unambiguous experimental support It is now clear that in a large number of cases embrittlement results from the creation of a barrier to dislocation propagation by formation of a hardened region at and near the boundary Whether this hardening occurs by a solution hardening mechanism or by changes in the dislocation density and structure at and near boundaries is not yet certain and the particular source of hardening may well vary from one case to another In the particular case of iron-sulphur, the extensive networks of dislocations adjacent to grain boundaries revealed in the transmission electron micrographs of Ainslie *et al* [6] are adequate to account for the observed boundary hardening

We are grateful to Messrs N G Ainslie, E A Aitken and J R Low for the loan of samples, to A J Peat for assistance, and to J R Low, A U. Seybolt and D Turnbull for helpful discussion

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Stress Concentration Effects in Tertiary Creep

In most cases, fracture under high-temperature creep conditions results from the formation and linking up of small voids on the grain boundaries within the metal A period of accelerating creep precedes the fracture and it is generally assumed that this acceleration is caused by the reduction in stress-carrying area through the formation of these voids[1,2]

It has recently been shown that the density of a material during creep decreases at an approximately linear rate up to the onset of tertiary creep but thereafter decreases at a continuously increasing rate until fracture ensues[3] If stress concentration is the cause of tertiary creep, the reduction in cross-section must have increased the stress to a value which gives a detectable change in the rate of deformation at this stage Clearly the dependence on stress of the rate of creep will then be an important factor in determining when this increase in creep-rate will be detectable and also in the rate of acceleration to fracture, that is in the fraction of the total time to rupture which is spent in tertiary creep

Some recent work[4] on nickel alloys containing only 0 012 wt per cent of tin is significant in this respect When tested at 400° C this material is extremely dependent on stress, a stress increase of 10 per cent giving an increase in minimum creep-rate and a decrease in fracture-life by a factor of 10^3 On the basis of a stress concentration mechanism for tertiary creep it might therefore be expected that a very rapid acceleration to fracture would be observed at an early stage of creep However, this is found not to be the case. The shape of the creep curves for this material is very similar to those for pure nickel at this temperature and similar to tests on the same material at higher temperatures where no large stress-dependent effects are observed

Considerable necking occurred before fracture at 400° C, but this must have occurred at a late stage of tertiary creep since only a small amount of necking would have been sufficient to give almost immediate fracture with a material having such a stress-dependent creep-rate

We conclude, therefore, that stress concentration, effected by void formation or necking, is not important in causing the acceleration in creep-rate before fracture

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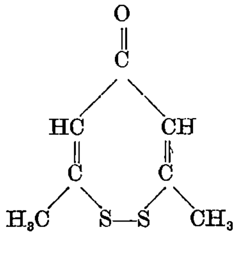
CHEMISTRY

Crystal Structure of 2,5-Dimethyl-Dithio-Furophthene, a Homologue of Thio-Thiophthene

DURING work on a series of sulphurated cyclic compounds[1], we have determined the structure of a substance to which formula I was previously ascribed



(I)

The substance crystallizes from toluene as yellow-orange prisms, often twinned, belonging to a monoclinic system, space-group $P2_1/c$, four molecules are in the unit cell of dimensions·

$$a = 4·44 \quad b = 10·78 \quad c = 17·20 \text{ Å}. \quad \beta = 97° 20'$$

Observed density 1·40 gm cm $^{-3}$· calculated 1·42

Single-crystal precession and Weissenberg photographs enabled us to carry out X-ray analysis on both (100) and (010) projections We determined the structure not with a heavy-atom method but using Sayre's direct method[2] The usual refinement process, based on a few Fourier and difference syntheses, gave the present values for the reliability index, $R = 0·21$ and $R = 0·13$ for $0kl$ and $h0l$ reflexions, respectively

Within experimental error, the molecule is planar, maximum perpendicular distance from mean plane being 0·04 Å. Fig 1 shows the molecular shape with bond lengths and angles, the corresponding shape of thio-thiophthene, as obtained after the last refinement, being also drawn for comparison (broken line)

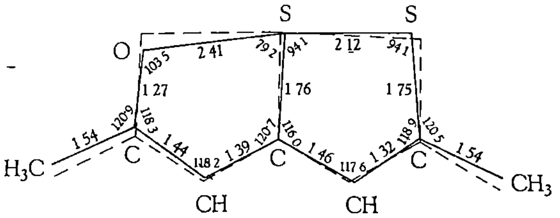


Fig 1

The oxygen–sulphur–sulphur system is seen to be almost linear, with the oxygen atom in the extreme position These results are in full agreement with the structure proposed in ref 3, and confirmed in ref 4 with the aid of nuclear magnetic resonance analysis Further chemical evidence was recently provided in ref 5

A comparison of this structure with the thio-thiophthene system is of great interest. The two molecules have about the same shape, structural configuration and crystal packing substitution of one sulphur atom with oxygen only causes the electronic arrangement to be slightly different In fact, we know that each sulphur–sulphur bond in thio-thiophthene may be regarded as made up from both fractional σ and π bonds, the bond orders being 0·707 and 0·439, respectively[6] The σ and π exchange integrals are, approximately, β and $\frac{1}{2}\beta$ (where β is the carbon–carbon π exchange integral), so that the bond is longer than the single one, even with a bond order sum greater than unity

Substitution of one sulphur atom by oxygen allows the same electronic interaction to take place only to a much less extent, owing to the different electronegativities of sulphur and oxygen, and to the much smaller overlap between the orbitals of the two atoms The sulphur–oxygen σ and π overlap integrals for a distance of 2·41 Å are, in fact, reduced to approximately one-third the values for the sulphur–sulphur interaction in the thio-thiophthene This reduction must also result in a corresponding increase of sulphur–sulphur overlap integral, in the oxygen–sulphur–sulphur system.

This discussion gives a satisfactory account of the following experimental data shortening of the sulphur–sulphur bond (2·12 Å) with respect to thio-thiophthene (the σ bond order is very close to unity), carbon–oxygen distance only slightly longer (1·27 Å) than the double-bond distance, rather distinct localization of double bonds in the carbon atoms chain, central carbon–sulphur bond length nearly the same as the other

From Fig 1 we can also notice in the present structure a reduction of valence angles not only in the right ring, but also in the left one This means that the abnormal sulphur oxygen length (2·41 Å), half-way between a single bond and Van der Waals distance, is chiefly to be considered a consequence of the reluctance toward distortion of the carbon atom valence angles From a geometrical point of view, it may be noted that the carbonylic group is anyway held in the molecular plane by its resonance with the dienic system We can say, therefore, that the essential features characterizing thio-thiophthene are present in this structure, but to a small extent, so that the actual electronic distribution is best represented by the formula shown in Fig 2, in which are also indicated, for each bond, the percentage of

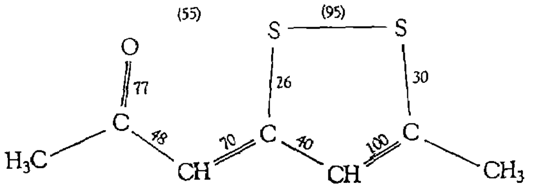


Fig. 2

double bond (or of single bond relatively to Van der Waals radii, for oxygen–sulphur and sulphur–sulphur distances)

For these reasons, the denominations suggested by Prof Richter, Beilstein Institute, Hamburg (cf ref 5), must be accepted as very rational and exact; for 2,5-dimethyl-thio-thiophthene it is:

meribicyclo-epidithio-(2,4)-eptadien-thion(6)

and for 2,5-dimethyl-dithio-furophthene it is:

epidithio-(2,4)-eptadien-on(6)

Further theoretical investigations, of the type performed by Giacometti and Rigatti[6] for thio-thiophthene, are under way, and will give a more exact description Some other compounds, homologous with thio-thiophthene, with sulphur atoms substituted by possible other group VI elements, are now under examination.

We gladly acknowledge the financial support by Shell International Research Maatschappij N.V , The Hague, Netherlands.

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Hydrogen Bonding in Alkyl Phosphonic and Alkyl Phosphonothionic Acids

In a recent series of papers[1], Peppard *et al* have examined alkyl and aryl phosphoric and phosphonic acids and their esters As a result of cryoscopic and infra-red measurements, they have concluded that, except in acetic acid solution, organophosphorus acids are dimeric, or polymeric, depending on the solvent, and that there is no evidence of the monomer in dilute solutions (*M*/1,000) in carbon tetrachloride They conclude that the hydrogen bonding in these acids is stronger than that in carboxylic acids These conclusions are in agreement with those reached by earlier workers[2-5], who all report the bonding to be unaffected by dilution in non-polar solvents In view of these findings, it has been suggested[3,6] that the hydrogen bonding may be intramolecular

Examination of more dilute solutions of ethyl methylphosphonic and ethyl methylphosphonothionic acids has shown that, if the dilution is great enough, these hydrogen bonds can be broken, and that the bonding is therefore inter- and not intra-molecular

Using carefully dried carbon tetrachloride as the solvent, path-lengths of up to 10 cm and double-beam compensation of solvent absorption, the results shown in Figs 1 and 2 were obtained With ethyl methylphosphonothionic acid (Fig 1), the initial bonded —OH absorption band at 3 27μ (3,058 cm^{-1}) decreases in intensity, relative to the C—H absorption band at 3 4μ (2,940 cm^{-1}), as the concentration of the solution decreases, and is replaced by a new absorption band at 2 78μ (3,600 cm^{-1}). In addition, a band at 4 35μ (2,300 cm^{-1}) progressively decreases in intensity and is replaced by a band at 4 65μ (2,150 cm^{-1})

In the case of ethyl methylphosphonic acid, the evidence is not so complete owing to experimental limitations No indication of breaking of hydrogen bonds is obtained in solutions more concentrated than *M*/3,000 Comparing the spectrum of a 1-cm path-length of this concentration with that of a 10-cm path-length of *M*/30,000 (Fig 2) shows that the absorption band at 3 8μ (2,630 cm^{-1}) has decreased in intensity relative to the C—H absorption at 3 4μ (2,940 cm^{-1}) and that a new band is appearing at 3 1μ (3,230 cm^{-1}) It was not possible to investigate this problem further as the spectrophotometer would neither accommodate a longer absorption cell nor produce reliable spectra from more dilute solutions over the same absorption path. It might be possible, however, to continue the observations using means such as electronic scale expansion The detection of a change in the intensity of the bonded OH absorption band, together with the appearance of a band at higher frequency, shows that the hydrogen

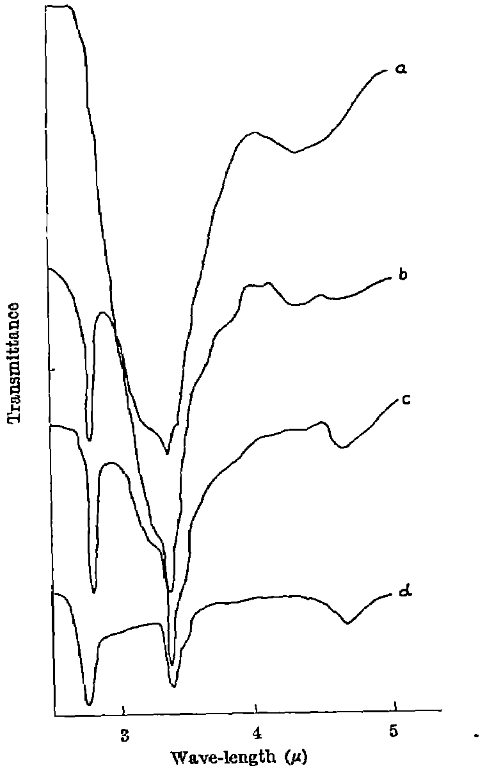

Fig 1 Spectra of ethyl methylphosphonothionic acid (*a*) Capillary film, undiluted (*b*) *M*/350 in carbon tetrachloride, 1 cm , (*c*) *M*/1,750 in carbon tetrachloride, 5 cm , (*d*) *M*/3,500 in carbon tetrachloride, 5 cm

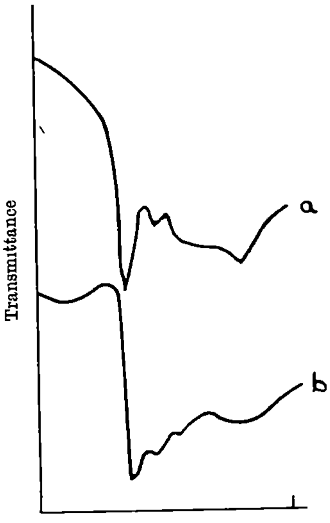

Fig. 2. Spectra of ethyl methylphosphonic acid in dilute solution. (a) *M*/3,000 solution in carbon tetrachloride, 1 cm.; (b) *M*/30,000 solution in carbon tetrachloride, 10 cm.

bonds are beginning to break in very dilute solution. It is therefore concluded that, as in the case of the phosphonothionic acid, the hydrogen bonds must be inter- rather than intra-molecular.

These experiments also throw some light on the assignment of the absorption bands associated with the $\mathrm{P(\!=\!S)OH}$ and $\mathrm{P(\!=\!O)OH}$ groupings. The $\mathrm{P{-}OH}$ group in $\mathrm{{>}P(\!=\!S)OH}$ compounds is characterized by absorption bands in the regions (*a*) 3,000–3,100 cm.$^{-1}$ and (*b*) 2,200–2,360 cm.$^{-1}$, whereas the same group in $\mathrm{{>}P(\!=\!O)OH}$ compounds has four characteristic bands in the regions (*a*) 2,525–2,725 cm.$^{-1}$, (*b*) 2,150–2,350 cm.$^{-1}$, (*c*) 1,600–1,700 cm.$^{-1}$, (*d*) 480–530 cm.$^{-1}$.

As bands (*a*) and (*b*) occur in both types, these must be vibrations associated solely with the P—O—H vibrations, band (*a*) being the O—H valency vibration. In view of the movement of band (*b*) to lower frequencies when the hydrogen bonds are broken, this band is assigned to a combination band involving the OH deformation vibration. Observations on deuteration, which will be reported in more detail elsewhere, have indicated that the O—H deformation vibration in P—O—H groups is in the vicinity of 1,250 cm.$^{-1}$. This is contrary to the assignments for this band given by Bellamy[2] and Corbridge[7], but in agreement with those of Ryskin and Stavitskaya[8]. The P—O—(H) vibration is found in the range 910–1,030 cm.$^{-1}$ (ref. 6) and the combination of the P—O—(H) vibration with the O—H deformation vibration would then result in a frequency in the range ascribed to band (*b*), which is therefore assigned to a combination of these vibrations.

Band (*c*) in $\mathrm{{>}P(\!=\!O)OH}$ compounds has been assigned[3,6] to the O—H deformation vibration. In the light of evidence from deuteration this assignment is no longer valid. As this band does not appear in $\mathrm{{>}P(\!=\!S)OH}$ compounds, it may be a combination band involving the P=O vibration. As this occurs in the region of 1,200 cm.$^{-1}$, this implies a frequency in the region 400–500 cm.$^{-1}$ for the other component. Band (*c*) is therefore tentatively assigned to a combination of the P=O valency vibration and of band (*d*), which is otherwise unassigned.

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Synthesis of the Toxic Principle of *Dichapetalum toxicarium* (18-Fluoro-*cis*-9-Octadecenoic Acid)

Dichapetalum toxicarium (*Chailletia toxicaria*, Don) a shrub occurring in Sierra Leone, produces a hard and woody fruit which is extremely toxic to warm-blooded animals. The pharmacological, medical, and historical aspects of this plant have recently been reviewed[1]. Peters and colleagues have concluded[2], on the basis of nuclear magnetic resonance, infra-red spectroscopy and ozonolysis, that the toxic principle is 18-fluoro-*cis*-9-octadecenoic acid (ω-fluoro-oleic acid), $F(CH_2)_8CH{=}CH(CH_2)_7COOH$. It was in order to confirm this conclusion that the following synthesis of ω-fluoro-oleic acid was undertaken.

$$F(CH_2)_8Br + NaC{\equiv}CH \rightarrow F(CH_2)_8C{\equiv}CH + I(CH_2)_7Cl \rightarrow$$
$$F(CH_2)_8C{\equiv}C(CH_2)_7Cl + NaCN \rightarrow F(CH_2)_8C{\equiv}C(CH_2)_7CN \rightarrow$$
$$F(CH_2)_8\,C{\equiv}C(CH_2)_7CONH_2 \rightarrow F(CH_2)_8C{\equiv}C(CH_2)_7COOH \rightarrow$$
$$F(CH_2)_8CH{=}CH(CH_2)_7COOH\ (cis)$$

Using *N*,*N*-dimethylformamide and xylene as solvents, 8-fluoro-octyl bromide[3] was converted to 10-fluoro-1-decyne in 60 per cent yield, b.p. 81–82° (10 mm.), n_D^{25} 1.4250, d_4^{20} 0.877 (found: C, 76.48; H, 11.02. $C_{10}H_{17}F$ requires C, 76.87; H, 10.97 per cent). Previous work[4] had indicated that the ω-fluoro-1-alkynes, in the presence of reactants such as sodamide and Grignard reagents, tended to lose fluorine rather than form the expected acetylenic organometallic derivative; however, by using metallic lithium in liquid ammonia, 10-fluoro-1-decyne formed 1-chloro-17-fluoro-8-heptadecyne in 89 per cent yield, b.p. 137° (0.25 mm.), n_D^{25} 1.4610, d_4^{20} 0.969 (found: C, 70.56; H, 10.38; Cl, 12.42. $C_{17}H_{30}ClF$ requires C, 70.68; H, 10.47; Cl, 12.28 per cent). Reaction of this last compound with sodium cyanide in dimethyl sulphoxide gave the corresponding nitrile in 93.5 per cent yield, b.p. 139° (0.025 mm.), n_D^{25} 1.4578 (found: C, 77.29; H, 10.77; N, 4.79

$C_{18}H_{30}FN$ requires C, 77 36, H, 10 82, N, 5 01 per cent)

Preliminary work using 15-cyano-1-fluoro-7-pentadecyne had indicated the difficulty of hydrolysing this type of nitrile, because the triple bond is susceptible to acidic reagents and the fluorine to vigorous alkaline treatment The required ω-fluorostearolic acid was ultimately obtained in 73 per cent yield by treatment of 1-cyano-17-fluoro-8-heptadecyne with hydrogen peroxide and alkali followed by hydrolysis of the resultant amide with alcoholic potassium hydroxide, m p 52–53° from aqueous methanol (found C, 72 50, H, 10 28 $C_{18}H_{31}FO_2$ requires C, 72 31, H, 10 47 per cent) Stereospecific partial hydrogenation of this fluoroacetylenic acid using Lindlar catalyst gave a 99 per cent yield of ω-fluoro-oleic acid, recrystallized from 30–60° petroleum ether at 2° (found C, 71 92, H, 11 21, F, 6 16 $C_{18}H_{33}FO_2$ requires C, 71 95, H, 11 07, F, 6 32 per cent) The overall yield from 8-fluoro-octyl bromide was 36 per cent The physical properties of this acid are shown in Table 1 and are compared with those of a sample of the naturally occurring acid provided by Peters Hydrogenation of the synthetic ω-fluoro-oleic acid using platinum and hydrogen formed ω-fluorostearic acid, m p 77–78° from methanol, mixed with authentic ω-fluorostearic acid[5], m p 76–77°

Table 1

PROPERTIES OF NATURAL AND SYNTHETIC ω-FLUORO-OLEIC ACID

		Natural	Synthetic	Interpretation
Melting point		12 4–13 5°	15 8–16 8°	
n_D^{25}		1 4616	1 4598	
Infra-red (cm $^{-1}$)*		3,007	3,005	CH stretching of CHR=CHR′ (*cis*)
		1,710	1,711	C=O stretching of COOH
		1,413	1,412	CH in-plane deformation of CHR=CHR′ (*cis*)
		1,048	1,046	C—F stretching
		1,012	1,015	
		726	726	CH out-of-plane deformation of CHR=CHR′ (*cis*)
Nuclear magnetic resonance (c /s)†	ν‡	258	259	FCH_2-
	J_{HF}	47	47	
	J_{HH}§	6	6	
	ν‡	317	316	—CH=CH—
	J_{HH}§	5	5	

* Determined using a Beckman *IR*-7 infra-red spectrophotometer, the absence of a band at 980–960 cm $^{-1}$ indicates the *cis* isomer, elaidic acid showed a strong band at 970 cm $^{-1}$ resulting from CH out-of-plane deformation of CHR=CHR′ (*trans*)

† Determined using a Varian 60 Mc /sec *V*-4302*B* spectrometer

‡ c /s from tetramethylsilane

§ Spin–spin interaction with adjacent methylene group

The proton resonance spectral data are listed in Table 1 The ω-CH_2 group appeared as a doublet of triplets centred at 258 c /s for the natural material and at 259 c /s for the synthetic acid, the splitting is due to spin–spin interaction with the terminal fluorine and the adjacent methylene group The coupling constants were the same in each case The olefinic protons absorbed at 317 c /s and 316 c /s for the two samples, again with identical coupling constants, the olefinic protons in a sample of oleic acid also gave exactly the same results (ν = 316 c /s , J = 5 c /s) The results determined for the natural product are in good agreement with those previously reported[2]

There can thus be little doubt that the toxic principle of *Dichapetalum toxicarium* is indeed ω-fluoro-oleic acid This acid is only the second known naturally occurring organic fluorine compound, the first being fluoroacetic acid It is not improbable that there is some biogenetic relationship between the two compounds Further work on this and other ω-fluoro unsaturated acids is in progress

We thank Sir Rudolph Peters for kindly providing us with a sample of the naturally occurring acid, Allied Chemical Corporation, General Chemical Division, Morristown, N J , for financial assistance, and Dr J B Stothers of this Department for help with the nuclear magnetic resonance spectra

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Effect of Strong Electrical Fields on the Boiling Points of some Alcohols

Katti and Chaudhri[1] have published a set of measurements on the effect of electrical fields on the boiling points of methanol, ethanol, and isopropanol This is an effect which can be calculated from the first principles of thermodynamics

A boiling liquid in an electrical field does both electrical and pressure-volume work, the conservation of energy equation is $dU = TdS - PdV + EdP'$, where P is the pressure, P' the polarization and E the electric field Hence the chemical potential of the liquid may be written as

$$d\mu_1 = -s_1 dT + v_1 dP - p_{1'} dE$$

where small italics stand for gram molecular quantities The condition of phase equilibrium is $d\mu_1 = d\mu_g$, hence $(dT/dE)_P = -\Delta p_{1'\to g}/\Delta s_{1\to g}$. The equation is exactly analogous to the Claperon equation

For such liquids as methanol, $p' = (\varepsilon - 1)vE$, so that

$$\frac{dT}{T} = \frac{(\varepsilon_1 - 1)v_1 - (\varepsilon_g - 1)v_g}{\Delta h} E dE$$

If the electrical work stored in the liquid is small compared with the heat of vaporization at zero field, and the change in boiling points is so small that one can ignore changes in the dielectric constants of liquid and vapour, then this equation may be integrated Approximating $\ln T/T_0$ as $\Delta T/T_0$, we find that

$$\Delta T = T_0 \frac{(\varepsilon_1 - 1)v_1 - (\varepsilon_g - 1)v_g}{2\Delta h} E^2$$

This equation is valid for a system in which all the material, liquid and vapour, remains within the field If experiments are done in a constant-volume container (as were Katti and Chaudhri's), a term must be added to the conservation of energy equation to account for the work done in removing vapour from the field This has the effect of changing v_g to v_1 Since $\varepsilon_g - 1$ is very small compared to $\varepsilon_1 - 1$, this correction is not important, even though the molar volume of the gas is much greater than that of the liquid

It should be noted that Katti and Chaudhri used an a c field They did not give the frequency, but it was presumably low enough for the dielectric constants to be approximately equal to their d c values and high enough for their Beckmann thermometer to respond to the average temperature In this case one can calculate the expected change in temperature, taking E as its root mean square value.

Since $\Delta T = C E^2$, where C is a combination of the molecular constants, and using standard values[2] one finds that C in M K S units equals 5 3, 5 5 and 5 7 × 10^{-17} for methanol, ethanol, and isopropanol respectively. Hence, for example, methanol at 5 kV /cm will show a rise of boiling point of 1 3 × 10^{-5} deg C At 5 kV /cm Katti and Chaudhri observed boiling point depressions of 2 0, 0·7, and 0 4 deg. C for methanol, ethanol, and isopropanol, respectively, in complete disagreement with theory

I thank Dr E R Nightingale for helpful discussions, and Esso Research and Engineering for permission to publish this communication

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Since the publication of our communication in *Nature*, several attempts have been made to explain this phenomenon and to calculate the magnitude of the lowering of the boiling points These attempts naturally deal with the application of thermodynamics to the mechanical effects arising out of the application of strong electrical fields to dielectrics These models, however, predict very small lowering of the boiling points (of the order of 10^{-5} deg. C. for fields of the order of 5 kV /cm)

In fact we ourselves were led to this investigation nearly two years ago because of a suggestion by Prof D S Kothari based on the Clapeyron equation with change of pressure replaced by $KE^2/8\pi$ We did not refer to this in our communication because a reference to it was made in an earlier book on physical chemistry and secondly because the sign and the magnitude of the observed changes in the boiling points are not in agreement with this model

The main purpose of our communication was to point out the unusually large depression of the boiling points of the liquids under the experimental conditions used More experimental work is clearly necessary, and we hope to report on further results in due course

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The Optical F Absorption Band

In a recent communication[1] a new model for the optical F absorption band was proposed for the alkali metal azides From a study of irradiation-produced bands in the visible and infra-red region of the absorption spectrum of the azides, it was shown that the anion (N_3^-) was intimately related to the F absorption band and it was suggested that the F band was caused by a 'charge-transfer' complex of the type discussed extensively in the chemical literature, especially by Mulliken[2] The 'charge-transfer' complex consists of a dative bond being established between one electron donor and an electron acceptor with a concomitant electronic absorption belonging to the complex as a whole

Table 1. ENERGY DIFFERENCE BETWEEN FIRST ULTRA-VIOLET PEAK AND THE F BAND*

	$h\nu_1 - h\nu_F = C$		$h\nu_1 - h\nu_F = C$
LiF	12 4 −5 0 = 7 4	LiBr	7 2 −2 7 = 4 5
NaF	10 6 −3 6 = 7 0	NaBr	6 78−2 3 = 4 48
KF	9 95−2 7 = 7 25	KBr	6 77−2 0 = 4 77
LiCl	8 8 −3 1 = 5 7	RbBr	6 6 −1 8 = 4 8
NaCl	7 95−2 7 = 5 25	KI	5 8 −1 8 = 4 0
KCl	7 76−2 2 = 5 56	RbI	5 7 −1 6 = 4 1
RbCl	7 5 −2 0 = 5 5		

* $h\nu_1$, energy of first ultra-violet band from Eby, J E , Teegarden, K J , and Dutton, D B , *Phys Rev* ,116, 1099 (1959), $h\nu_F$ energy of the F band from Dekker, A J , *Solid State Physics* (Prentice Hall, 1959), $h\nu_1$, for LiF estimated from H C Kremers, *Indust and Eng Chem* , 32, 1478 (1940)

For the azides, the following mechanism was proposed for the formation of the 'charge-transfer' complex and the resulting absorption band Under ultra-violet irradiation an interstitial metal atom is ionized, this metal ion now moves to a position of lower potential energy, that is, to a cation vacancy and as a consequence the equilibrium crystal field is so perturbed (in an unspecified manner) as to set up a 'charge-transfer' complex between that cation and a neighbouring anion. Thus in the proposed model the F absorption band depends on the existence of cation vacancies rather than anion vacancies

I feel that the phenomena in the halides cannot be essentially different from those in the azides If one accepts the importance of cation vacancies then several phenomena in the halides can be understood in a straightforward manner. For example, the following experimental facts strongly suggest the importance of the cation vacancy The electrical conductivity of crystals is decreased by darkening[3] Plastic strain which enhances conductivity enhances darkening by X-ray irradiation[3] Divalent cation impurities which increase ionic conductivity enhance colorability by irradiation[3].

The role of the anion in the F band in the halides is not easily demonstrated as in the azides, since there is no infra-red vibration associated with it except for the lattice vibration However, I suggest that the correlation between the F band and the first ultra-violet absorption band in the halides, first pointed out by Glasner and Tompkins[4], be interpreted in a manner similar to that in the azides Table 1 lists the difference in energy between the first ultra-violet absorption band in uncoloured crystals and the F band produced in the crystals It can be seen that the energy difference is a constant for any particular anion It is generally accepted[5] that the first ultra-violet absorption peak in the halides is caused by the removal of an electron from a halogen ion to a neighbouring metal ion Our suggested model for the F band is the *partial* removal of an electron to a neighbouring metal ion so that it is not surprising that there exists a relationship between the ultra-violet band and the F band Further, a colourless electronegative ion with an absorption band in the ultra-violet, under a perturbing influence (the formation of a bond with increasingly greater covalent character), would expect to have an absorption band increase in wave-length proportional to the degree of perturbation[6] One might expect that any such perturbation would have the greatest effect on the most ionic system If we use Pauling's function[6], $1-\exp(-\frac{1}{4}(X_a-X_b)^2)$ (where X_a and X_b are the electro-negativities of the atoms in the bond), as a measure of the ionic character for the halides, then the fluorides appear to be most ionic and the iodides least so and the difference in energy between the ultra-violet band and the *F* band varies in the expected manner My proposed model for the *F* absorption band is a point defect where just such a phenomenon is occurring

I cannot justify the imposition of a special site for a cation vacancy which afterwards captures a metal ion except to say that perhaps the ion does not completely enter the vacancy or that there is incomplete relaxation about the vacancy caused by other defects in the neighbourhood

It has been shown[7] that normal sodium chloride contains about 10^{17} interstitial sodium atoms/c c and thus the model yields a plausible mechanism for the formation of *F* centres by low-energy irradiation The 'charge-transfer' complex also allows one to understand more easily the dichroism observed in *F* centres[8]

Perhaps the most serious drawback to the model is that no explanation is given for the observed electron spin resonance in coloured crystals I suggest that the electron which is removed from the interstitial metal atom is trapped at another centre giving rise to the electron spin resonance The optical *F* absorption band and electron spin resonance appear simultaneously but come from two different points in the crystal.

This work was initiated at the Convair Scientific Research Laboratory, San Diego, California

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BIOCHEMISTRY

Particle Involvement in Fatty Acid Synthesis in Liver and Yeast Systems

THE synthesis of fatty acids from acetate has been shown to occur in particle-free supernatant fractions obtained from a variety of mammalian tissues[1-3] An examination of the literature[4-7] reveals that such synthesis is significantly increased in the presence of a microsomal fraction (While the term microsome may be used to describe the particle fraction prepared from liver, it is premature to refer to yeast particles by this name since the cytological fractionation of the yeast cell is less well established As used here, the term particles refers to the fraction sedimented, after prior removal of the mitochondrial fraction, at 100,000*g* for 45 min 'Supernatant' refers to the solution obtained after removal of the 100,000*g* pellet) We have shown, for example, that the addition of rat liver microsomes, in appropriate amounts, results in an increase in the incorporation of acetate carbon into fatty acids many times greater than that observed with the rat liver supernatant fraction alone[8] Similar observations have been made with a yeast system[9]

Table 1 EFFECTS OF ADDITION OF PARTICLES ON FATTY ACID SYNTHESIS FROM ACETATE IN LIVER AND YEAST SYSTEMS

Supernatant alone	After addition of liver microsomes (ml)				After addition of yeast particles (ml)			
	0 025	0 05	0 10	0 30	0 025	0 05	0 10	0 30
Liver, 13 6	25 1	46 2	32 6	7 6	41 1	33 4	23 0	14 2
Yeast, 10 3	13 5	13 2	7 3	3 8	27 3	34 8	29 4	24 9

40 μmoles of potassium phosphate buffer (*p*H 7 5), 15 μmoles of reduced glutathione (K^+ salt) 18 μmoles of $MgCl_2$, 0 25 μmole of $MnCl_2$, 2 5 μmoles of $KHCO_3$, 1 25 μmoles of potassium acetate-1-^{14}C (3 × 10^5 c p m), 12 μmoles of ATP (K^+ salt), 0 03 μmole of CoA, 0 125 μmole of TPN, and 18 8 μmoles of potassium citrate, all contained in 0 5 ml , were incubated with 0 4 ml of a particle-free supernatant fraction (11 9 mgm protein for liver, 8 8 mgm protein for yeast) plus the amount of particle suspension (in 0 1 *M* phosphate buffer, *p*H 7 5) recorded above (55 mgm per ml protein for liver, 67 mgm. per ml. protein for yeast) for 2 hr at 37° C with air as the gas phase The preparation of the liver and yeast fractions in 0 1 *M* phosphate buffer (*p*H 7 5) has been described (refs 9, 14) The fatty acids were isolated and assayed for carbon-14 activity as described in ref 15 All values above represent mμmoles of acetate converted to fatty acids per mgm supernatant protein

The failure of other workers to observe particle stimulation in mammalian preparations[1,10]—as well as the report that removal of these particles actually increased synthesis[7]—may be explained by our present findings (Table 1), which show that microsomes increase fatty acid synthesis over only a narrow range of concentrations Indeed, additions of excess amounts of particles reduced synthesis below that observed with the supernatant fraction alone Since adenosine triphosphate (ATP) is an absolute requirement for fatty acid synthesis from acetate, our findings of high ATPase activity in liver microsomes offers a reasonable explanation for this inhibitory action of the microsomes The inhibition observed with moderately large amounts of microsomes can be relieved by increasing the ATP concentration in the incubation medium

Table 1 also shows that yeast particles can be substituted for liver microsomes in the experiments with liver supernatant fractions. Here again, fatty acid synthesis from acetate reached its optimum in the presence of specific amounts of yeast particles, but dropped when those amounts were increased The addition of liver microsomes to the yeast supernatant fraction failed to augment fatty acid synthesis, but their inhibitory property at high levels was retained Fatty acid synthesis in the yeast system was less sensitive to large amounts of yeast particles

The addition of particles also increased the incorporation of the carbon-14 of acetyl-1-^{14}C-coenzyme A (CoA) into fatty acids, but did not affect fatty acid synthesis from malonyl-CoA (Table 2) These findings suggest that particles exerted their effect before the formation of malonyl-CoA in both systems Evidence that this may be the case in the yeast system comes from the observation that yeast particles are rela-

Table 2 EFFECT OF PARTICLES ON INCORPORATION OF THE CARBON-14 OF ACETATE-1-^{14}C, ACETYL-1-^{14}C-COA AND MALONYL-2-^{14}C-COA INTO FATTY ACIDS BY RAT LIVER AND YEAST PREPARATIONS

Labelled substrate	Percentage of carbon-14 incorporated into fatty acids			
	Rat liver		Yeast	
	Supernatant alone	Supernatant + microsomes	Supernatant alone	Supernatant + particles
Acetate-1-^{14}C	5 9	32 3	3 7	23 2
Acetyl-1-^{14}C-CoA	6 1	24 6	4 1	17 1
Malonyl-2-^{14}C-CoA	44 0	51 6	38 5	46 2

60 μmoles of glycylglycine-KOH buffer (*p*H 7 5), 15 μmoles reduced glutathione (K^+ salt), 18 μmoles of $MgCl_2$ 0 25 μmole of $MnCl_2$, 2 5 μmoles of $KHCO_3$, 12 μmoles ATP (K^+ salt), 0 125 μmole of TPN, 18 8 μmoles of potassium citrate and either (*a*) 0 12 μmole of potassium acetate-1-^{14}C (4 5 × 10^5 c p m) plus 0 12 μmole of CoA or (*b*) 0 12 μmole of acetyl-1-^{14}C-CoA (9 4 × 10^4 c p m) or (*c*) 0 12 μmole of malonyl-2-^{14}C-CoA (2 3 × 10^5 c p m) plus 0 02 μmole of acetyl-CoA, all contained in 0 5 ml , were incubated with 0 4 ml of particle-free supernatant fraction (7 8 mgm protein for liver and yeast) and 0 1 ml of particle suspension (2 1 mgm protein for liver and 5 4 mgm protein for yeast), as described in Table 1

Table 3 Incorporation of the Carbon-14 of Acetate-1-^{14}C, Acetyl-1-^{14}C-CoA and Malonyl-2-^{14}C-CoA into Fatty Acids by Rat Liver and Yeast Particles incubated Alone

Labelled substrate	Percentage of carbon-14 incorporated into fatty acids	
	Liver microsomes	Yeast particles
Acetate-1-^{14}C	0 2	20 3
Acetyl-1-^{14}C-CoA	0 3	14 7
Malonyl-2-^{14}C-CoA	29 0	15 8

60 μmoles of glycylglycine-KOH buffer (pH 7 5), 15 μmoles of reduced glutathione (K^+ salt), 18 μmoles of $MgCl_2$, 0 25 μmole of $MnCl_2$, 2 5 μmoles of $KHCO_3$, 12 μmoles of ATP (K^+ salt), 1 μmole of TPNH, and either (*a*) 0 12 μmole of potassium acetate-1-^{14}C (4 5 × 10^5 c p m) plus 0 12 μmole of CoA or (*b*) 0 12 μmole of acetyl-1-^{14}C-CoA (9 4 × 10^4 c p m) or (*c*) 0 12 μmole of malonyl-2-^{14}C-CoA (2 3 × 10^5 c p m) plus 0 02 μmole of acetyl-CoA, all contained in 0 5 ml , were incubated with 0 1 ml of particle suspension (2 1 mgm protein for liver and 5 4 mgm protein for yeast), as described in Table 1

tively rich in acetyl-CoA carboxylase activity[11] However, the results reported here indicate that this explanation is not tenable for the liver system

Particles from liver and yeast were incubated with acetate, acetyl-CoA, or malonyl-CoA, under the conditions shown in Table 3 Liver particles, by themselves, incorporated into fatty acids appreciable amounts of the carbon-14 of malonyl-2-^{14}C-CoA, but not of acetate-1-^{14}C or acetyl-1-^{14}C-CoA Under identical conditions, yeast particles converted significant amounts of all three substrates to fatty acids That yeast particles have an enzyme system capable of the complete conversion of acetate to fatty acids receives support from our observation that they contain acetokinase activity equivalent, on a protein basis, to that found in the supernatant fraction (Klein, H P , and Den, H , unpublished observations) Liver particles, on the other hand, presumably lack acetyl-CoA carboxylase activity, and may be devoid of acetokinase activity With malonyl-CoA as substrate, if no added co-factor is rate limiting, an additive effect would be expected when the two fractions of each system are combined But this was not observed (Table 2) The relation of this fatty acid-synthesizing system in the particles to fatty acid synthesis in the system composed of the supernatant fraction plus particles remains to be elucidated

Although the increase in fatty acid synthesis from acetate and from acetyl-CoA induced by liver microsomes is not due to their ability to convert acetyl-CoA and bicarbonate to malonyl-CoA, the possibility exists that malonyl-CoA is supplied through an interaction between the particles and the supernatant fraction It is conceivable that some compound in the system may donate a carboxyl group to acetyl-CoA without passing through a bicarbonate pool (cf Swick and Wood[12]) We have reported[8] that, while glucose-6-phosphate or malate will produce adequate amounts of reduced triphosphopyridine nucleotide (TPNH) for fatty acid synthesis from acetate in mammalian systems, these compounds do not stimulate fatty acid synthesis as effectively as do citrate, isocitrate, and aconitate These tricarboxylic acids, which yield amounts of TPNH comparable with those formed from malate or glucose-6-phosphate, are distinctly superior in their capacity to augment fatty acid synthesis from acetate or acetyl-CoA (The addition of liver microsomes to the supernatant fraction oxidizing citrate-6-^{14}C will increase the rate and level of $^{14}CO_2$ production) It is therefore reasonable to infer that the tertiary carboxyl group of citrate may be involved in a transcarboxylation reaction with acetyl-CoA leading to the formation of malonyl-CoA The supernatant fraction, containing isocitric dehydrogenase and aconitase, would initiate this reaction, which would then be completed by particles through a transcarboxylase reaction Numa *et al* [13] were unable to demonstrate transcarboxylase activity in rat liver preparations, but their examination of transcarboxylase activity was confined to the particle-free supernatant fraction only

This work was aided by a grant to one of us (I L C) from the United States Public Health Service and the Life Insurance Medical Research Fund, and to another (H P K) from the National Science Foundation

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Comparative Effects of 2,4-Dinitrophenol on the Actomyosin Adenosine Triphosphatase from Fresh, Frozen and Freeze-dried Beef

In our work on biochemical alterations in beef consequent upon freeze-drying the evidence obtained so far indicates that the changes are probably subtle Previously[1] we reported that there is little change between the salt solubility of frozen and freeze-dried beef and that only small differences occurred in the amount of sedimentable actomyosin No consistent difference appeared in the adenosine triphosphatase (ATPase) activity of actomyosin extracts from these materials, and the electrophoretic patterns shown by sarcoplasmic protein extracts were almost identical for frozen and freeze-dried beef, under the conditions used With contractile protein extracts there was noticeable alteration in the freeze-dried pattern indicating denaturation

In present work reported here, designed to detect ageing changes in beef, I have compared the stimulatory effects of 2,4-dinitrophenol (DNP) on actomyosin ATPase preparations from fresh, frozen and freeze-dried beef While there were no demonstrable changes ascribable to ageing, I observed a consistently greater stimulation by DNP of the actomyosin ATPase from fresh beef than from frozen and freeze-dried beef

Actomyosin extracts were prepared in two ways from semitendinosus and longissimus dorsi muscles. Precipitated actomyosin was prepared by the conventional technique of extraction with 0.53 ionic strength buffer pH 8.2 and dilution to ionic strength 0.08. Actomyosin obtained indirectly was prepared as previously described[1]. The only procedural change was the substitution of a Virtis blender (1½ min.) for the Waring blender.

Beef carcasses (hindquarters or short hips right and left sides) were received on the dates shown in Table 1. Muscle designated as 'not aged' was excised within 18 hr. of receipt. From this, samples labelled 'fresh' were taken for immediate analysis. The remainder was frozen in air at −29° C for periods of 24–48 hr. The frozen muscle was cut into slices 3/4 in. thick. One portion was kept frozen and the other freeze-dried by radiant heating at a chamber pressure between 0.4 and 0.5 mm. mercury[2]. Analyses of the frozen samples were begun during this 48-hr. period. Freeze-dried samples were vacuum-packed immediately on removal from the drying chamber, and stored at −29° C. Analyses of the latter were always made within 2 weeks of freeze-drying.

Samples designated as 'aged' were taken from the opposite hind limb, which was hung (aged) for 3 weeks at +4° C. At the end of the ageing period the muscles were excised and treated in the same manner as the unaged samples.

The ATPase activities of the extracts were determined at 30° C in a manner similar to that described previously[1], the final concentration of reactants being calcium chloride 0.005 *M*, DNP 0.004 *M*, ATP 0.0025 *M*, potassium chloride 0.10 *M*, *tris*-(hydroxymethylaminomethane) 0.05 *M*, pH 8.1. The concentration of enzyme giving a linear reaction-rate for the 10-min. period was found to be between 0.200 and 0.300 mgm. nitrogen.

The results are shown in Table 1.

Table 1. COMPARISON OF DNP STIMULATION OF ACTOMYOSIN ATPASE FROM FRESH, FROZEN, FREEZE-DRIED BEEF

	Precipitated actomyosin			Indirect	
	Without DNP	Percentage stimulation with DNP		Without DNP	Percentage stimulation with DNP
S.T. aged 29/3/60			S.T. aged 7/1/60		
Fresh	35.5	104.2	Fresh	60.2	62.0
Frozen	57.2	37.2	Frozen	66.2	45.7
F.D.	40.7	9.8	F.D.	44.6	41.2
S.T. not aged 29/3/60			S.T. not aged 7/1/60		
Fresh	39.9	71.0	Fresh	54.0	53.2
Frozen	58.4	7.0	Frozen	47.6	25.6
F.D.	43.1	55.0	F.D.	43.1	35.0
S.T. aged 7/1/60			S.T. aged 29/3/60		
Fresh	48.7	53.6	Fresh	54.1	61.2
Frozen	64.1	14.2	Frozen	45.1	7.5
F.D.	61.9	3.3	F.D.	55.6	1.6
S.T. aged 16/11/59			S.T. not aged 29/3/60		
Fresh	36.5	41.0	Fresh	54.0	53.2
Frozen	32.0	32.2	Frozen	47.7	30.2
F.D.	52.1	15.5	F.D.	43.1	35.0
L/D aged 29/3/60			L/D aged 29/3/60		
Fresh	44.2	57.5	Fresh	46.1	74.0
Frozen	53.1	5.8	Frozen	52.3	20.2
			L/D not aged 29/3/60		
			Fresh	41.2	126.5
			Frozen	55.5	11.5

Activities expressed in μgm. P/min./mgm. N.
S.T., Semitendinosus muscle; L/D, longissimus dorsi muscle; F.D., freeze-dried.

It is well known that DNP stimulates the calcium-activated ATPase of myosin and actomyosin[3-5]. However, no previous report appears to be available concerning the effect of DNP on actomyosin from post-rigor muscle. The mechanism by which stimulation is achieved is not agreed upon. At temperatures above 16° C, DNP also stimulates the cytidine triphosphatase (CTPase) activity of myosin but inhibits the inosine and guanosine triphosphatase (ITPase, GTPase) activity[6,7]. It has been postulated that retardation of hydrolysis of the former two enzymes results from the interaction of —SH or other groups in the enzyme and the 6-NH_2 group common to both adenine and cytosine. This retardation is relieved or removed by the action of DNP on the rate-retarding group of the protein[6-8]. There is no such retardation with the 6-OH base present, for example inosine, and DNP does not stimulate hydrolysis.

Gilmour and Griffiths[5] have suggested that since myosin aggregates at 25° C, there is consequent masking of the active centres. At 0° C there is no masking and no stimulation by DNP. At the higher temperature, DNP is thought to achieve its effect by splitting the polymer and restoring the enzyme to the state in which it exists at 0° C.

The finding of Perry and Chappell[4] that stimulation of myosin ATPase is depressed by an increasing actin/myosin ratio in synthetically prepared actomyosins also bears on the interpretation of these results. The mechanism by which actin can reverse the DNP effect is not understood, but it is presumed that actin can alter the nature or sequence of events at the active centres of myosin.

In the light of the foregoing, the results presented in Table 1 may be interpreted in either of two ways. The depressed stimulation shown by the frozen and freeze-dried samples could be due to unmasking of the active centres during freezing and drying. This would imply the existence of some depolymerizing process as a result of freezing or drying. It is highly unlikely that depolymerization occurs[1]. Moreover, the DNP–ATPase activities of the pellet from actomyosin fractionated at high speed (Cole, unpublished results) show no consistent differences from those of the supernatant liquid. If particle-size affected the DNP–ATPase this would be reflected in greater stimulation of pellet enzyme material.

A second and more logical interpretation of the results reported above can be based on a changed or changing active myosin/actin ratio. A preferential denaturation of myosin in the actomyosin complex would decrease this ratio and thus decrease the stimulatory effect of DNP.

I wish to thank Dr. W. R. Smithies for having the samples of freeze-dried meat prepared and to acknowledge the technical assistance of Mrs. Helen Kent.

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Formation of Auxin-bound Proteins

THE occurrence of auxin–protein complexes has been recognized for a long time, but very little is known about the mechanism of the binding process According to Siegel and Galston[1] energy for the coupling of auxin to protein is supplied by adenosine triphosphate, and the process can be inhibited by several metabolic inhibitors Galston *et al* [2,3] have observed, using indole-3-acetic acid (IAA) labelled with carbon-14, that auxin is not bound appreciably to any of the cell organelles but that the particle-free supernatant contains macro-molecules to which auxin is bound. Masuda[4] has recently put forward the suggestion that IAA may render ribonucleic acid (RNA) separate from ribonucleoprotein by becoming complexed with the protein moiety. During the course of an investigation on the binding of auxin to protein in growing tissues it was observed that several amino-acids stimulated the binding process This communication summarizes some of the observations made concerning the inter-relationship between the binding process and protein synthesis

The auxin used was naphthalene acetic acid-1-^{14}C (NAA-^{14}C) obtained from Tracerlab Inc , Waltham, Mass , United States The specific activity of this preparation was rather low (6 7 μc./mgm) , in the experiments described here, concentrations lower than 5×10^{-4} *M* could not be used To study the effect of amino-acids on the binding of NAA-^{14}C to protein, pea-internode sections (var Duke of Albany) were allowed to metabolize different amino-acids in phosphate buffer of *p*H 6 0 in the presence of NAA-^{14}C, and the proteins precipitated by trichloracetic acid (TCA) It can be seen from Table 1 that all the amino-acids used promoted the binding of NAA-^{14}C to protein, and the stimulation was particularly noteworthy in the case of aspartic acid With aspartic acid, glutamic acid and leucine, incorporation of carbon-14 into the TCA-precipitable proteins was higher at 10^{-3} *M* than at 10^{-6} *M* It is not known, however, whether these amino-acids or the auxin-amino-acid conjugates—if such conjugates were formed in the present case—participated in the formation of the auxin-bound protein

Table 1 EFFECT OF AMINO-ACIDS ON THE BINDING OF NAA-^{14}C TO THE 'PROTEIN' of PEA (VAR DUKE OF ALBANY) INTERNODES

Treatment	Per cent incorporation of carbon-14 after 3 hr
Control	100
Aspartic acid 10^{-6} *M*	215
Aspartic acid 10^{-3} *M*	333
Glutamic acid 10^{-6} *M*	105
Glutamic acid 10^{-3} *M*	243
Serine 10^{-6} *M*	115
Serine 10^{-3} *M*	129
Leucine 10^{-6} *M*	180
Leucine 10^{-3} *M*	214
Alanine 10^{-6} *M*	158
Alanine 10^{-3} *M*	158
Glycine 10^{-6} *M*	158
Glycine 10^{-3} *M*	154

If new protein is formed, it may be expected that chloramphenicol—which has been reported to be an inhibitor of protein synthesis—would inhibit the process of formation of the auxin-bound protein Accordingly, internode sections were incubated with three different concentrations of chloramphenicol The results are shown in Fig 1 It is surprising to note that even at 10^{-3} *M* no inhibition was observed, 10^{-5} *M* chloramphenicol brought about a stimulation Some inhibition was observed if the NAA-^{14}C was added 1 hr after pre-incubation with chloramphenicol.

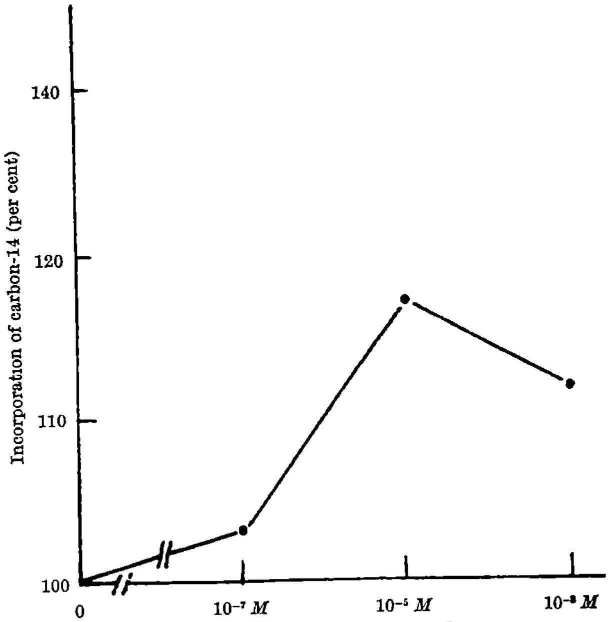

Fig 1 Effect of chloramphenicol on the binding of NAA-^{14}C to protein of internodes of peas

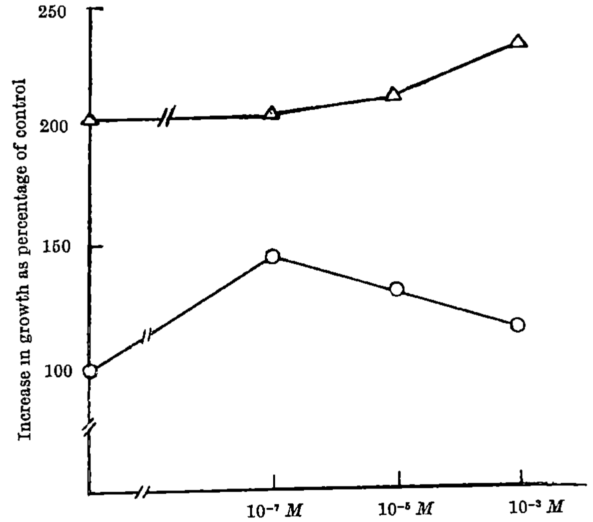

Fig 2 The effect of different concentrations of chloramphenicol on the growth of *Avena* coleoptiles in presence (△) or absence (○) of 10^{-6} *M* IAA

Since the binding of auxin to protein is usually supposed to be related to the growth process, it was necessary to see whether chloramphenicol inhibited auxin-induced growth. The results are shown in Fig. 2 Chloramphenicol not only fails to inhibit growth, but also has no marked effect on the incorporation of ^{14}C-labelled amino-acids into the proteins At 10^{-3} *M* some inhibition was observed in the case of glycine-2-^{14}C (Table 2)

In subsequent experiments the effect of ribonuclease (bovine pancreas RNase, Sigma Chemical Co , St Louis, Mo , U S A.) on the binding process was investigated *Avena* coleoptiles were used, since from the work of Masuda[5] it appears that RNase penetrates coleoptile sections The coleoptiles were treated with RNase (100 μgm /ml) for 2 5 hr ,

Table 2 EFFECT OF CONCENTRATIONS OF CHLORAMPHENICOL ON THE INCORPORATION OF GLYCINE-2-^{14}C AND LEUCINE-1-^{14}C INTO THE 'PROTEIN' OF PEA (VAR DUKE OF ALBANY) INTERNODES

Amino-acid	Concentration of chloramphenicol	Per cent incorporation of carbon-14 after 3 hr.
Glycine-2-^{14}C ($5 \times 10^{-4}M$)	0	100
	10^{-6} *M*	139
	10^{-3} *M*	84
Leucine-1-^{14}C ($5 \times 10^{-4}M$)	0	100
	10^{-6} *M*	133
	10^{-3} *M*	98

Table 3 EFFECT OF RNASE ON THE BINDING OF NAA-^{14}C TO THE 'PROTEIN' OF PEA (VAR DUKE OF ALBANY) INTERNODES

Treatment	c p m /mgm of 'protein' after 3 hr.
Control	600
RNase (100 μgm /ml)	809

washed and incubated again for 3 hr in darkness at 28° C. in the presence of NAA-^{14}C From Table 3 it is apparent that treatment with RNase has no inhibitory effect On the other hand, some stimulation was observed No inhibition was found even when the period of incubation with NAA-^{14}C was reduced to 1 hr—a period much shorter than that required by the coleoptiles to overcome the inhibitory effect of RNase[4] The process of binding of auxin to protein, thus, does not appear to be an RNA-controlled process. Whether the stimulation in binding due to RNase treatment is caused by the liberation of nucleotides[5] which help in the binding of auxin to protein, or due to an increased availability of sites for the binding of auxin owing to the separation of RNA from ribonucleoprotein, is not known at present

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Effect of Indole-3-Acetic Acid (IAA) on Phosphorus Metabolism in the *Avena* Coleoptile

IT is generally believed that energy-rich phosphate compounds act as a store-house of energy for different reactions during growth The auxin-induced stimulation of respiration is presumed to be due to an increased availability of phosphate acceptors due to the rapid utilization of nucleotides during the growth process[1-3] Rhodes and Ashworth[4] have speculated on the mechanism by which growth substances may be metabolized for the generation of energy-rich phosphate bonds, but an unequivocal demonstration of the stimulation of nucleotide synthesis is not available Ormrod and Williams[5] and Key *et al*[6] have recently observed increased phosphorylating activities in plants sprayed with 2,4-dichlorophenoxyacetic acid Marre and Forti[7] had noted a rapid increase in high-energy phosphate level in auxin-treated pea internode segments during the first 30 min, followed by a decline The method of estimation of adenosine triphosphate (ATP) adopted by them, which involves the adsorption of adenine nucleotides on charcoal followed by elution with hydrochloric acid, has several limitations when applied to tissue sections Since numerous metabolic reactions go on in the cell at the same time, and a large number of them concern the participation of nucleotides, particularly reactions mediated by the kinase type of enzyme, and the phosphate residues are transferred to a variety of substrates, estimation of the level of adenine nucleotides at any given time is not the true measure of the total ATP formed during the experimental period A more correct approach is to estimate the phosphate esterified to organic compounds including the nucleotides Phosphate labelled with phosphorus-32 may be conveniently used for this purpose and the present communication summarizes the findings of an investigation directed towards this objective

Five-mm sections of 4-days old *Avena* coleoptiles (var *N P* 1) were incubated with radioactive disodium hydrogen phosphate (Na$_2$H^{32}PO$_4$) and different concentrations of IAA in *M*/150 phosphate buffer of *p*H 6 0 for 1 and 2 hr. The tissues were extracted with 80 per cent ethanol and the concentrated extract chromatographed two-dimensionally with phenol/water as the first, and butanol/acetic acid/water (4 1 1) as the second, solvent for 48 hr., or unidimensionally in 69 per cent butyric acid in 0 85 per cent sodium hydroxide, followed by autoradiography with Ilford X-ray films The sum of the ^{32}P-activities in the radiation spots other than inorganic phosphate was taken as a measure of phosphate-^{32}P esterified. It may be observed from Fig 1 that with low concentrations of IAA in the experimental solution, esterification of ^{32}P was increased considerably, at 10^{-3} *M*, a concentration which was inhibitory to growth, esterification was also inhibited The stimulation in esterification

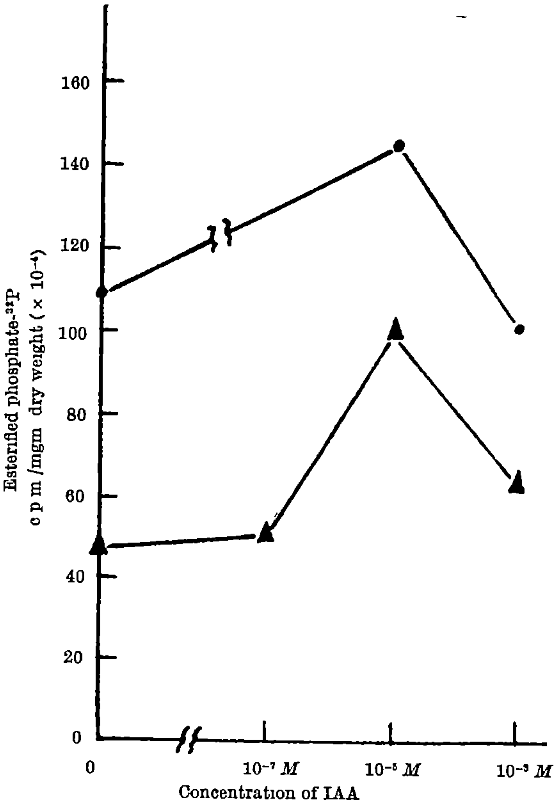

Fig 1 Effect of different concentrations of IAA, in presence (▲) or absence (●) of DNP (10^{-5} *M*) on esterification of phosphorus-32 (from Na$_2$H^{32}PO$_4$) in the 80 per cent ethanol-soluble fraction of *Avena* coleoptile sections, experimental period 2 hr

observed at the end of 1 hr was greater than that after 2 hr Similar results were obtained with rice coleoptiles

When 10^{-5} M 2,4-dinitrophenol (DNP), the uncoupling agent of phosphorylation, was included in the experimental solution it was found that DNP itself inhibited the esterification of phosphate-^{32}P by more than 50 per cent The inhibitory effect was countered by low concentrations of IAA IAA at 10^{-7} M had very little effect, but the higher concentration of 10^{-5} M considerably diminished the inhibitory effect due to DNP IAA at 10^{-3} M, which inhibited esterification, failed to oppose the effect of DNP to any great extent

The effect of IAA and DNP on individual compounds is shown in Table 1 Phosphate-^{32}P was metabolized mainly to hexose phosphates, ATP and ADP, and an unidentified compound designated 'compound X' in Table 1 While changes in activity in the sugar phosphates and adenine nucleotides reflected the pattern observed in investigation of phosphate esterification, incorporation of phosphorus-32 into compound X increased with increasing concentrations of IAA DNP completely inhibited its formation, it reappeared only when DNP was combined with 10^{-3} M IAA

Table 1 Effect of IAA and DNP on the Incorporation of Phosphorus-32 from $Na_2H^{32}PO_4$ into the 80 per cent Ethanol-soluble Phosphorylated Compounds of *Avena* Coleoptiles Specific Activity (c p m /mgm × 10^{-3}) after Inhalation for 2 hr

Treatment	Hexose phosphate	ADP + ATP	'Compound X'
Control	154 6	545 4	75 1
IAA 10^{-5} M	269 6	721 8	162 7
IAA 10^{-3} M	203 9	365 5	164 7
DNP 10^{-5} M	97 1	235 0	0
DNP 10^{-5} M + IAA 10^{-7} M	89 2	185 0	0
DNP 10^{-5} M + IAA 10^{-5} M	152 7	322 2	0
DNP 10^{-5} M + IAA 10^{-3} M	120 8	316 4	26 8

Results obtained in this work reveal an increased incorporation of phosphorus-32 in both the alcohol-insoluble fraction and in the esterified organic compounds in the alcohol-soluble fraction in the presence of low concentrations of IAA which promote the growth of coleoptiles If the phosphorylase and phosphatase activities of these tissues are not very high, this may be taken to reflect largely, if not entirely, the formation of energy-rich nucleotides as affected by auxins The inhibition by DNP and its counter-action by IAA provide further evidence in support of the contention that application of auxins leads to metabolic changes resulting in an increased synthesis of energy-rich nucleotides utilized in various reactions involved in the growth process

Our thanks are due to Dr D M Bose, director, and Dr P K Bose, head of the Department of Chemistry, Bose Institute, for their interest and encouragement

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Quantitative Aspects of the Re-utilization of Protein-bound Amino-acids *in vivo*

The extent of the re-utilization of protein-bound amino-acids in the formation of new protein has been a subject of interest for many years[1-3] The main difficulty with some of the work reported in the literature has been that the half-life of the protein into which the re-utilized amino-acid residue was traced was short Thus, when one injects a labelled soluble protein and determines the uptake of the label into liver or serum proteins (the half-lives of which are a few days in extent) one deals with a situation in which the uptake, transfer and loss of label is rapid and, furthermore, where the 'recipient' protein continually recirculates some of the amino-acid to itself In these circumstances one is unable to assess reliably the significance or extent of the re-utilization

In this communication we describe the quantitative re-utilization of amino-acids from serum protein to globin Globin is a protein which, contained in the red cell, enjoys a life-span of about 55 days in the rat Thus, over a reasonably short period no globin formed during that interval of time is broken down

22 hr after injecting a rat with 1 mc (about 20 mgm) of glycine-2-^{14}C, the animal was bled and serum albumin and serum globulin were prepared by methods described in ref 3 20 mgm of either labelled serum albumin or serum globulin containing 65 mμc / mgm were injected intravenously into recipient rats Animals were killed 1, 3, 6, 9, 12 or 15 days after the injection and globin was prepared from washed erythrocytes by the method in ref 4 Dry protein powders[3] of the globin and also of aliquots of the injected serum protein were hydrolysed in 6 N hydrochloric acid for 24 hr and the hydrolysate was then treated with 2,4-dinitrofluorobenzene according to Levy[5] The dinitrophenyl glycine was separated from the other dinitrophenyl-amino-acids on a 'Celite' column and purified as described in ref 6 Samples of the injected protein and of the dinitrophenyl glycine from injected protein and from globin were counted using a D-47 Nuclear-Chicago flow-gas counter with 'Mylar' window When sample weights differed from 30 mgm a self-absorption correction was applied In addition, the counts obtained for dinitrophenyl glycine samples were recalculated for free glycine

In preliminary experiments we found that the half-life of serum albumin is 3 days and of serum globulin is 4 days in the Sherman strain rats used in these experiments Furthermore, it was established that about 31 per cent of the radioactivity of serum albumin or serum globulin is due to glycine when these proteins are prepared as described here and the remainder of the activity is in serine In experiments to be reported in full elsewhere it was also found that the conversion of labelled serine to labelled glycine, in recipient animals injected with glycine-serine labelled serum proteins, does not materially affect the results of the calculation presented here

Using the data obtained in the above experiments it is possible to calculate the percentage serum albumin and serum globulin glycine re-utilized in globin synthesis The percentage of the total glycine required for globin synthesis which has its origin in serum protein glycine can also be determined

The calculation is based on the counts/min /mgm of globin glycine found at time t after injection, the total serum protein-bound glycine activity injected into the rat, the assumption that 3 4 per cent of the body-weight corresponds to the total packed red cell volume, that there are 314 mgm of hæmoglobin/ml packed cells[7] and that the life-span of red cells is 55 days in the rat[8] Furthermore, the glycine content of globin is assumed to be 4 8 per cent[9], that of serum globulin 4 3 per cent and that of serum albumin 1 6 per cent[9,10], the molecular weight of hæmoglobin is taken at 68,000 and that of globin at 65,400 (ref 11) The sample calculation presented here is one which is based on a rat injected with labelled rat serum globulin and killed 12 days later

The final calculated results are typical of those obtained in rats injected with serum globulin and killed at any of the times after injection mentioned here

A sample calculation of the re-utilization of serum globulin glycine in globin biosynthesis is as follows

(1) Total glycine radioactivity released on breakdown of injected labelled serum globulin from time 0 to time t (t = 12 days) Counts/min injected × percentage activity in glycine × fraction degraded at time t = 196,500 × 31 per cent × 87 5 per cent = 53,300

(2) Total mgm glycine released a day on breakdown of serum globulins Rat weight (gm) × ml serum/gm body-weight × mgm serum globulin/ml × percentage glycine in serum globulin × fraction of serum globulin degraded a day = 283 × 0 034 × 32 2 × 4 3 per cent × 16 per cent = 2 13

(3) Total mgm glycine required in globin synthesis a day in this rat Weight of rat (gm) × ml packed red cell volume per gm body-weight × mgm hæmoglobin/ml × $\frac{\text{M W globin}}{\text{M W hæmoglobin}}$ × per cent glycine in globin × $\frac{1}{\text{life span of red cells}}$ = 283 × 0 034 × 314 × $\frac{65{,}400}{68{,}000}$ × 4 8 per cent × $\frac{1}{55}$ = 2 54

(4) Total radioactivity incorporated into total globin glycine from time 0 to time t (t = 12 days) Counts/min /mgm globin glycine × total mgm globin glycine = counts/min /mgm globin glycine × mgm glycine required in globin synthesis a day × life-span of globin = 4 38 × 2 54 × 55 = 612

(5) Percentage serum globulin glycine radioactivity incorporated into globin glycine

$$\frac{\text{total counts/min in globin glycine}}{\text{total glycine counts/min released from serum globulin}} \times 100 = \frac{612}{53{,}300} \times 100 = 1\ 15$$

(6) Weight of serum globulin glycine incorporated into globin glycine over this 12-day time-period mgm serum globulin glycine released per day × days × fraction serum globulin glycine counts/min incorporated in globin = 2 13 × 12 × 0 0115 = 0 294

(7) Percentage of total glycine required in globin synthesis which has its origin in serum globulin glycine·

$$\frac{\text{Weight of serum globulin glycine incorporated into globin}}{\text{Total mgm. glycine incorporated into globin in 12 days}} \times 100 = \frac{0\ 294}{2\ 54 \times 12} \times 100 = 0\ 96$$

Thus about 0 96 per cent of the total glycine required in globin synthesis has its origin in serum globulin and approximately 1 15 per cent of serum globulin glycine is used for globin synthesis Similar experiments with rats injected with labelled serum albumin indicate that 0 57 per cent of the total glycine required in globin synthesis has its origin in serum albumin and approximately 1 per cent of serum albumin glycine is used for globin synthesis This contribution by serum proteins of 1 5 per cent of the total glycine required in globin synthesis must be deemed significant in view of the fact that serum proteins constitute only 2 per cent of the rat's total proteins.

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Isolation of Echinochrome *A* from the Spines of the Sea Urchin, *Stomopneustes variolaris* (Lamarck)

I have reported the presence of echinochrome *A* (7-ethyl-2,3,5,6,8-pentahydroxy-1,4-naphthaquinone) in the spines of the sea urchin, *Echinarachnius* (Scaphechinus) *mirabilis*[1] and *Diadema setosum*[2]. Recently it was found that the principal naphthaquinone pigment in the almost black spines of the sea urchin, *Stomopneustes variolaris* (Lamarck) (Japanese name, kuro-uni), which was collected at Shirahama in Wakayama Prefecture, was identical with echinochrome *A* The isolation and purification of the pigment have been carried out by the identical method used in the case of *Diadema setosum*

The pure pigment crystallized from dioxane–water in dark red-brown needles and melted at 215° The yield of the pure material was about 26 mgm from each 100 gm of the spines Absorption maxima were at 256, 340, 466, 489, 525 mμ in chloroform and the percentage of carbon and hydrogen was 53 63 and 3 40 respectively (calc for $C_{12}H_{10}O_7$ C, 54 14, H, 3 79)

The trimethyl derivative of the pigment was obtained as long, red needles when it was methylated with diazomethane and crystallized from dioxane–water It melted at 130° (sealed tube) and showed absorption maxima at 249, 323 5, 478, 503, 538 mμ in chloroform The percentage of carbon and hydrogen was 58 53 and 4 96 respectively (calc for $C_{12}H_7O_4$-

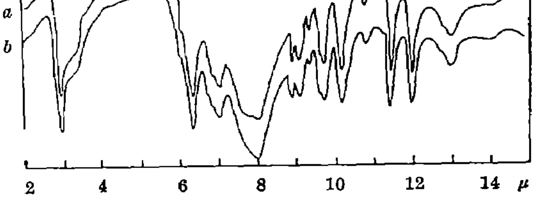

Fig 1 Infra-red spectra *a*, free pigment of *Stomopneustes variolaris*, *b*, echinochrome *A* of *Diadema setosum* (potassium bromide)

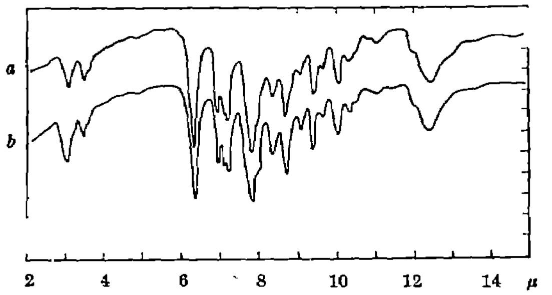

Fig. 2 Infra-red spectra *a*, trimethyl derivative of the pigment of *Stomopneustes variolaris*, *b*, trimethyl echinochrome *A* of *Diadema setosum* (potassium bromide)

$(OCH_3)_3$: C, 58 35, H, 5 29) It was soluble in aqueous sodium hydroxide giving a blue colour

The yellow dehydro-derivative having absorption maxima at 257·5, 319, 392 mμ (in aqueous methanol) was obtained when the free pigment was dehydrogenated with the peroxidase–hydrogen peroxide system. The original red pigment was regenerated when the dehydro derivative was treated with hydrogen sulphide.

The colour reactions and the ultra-violet, visible and infra-red absorption spectra of the free pigment and of the trimethyl derivative were in fair agreement with those of authentic specimens of echinochrome *A* and trimethyl echinochrome *A*, respectively. No depression of the melting point was observed when the mixed melting point examination was carried out with authentic specimens of echinochrome *A* and trimethyl echinochrome *A*, respectively

I wish to thank Prof Y. Nakamura and Prof T. Saito, of Hokkaido University, for their encouragement and guidance I am also grateful to Dr. H Utinomi, of Seto Marine Biological Laboratory, Kyoto University, for collecting samples, and to Dr M Fujino, of Takeda Research Laboratory, for the elemental and infra-red analysis

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PHYSIOLOGY

Inhibitory Phenomena and 'Habituation' at the Neuronal Level

In behavioural habituation, according to Thorpe[1], the animal "learns not to respond to stimuli which tend to be without significance in the life of the animal" It does so by a mechanism which determines a "relatively permanent waning of a response as a result of repeated stimulation" Different authors conceive that the phenomenon of habituation may be correlated with changes in the central nervous system (*a*) development of inhibitory connexions, in a way analogous to the extinction of a conditioned reflex[2], (*b*) development of inhibitory influences acting on different synaptic relays of the sensory pathways, which, by producing 'afferent neuronal habituation', would lead to behavioural habituation[3]

We have attempted to analyse the nature of these changes in the snail, in which behavioural habituation was described long ago (Humphrey, 1933, quoted by Thorpe[1]) In electrophysiological investigations with intracellular microelectrodes on the ganglion cells of the snail and other molluscs, Tauc[4,5] has recently described postsynaptic inhibitory potentials, and inhibitory effects of long duration, which might be relevant in an interpretation of the mechanism of habituation

Our experiments were performed in an isolated nervous system preparation of the land snail (*Helix*) The whole periœsophageal ring of nervous ganglia and commissures, together with the mantle nerves, was cut loose and partially desheathed It was mounted in a 'Lucite' chamber and covered with Cardot's solution The mantle nerves were threaded through small holes into a neighbouring chamber, and mounted on bipolar platinum–iridium electrodes and covered with mineral oil Intracellular electrical recording was carried out with conventional glass micropipettes filled with 2 5–3 *M* potassium chloride

In the course of exploratory recording of the electrical activity of different cells in the parietal ganglia, we concentrated our observations on a group of two or three big cells (150–225μ in diameter) at the medial side of the right parietal ganglion, just left of the root of the internal right mantle nerve We selected these cells because of their size, easy recognition, and stability of response (a couple of hours)

When a micropipette penetrates the cell membrane, a resting potential of 40–60 mV is recorded, with an occasional burst of spikes After some minutes of impalement, these neurones exhibit a low-frequency discharge of impulses, with slow and irregular changes in frequency, or with alternating periods of activity and silence We speak of this as 'spontaneous' activity not prejudging if it is due to random synaptic bombardment or autorrhythmic activity generated in the impaled cell itself Not only full-sized spike potentials (with an overshoot of 20–40 mV) but also abortive or incipient spikes, and slower or faster oscillations of the membrane potential, in a depolarizing or hyperpolarizing direction, may be recorded

If the ipsilateral mantle nerve is stimulated with electrical pulses at rather low frequencies (1–5 sec.$^{-1}$), each stimulus may at the beginning drive a full-sized spike potential in the neurone (Fig 1) After a certain number of stimuli, both the driven responses, and the 'spontaneous' impulses as well, are blocked and replaced by an oscillating type of hyperpolarizing activity, which either builds up gradually during stimulation or appears abruptly after some stimuli. In many cases, with adequate amplification and time base, each afferent impulse can be seen to be followed by a hyperpolarizing potential with quite a regular latency These hyperpolarizing effects have been

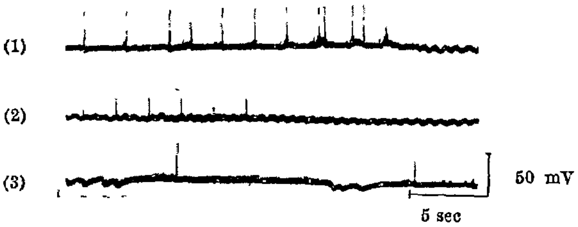

Fig 1 Electrical activity of snail ganglion cell (continuous record except for 18 sec cut between (2) and (3)) (1) Neurone discharges 'spontaneously' Stimulation of ipsilateral mantle nerve at a frequency of 5/sec 'Driven' spikes, preceded by slight positive potentials, can be observed after the first seven stimuli Spike activity is then blocked, being replaced by oscillating hyperpolarizing potentials These continue despite cessation of stimulation, for 40 sec, before a 'spontaneous' spike reappears (2)–(3) (3) Full-sized spikes intermingled with incipient spikes and positive potentials

recorded both with ipsilateral and contralateral stimulation In some instances the inhibitory effects are established after some stimuli, but without the remarkable oscillating behaviour already described

When stimulation is discontinued, hyperpolarizing oscillations and inhibitory effects gradually subside, and after 20–100 sec, 'spontaneous' activity reappears, the membrane potential shifts towards negativity, positive potentials may appear intermingled with full-sized or incipient spikes, until the resting pattern of activity is finally re-established (Fig 1) After some minutes of rest, renewal of stimulation is followed by earlier inhibitory effects, block being obtained with fewer stimuli, and eventually with a single stimulus These blocking effects of long duration, especially when correlated with hyperpolarizing responses, suggest a mechanism different from fatigue—rather a mechanism of active inhibition It is also improbable that fatigue could result from such a low frequency of stimulation, on occasions lower than the frequency of 'spontaneous' activity itself

It is rather difficult to justify the use of the term 'habituation' to describe our observations, because of the conditions in which the experiments were carried out—with an isolated nervous system preparation, electrical stimulation of nerve trunks, and the short period of observation possible Nevertheless, they confirm the existence of an inherent inhibitory mechanism in the nervous system of the snail, a mechanism which, put into action by repeated stimulation, could be the basis for a process analogous to habituation, at the neuronal level Finally, as our observations seem to conform so neatly with Moruzzi's vivid suggestion regarding the mechanism of habituation, namely, that "monotonous repetition of an identical stimulus would bring about, by a phenomenon of temporal facilitation—an avalanching increase of activity in inhibitory neurons", we communicate these results under this rather provoking title, and as a schematic model for describing the phenomenon of habituation and discussing its hypothetical mechanism

This work was supported financially by the Commission for Promotion of Research, Faculty of Medicine, University of Chile

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Effects of Intensity of Driving Stimulus on the Shape of Membrane Action Potential of the Heart

An investigation of the influence of driving stimuli on the membrane action potentials of toad cardiac muscle fibres has been made Endocardial surfaces of excised hearts were exposed and the transmembrane potentials of atrial and ventricular fibres recorded by means of glass-pipette microelectrodes.

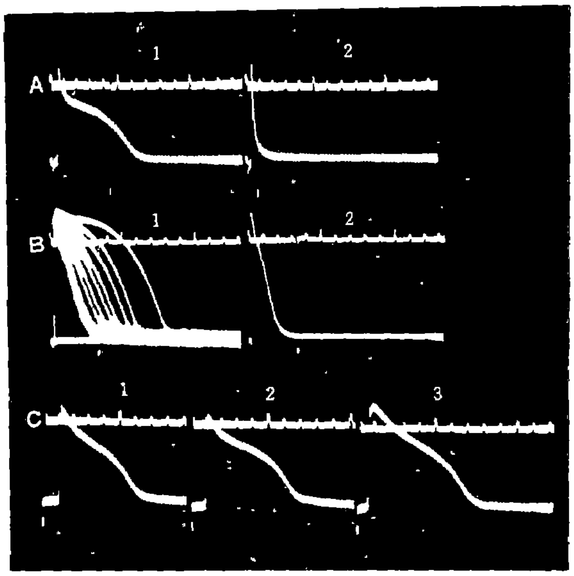

Fig 1 *A*-1, Duration of action potential of an atrial fibre when using a threshold driving stimulus (7 V), and *A*-2 the change in duration when driving stimuli were of twice threshold strength *B*-1, The progressive shortening of the action potential when driving of quiescent atrial fibres was begun—the first ten beats *B*-2 shows the steady state attained *C*, Control atrial fibre action potential (1), after atropinization (terminal concentration of 1 in a million) (2), and when twice threshold driving stimuli were used on the atropinized tissue (3) Time is indicated in 100 (small pips) and 500 (large pips) msec intervals Calibration of voltage on the left base indicates −100 mV from the extracellular potential (time-base)

As shown in Fig 1*A*, duration of the action potentials of atrial fibres was reduced from 500 msec to 59 msec or by 88 per cent, when the intensity of the driving stimulus was made twice the threshold strength (Fig 1 *A*–2) This effect was reversible and could be reproduced at will Thus, by properly adjusting the intensity of driving stimuli the duration of induced action potentials could be controlled

It was occasionally observed that similar changes in duration occur without the use of supraliminal stimuli When atrial fibres, which had been quiescent for some time (about 2 min), were driven with threshold stimuli, the action potential shortened progressively (Fig 1 *B*–1) and attained a steady state of duration at about 22 per cent of the initial duration (Fig 1 *B*–2) that of the first driven response

Shortening of the duration of the action potential brought about by the procedure mentioned above was quite similar to the effect of acetylcholine or vagal stimulation All three effects were antagonized by atropine These findings suggest that a substance like acetylcholine is liberated by the driving stimulus and that the amount of the substance discharged is increased with an increase in the intensity of the stimulus

In both atrial and ventricular fibres, the degree of reduction brought about by an increase in the intensity of driving stimuli was almost the same as that caused by maximal vagal stimulation This result and the fact that the reduction was not uniform around the driving cathode suggest the possibility that liberation of acetylcholine occurs from intramural nerve endings or ganglia stimulated directly by the electrical drive itself

The next problem is whether or not autonomimetic substances other than acetylcholine are also liberated by driving stimuli The duration of the action potential elicited by threshold driving stimuli was prolonged to some extent by administration of atropine (Fig. 1 *C*–2). After atropinization, increase in the intensity of driving stimuli still brought about certain changes in shape of the action potential, namely, a prolongation of its duration, rather than a shortening, an increase in amplitude of the overshoot, a slowing of the terminal phase of depolarization and initial phase of repolarization (Fig. 1 *C*–3). Since similar effects were obtained both in atrial and ventricular fibres by administration of epinephrine or norepinephrine as well as by sympathetic nerve stimulation, it is conceivable that increase in the intensity of the driving stimuli results in liberation of a sympathomimetic substance. This liberation might result from stimulation of intramural nerve fibres by driving stimuli as in the case of liberation of acetylcholine. In preparations not atropinized, these effects were not observed, probably because they were masked by those of the acetylcholine which was simultaneously liberated.

It is obvious that in electrically driven hearts the duration and shape of the membrane action potential can be widely varied, depending on the intensity of the driving stimuli. The possible liberation of the autonomimetic substances should always be taken into account in interpreting the results of experiments which involve electric stimulation of cardiac tissue.

Our thanks are due to Dr. Chandler McC. Brooks for reading the manuscript and making valuable suggestions.

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Interrelationship between Fat and Sugar Metabolism in Infant Rats

Rat milk contains a large amount of fat and protein[1]. The amount of sugar in this food is just sufficient to cover the energy needs of the infant rat for about 2 hr., and it is thus obvious that the energy required for the remaining 22 hr. of the day must come either from fat or protein. This is underlined by the fact that liver and muscle glycogen content, although falling transiently shortly after birth, soon return to higher levels[2].

In order to gain further insight into the ways in which individual nutrients are utilized by the infant rat the following experiments were performed. Rats aged 5 (suckling period) and 30 days (end of weaning) and adult animals (body-weight 200 gm.) of both sexes were starved for 24 hr., the 5-day-old animals at 30° C., the older ones at room temperature. They were then given 1·1 ml. of olive oil/100 gm. body-weight via a stomach tube, and were killed at different intervals. Liver glycogen content[3] and blood glucose-levels[4] were determined. It is evident from Fig. 1 that administration of olive oil to animals aged 5 days resulted in a rapid increase in the level of liver glycogen, while no such rise was observed in animals aged 30 days, or in adult rats. Blood glucose-levels remained unchanged in all age-groups.

The administration of 1·1 ml. oleic acid/100 gm. body-weight to 5-day-old rats resulted in a much smaller, and variable, rise in liver glycogen content and the blood glucose-level was slightly raised.

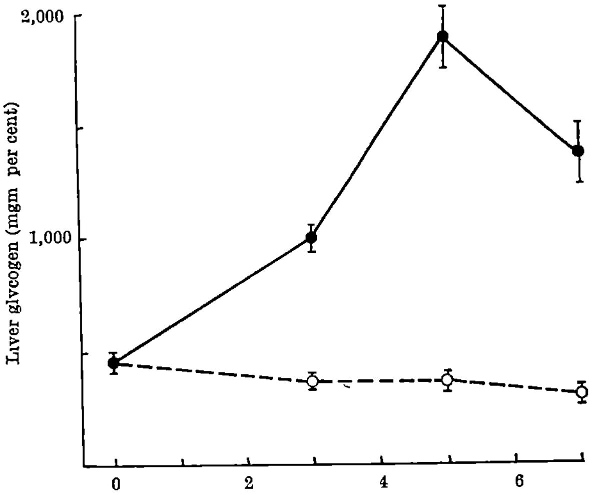

Fig. 1. Liver glycogen content in rats starved for 24 hr. and then given 1·1 ml. olive oil/100 gm. body-weight via stomach tube. Full line, rats aged 5 days, interrupted line, rats aged 30 days (the same type of curve was obtained for adult animals and for 5-day-old rats not given oil). Vertical lines indicate standard errors, each point is the average of at least 8 rats

It thus appeared that the glycerol contained in olive oil was responsible for the large rise in liver glycogen content found in 5-day-old rats. Administration of 2 ml. of a 2·5 per cent glycerol solution per 100 gm. body-weight, or administration of a mixture of oleic acid and glycerol corresponding to 1·1 ml. olive oil/100 gm., however, did not cause a rise in liver glycogen content, although hyperglycæmia was regularly found.

These results may be explained in several ways. Either fat gives rise to carbohydrate or fat spares sugars to a greater extent in the younger age-group. The first possibility can be rejected on theoretical grounds[5]: the second, however, may also be objected to since, following olive oil administration, glycogen accumulates in the liver and must be synthesized from some substance other than protein or amino-acids following the finding that gluconeogenesis from casein hydrolysate is much lower in sucking rats than in older animals[6]. Finally, there remains the possibility that triglycerides are transported differently in infant rats, as is also indicated by other results[7], and are transferred across the intestinal wall as such and broken down elsewhere, the glycerol being used for synthesis of liver glycogen.

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Generalized Skeletal Response on Local Trauma in Young Rats studied by means of ^{45}Ca-Metabolism

In connexion with following-up deviations in metabolism of calcium in bones in young animals after local injury by Roentgen irradiation, we wished to be convinced about the effect of other kinds of physical traumatism on the metabolism of calcium in bones For this purpose we chose electric current for several reasons in the first place, almost nothing is known about deviation in metabolism of bones injured by electric current, and secondly, because the entire question of the effect of such trauma on bones has been relatively little studied Electro-trauma is quite easily carried out It is also known that the majority of wounds in electro-trauma quickly heal without suppuration so that when experimenting it is possible to exclude undesired suppuration as a complication which could misrepresent the observed results In case of other surface injuries, for example, radiation damage, torpid ulcers arise which are the seats of infection, causing local irritation which may meet with a response in the entire organism

In our experiments we used male rats of the Wistar strain about 10 weeks old, each weighing 120–130 gm We wounded them in the upper part of the shank on the back right limb by discharging a condenser, the capacity of which was 80 μF , loaded at a potential of 2,000 V

Thus only one discharge penetrated across the limb The rats were then divided into groups of five, and 20 μc of $^{45}CaCl_2$ in 1 ml physiological salt solution were applied intraperitoneally after 4, 10, 17, 24 and 38 days after injury The rats were killed 48 hr after addition of the isotope, and the tibias of the injured limbs dissected The control limbs of the injured animals and the tibias of a control group of animals treated with isotope but not subjected to electro-trauma were also removed Besides the tibia, we also took out the shoulder blade of all animals With all the bones we recorded the weight of the dry bone and the ash after burning, measured the entire contents of bone calcium, and determined the specific activity of the calcium-45

Four days after the injury, differences appeared in the weight of the dry bone, ash and calcium content of the tibias of the injured animals in comparison with the control group (Fig 1) All values showed a tendency to fall, followed by a much slower incline and restitution than in the uninjured animals At the same time certain differences in weight appeared even in uninjured limbs of wounded animals, showing that a general reaction results from a local trauma These changes occurred not only on the bones of the limbs but were also evident on the scapula, which is a bone of differently growing type from that of the limb, and is situated far from the injured place

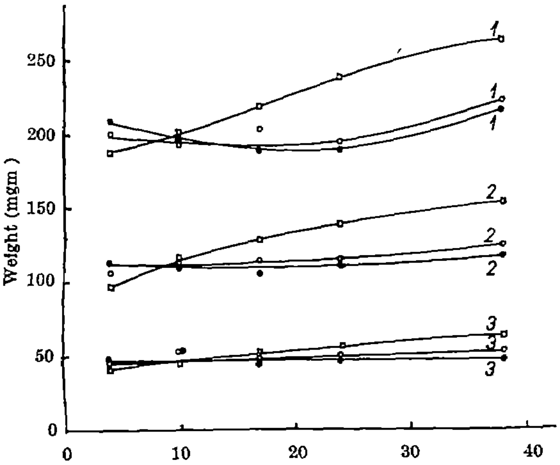
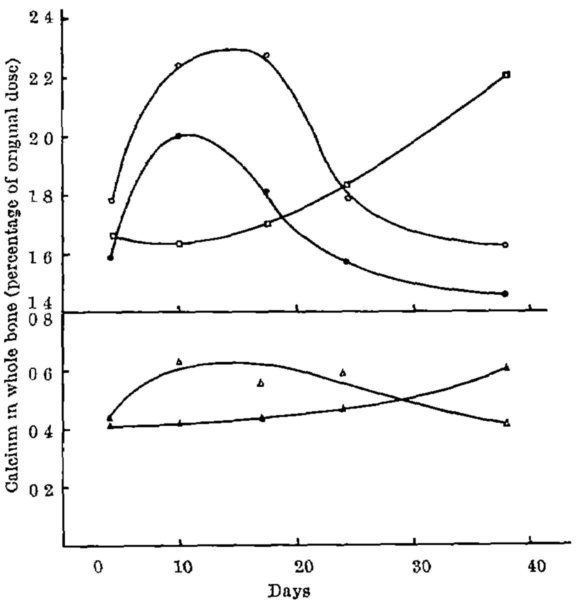


Fig 1 Weight of dry bone (1), ash (2) and total bone calcium (3) of rat tibias □, Control group, ○, traumatized tibia, ●, non-traumatized tibia



Figs 2 and 3 48-hr accretion of calcium-45 in the tibia (above) and scapula (below) □, Tibia in control group, ○, traumatized tibia, ●, non-traumatized tibia, ▲, scapula of control group, △, scapula in traumatized animals

Distinct and important changes are evident when tracing the specific activity by measuring the values of calcium-45 in injured and uninjured bones Fig 2 shows the 2-day accretion of calcium-45 in the tibia and Fig 3 in the scapula It shows that in the bones of injured animals an increased accretion of isotope at first took place in all bones, again indicating the general response of calcium metabolism in the skeleton When comparing Fig 1 and the weights, it is surprising to find that while the weight of dry bone, ash and total bone-calcium content show a decline, the specific activity increases

Calcium metabolism depends on a number of factors[1–4] the most important of which are accretion, resorption and calcium exchange In comparing the results of our experiments, which follow accretion for 2 days, changes are evident in accretion values of calcium-45 in injured and uninjured bones of the experimental animal, changes which are expressions of local and general reactions as a result of local traumas In comparing the results with the values of weight, it is shown that, after injury, not only the accretion is increased, but also the resorption of calcium (even more than accretion), so that the injury causes deep interference in the metabolism of calcium in the entire skeleton

One other interesting inference, which follows from measuring and observing these values, is that in young animals even at the end of the experiment (about 40 days after injury) the conditions of metabolism had not become normalized Although the skin wounds after electro-trauma were healed without complications within ten days of the injury—healed in such a way that no traces of the transit of current were seen macroscopically—the disturbance of calcium metabolism and the response to local trauma remains as is shown by the dry-weight, ash, and calcium content of measured bones Hence, the metabolic changes which result from local injuries to growing organism can persist for some time after healing. A similar reaction was noticed not only after electro-trauma injury, but also as a result of radiation injury[3], showing that this general response is the natural reaction of growing organisms to local trauma

Details of the conditions of accretion and resorption of radiocalcium after local electro-trauma has been published in *Archiv fur orthopadische und Unfall-Chirurgie*[5]

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Number of Recirculations of Chick Embryo Erythrocytes

It has recently been shown by Allison[1] on the grounds of cardiac output × erythrocyte life-span/blood volume that in spite of quite different erythrocyte life-spans in man, dog and rat the average number of times an erythrocyte is oxygenated and deoxygenated, that is, the number of recirculations, is in all three species of the same order, about 180,000 times It might be of interest to determine if this relation is valid for embryonic erythrocytes also, and for their various lines respectively Unfortunately, only incomplete data or none at all are available for mammalian embryos, though values covering nearly the whole incubation period have been published for chick embryos

Using Allison's formula it was found that the number of recirculations of nucleated adult hen's erythrocyte is between 147,150 and 186,300, that is, in the range valid for non-nucleated erythrocytes of adult mammals[2]

Allison's formula in its original form is generally not suitable for calculation of embryonic erythrocyte recirculations because the cardiac output and the increase in blood volume and the absolute number of erythrocytes of one generation changes as development proceeds For this reason we suggest the following procedure for each day of incubation the number of recirculations is multiplied by the corresponding absolute number of erythrocytes of one line present in circulation (taken from their survival curve) The sum of these products over the whole incubation period will be divided by the maximal number reached by the erythrocyte line during incubation Thus the average number of recirculations one erythrocyte undergoes will be obtained

For each day of incubation starting with the second, the number of recirculations was calculated from Hughes's[3] data for stroke volume (data published by Hughes are for both ventricles together, and must therefore be gradually reduced starting with the fourth day to one-half from the seventh day onwards because of the development of allantoic circulation) multiplied by heart-rate value[4] The product yielded the cardiac output/min Thereafter the cardiac output values were multiplied by 1,440 min (24 hr) and divided by the corresponding blood volume[5]. So the average number of recirculations of the entire circulating blood/day of development was estimated The average number of recirculations in a day's interval increase from 362 between the second and third day of incubation to 6,145 between hatching (20th day) and the first post-embryonic day (Fig 1*a*) Because the results on the stroke volume[3] cover the period from day 2 5 to day 17 only, the border values on both sides have had to be extrapolated (dotted line in Fig 1*a*). The error of this extrapolation is, however, of little bearing because the corresponding values in both survival curves (Fig 1*b*, *c*) are low

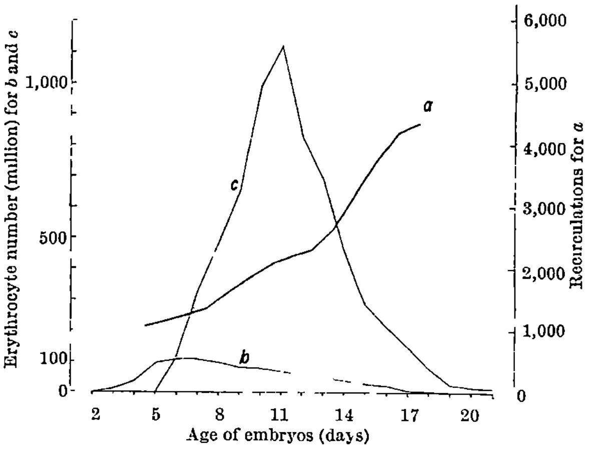

Fig. 1 (*a*) Number of recirculations from the second day of incubation until the first postembryonic day, survival curves of primitive (*b*) and definitive erythrocytes with round nuclei (*c*) during the same period

Both survival curves (absolute number of erythrocyte lines concerned present in circulation) are approximately of triangular shape and are practically limited to the embryonic period That of the primitive erythrocytes[6], the first generation of erythrocytes (E I) starts on the second day of incubation with a maximum on the seventh day, while that of the definitive erythrocytes with round nuclei[7], the second generation of embryonic erythrocytes (E II$_1$), which appears as late as on the fifth day, reaches its maximum on the eleventh day

From these results the average number of recirculations for a primitive erythrocyte was calculated to be 14,452 and quite a similar number (13,935) was found for a definitive erythrocyte with round nucleus These values are most probably maximal, because the data for stroke volume probably err, according to Hughes[3], on the high side That means that the average number of recirculations of two generations of chick embryo erythrocytes is considerably lower than in hens. being less than 10 per cent of adult values This is not proportioned to the ratio of average erythrocyte life-spans concerned—30 days in hens[8] as compared with 8 5 days[6] for E I and 5 7 days[7] for E II$_1$ respectively in chick embryos. This indicates that there will be perhaps some other factor or factors besides that of the metabolic wear connected with ionic fluxes across the erythrocyte membrane as suggested by Allison, which play their part in the extremely short life-spans of chick embryo erythrocytes. One of these factors might be the high degree of immaturity at which the chick embryo erythrocytes are poured out into the blood stream[9].

A full account of our calculations is to be published elsewhere[2].

Future investigations will show if this relatively small number of recirculations holds good also for mammalian primitive erythrocytes. There are indications that their average life-spans are also about one-quarter of that found in adult mammals[10].

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PHARMACOLOGY

Stimulation of Uptake of Calcium-45 in the Adrenal Gland by Acetylcholine

RECENTLY, Douglas and Rubin[1] have suggested that acetylcholine, the physiological transmitter of sympathetic nerve effects at the adrenal medulla, evokes catecholamine secretion by causing calcium ions to penetrate the medullary cells. Our experiments with calcium-45 have now provided further evidence consonant with this idea.

Adrenal glands from nine cats were perfused *in vitro* with Locke's solution. One gland from each animal was used to determine 'resting' uptake of calcium and the contralateral gland stimulated with acetylcholine. Both glands were perfused with Locke's solution containing calcium-45 for about 10 min during which time one was given acetylcholine in a concentration (10^{-5} gm./ml.) sufficient to cause a brisk secretion of catecholamines. Thereafter, they were perfused with Locke's solution containing only calcium-40 and measurements were made of the efflux of calcium-45. Loss of calcium-45 from the perfused glands was at first very rapid, but it slowed after about 50 min and the decline in activity in the glands thenceforth followed a simple exponential course. It was assumed, on the basis of the work of others on muscle[2,3], that by this time the extracellular calcium-45 had been completely washed out. One hundred minutes after beginning the washout, stimulated and unstimulated glands were separated into medulla and cortex and their radioactivity determined.

The glands exposed to acetylcholine contained more radioactivity than the unstimulated glands. On a weight basis, the medulla was several times more active than the cortex. Comparison of the stimulated and 'resting' medullæ indicated that during perfusion with acetylcholine the rate of calcium uptake was increased about eight-fold. A number of similar experiments were carried out with potassium, which is also known to evoke adrenal secretion. Potassium (56 m*M*) also caused an increased uptake of calcium-45, with the medulla again showing the greatest rate of uptake. This stimulant effect of potassium on calcium influx persisted in the presence of hexamethonium sufficient to suppress indirect effects due to stimulation of nerve endings present in the gland, and was presumably due to depolarization of the medullary cells.

We observed incidentally that acetylcholine and potassium also increased the rate of uptake of calcium-45 by the cortex, although to a lesser extent. This effect was too great to be accounted for by incomplete separation of medulla from cortex. It may be related to a stimulant effect of acetylcholine on adrenocortical secretion, such as Rosenfeld[4] observed in perfused calf adrenals.

The finding of increased uptake of calcium during exposure to acetylcholine or potassium is consistent with the idea that acetylcholine evokes catecholamine secretion by causing calcium ions to penetrate the adrenal medullary cells and that this penetration may be brought about by depolarization[1]. The finding also extends the remarkable parallelism previously noted[1] between the two sets of evidence implicating calcium in 'stimulus-secretion coupling' at the adrenal gland and in 'excitation-contraction coupling' in muscle.

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Pharmacodynamical Effects of Asarone and β-Asarone

DANDIYA *et al.*[1,2] have determined the effect on the central nervous system of *Acorus calamus*, a drug used in the Indian system of medicine for various ailments including some of the mental disorders. In the present work two fractions 'asarone' and 'β-asarone' (isolated by Baxter and Kandel) of the volatile oil of *Acorus calamus* have been investigated with regard to effects on the central nervous system and other pharmacodynamical actions which these drugs have.

The prolongation of hypnotic activity of (i) pentobarbitone, (ii) hexobarbitone, and (iii) ethanol was investigated in white mice. Asarone and β-asarone were given in different groups of animals intraperitoneally in doses of 50 mgm./kgm., followed 15 min later by one of the anæsthetic agents mentioned. The sleeping time was recorded in the manner described in refs. 1 and 2. It was observed that both asarone and β-asarone significantly ($P < 0.01$) prolonged the sleeping time of all the anæsthetic agents mentioned, for example, the mean sleeping time due to pentobarbitone alone, of 101.6 ± 14.5 min., was raised to 254.5 ± 18.8 min. when the animals were pretreated with β-asarone and to 206.4 ± 28.3 min. when the animals were pretreated with asarone.

It was also observed that the hypnotic potentiating property of β-asarone was significantly more ($P < 0.05$) than that of asarone in these doses against all these anæsthetic agents. Asarone or β-asarone in these doses exerted no effect on the spontaneous motor activity of the animals in contrast to the increased motor activity observed with all the anæsthetic agents mentioned However, the spontaneous activity of untreated animals was definitely reduced by asarone or β-asarone

Both these agents caused a significant fall in rectal temperature of mice ($P < 0\ 01$) The fall was maximum in the first 30–45 min and lasted 2–3 hr and the recovery to the normal was attained in about 5 hr

Anticonvulsant action of asarone and β-asarone was studied in ninety albino rats Convulsions were induced by electro-shocks, or 'Metrazol' or 'Picrotoxin' It was seen that β-asarone in an intraperitoneal dosage of 20 mgm /kgm failed to influence the electro-shock seizures Asarone in a dose of 20 mgm / kgm intraperitoneally caused reduction in the incidence, duration and severity of extensor tonic phase induced by electric shocks Asarone in a dose of 50 mgm /kgm gave protective action against shocks induced by 'Metrazol' when prevention of extensor tonic spasm and death were considered as the protective end-points The occurrence of both these end-points was markedly reduced in animals treated with asarone β-asarone, on the other hand, potentiated the lethal action of 'Metrazol' However, both the compounds potentiated convulsions induced by 'Picrotoxin' β-asarone itself in 50 mgm /kgm doses intraperitoneally caused mild generalized myoclonic convulsions in rats, this property was lacking in asarone

Effect on the spontaneous behaviour pattern was studied in eight cats according to the method in ref 3 Both these compounds reduced the sociability score Similar results were obtained with other tranquillizers like reserpine and chlorpromazine The influence on conditioned escape and avoidance responses on 44 rats was investigated The rats were trained to develop these two reflexes by the method in ref 4 (with slight modifications) It was seen that the escape phenomenon (the rat jumping out of the cage even before the presentation of the conditioned stimulus, namely, bell) was abolished in 25 per cent of the trials and conditioned avoidance behaviour was abolished in 10 per cent of the trials, after both asarone and β-asarone This percentage loss in these two reflexes was significant as compared with control data

Frog heart perfusion experiments revealed that both asarone and β-asarone cause stoppage of heart in diastole in quite low concentration However, β-asarone appeared to be more potent than asarone

Isolated pieces of rabbit intestine were fixed for investigating the influence of these compounds on the longitudinal movements and the tone of the intestine It was seen that both asarone and β-asarone reduced the tone and caused diminution in the longitudinal movements β-asarone was found to be much more potent than asarone Rhythmicity of the isolated uterine strip was reduced by both these agents

Anti-acetylcholine actions for both these agents were investigated on the following preparations (1) isolated guinea pig ileum, (2) isolated uterine strip of rat, (3) dog's tracheal muscle preparation, and (4) frog rectus Both asarone and β-asarone were found to have anti-acetylcholine actions on these preparations and here, too, β-asarone was more potent than asarone

So both asarone and β-asarone possess many pharmacodynamical actions quite similar to some of the well-established tranquillizers β-asarone appears to be more potent than asarone and the influence of these compounds on electro-shocks and 'Metrazol' shocks differs β-asarone itself causes generalized convulsions and potentiates the 'Metrazol' seizures, while asarone has a definite tendency to protect against 'Metrazol' convulsions and modify electro-shocks

The investigation was partly financed by the Indian Council of Medical Research, New Delhi

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HÆMATOLOGY

Haptoglobin and Transferrin Types in Peruvian Indians

RECENT reports on the incidence of the serum haptoglobin types of American Indians have included observations on natives of Alaska[1], south-western United States[2,3], southern Mexico and Guatemala[4], Venezuela[5], and Chile[3] The incidence of the Hp^1 gene, shown in Table 1, is usually higher than that found in the population of western Europe[4] (gene frequency 0 32–0 43, mean about 0 39) In several tribes, the occurrence of Hp^1 is as frequent as that reported in the various Bantus of Africa[4] (0 49–0 87) This finding is of particular interest when compared with the low incidence of Hp^1 (0 09–0 28) in Asiatic populations[2,6] Since genetic drift is often responsible for marked fluctuations in the concentration of given genes in comparatively isolated areas, more observations are necessary to confirm these apparent differences We have investigated the haptoglobin (and transferrin) types of two Peruvian Indian groups

Plasma specimens were obtained from 56 Aymara-speaking Indians of Jaillihuaya, who have little, if any, European or African admixture, and an additional

Table 1. DISTRIBUTION OF HAPTOGLOBIN TYPES IN NORTH, CENTRAL AND SOUTH AMERICAN INDIANS

Population	No	Haptoglobin phenotype (per cent)				Hp^1 gene frequency
		1–1	2–1	2–2	0*	
Alaska (ref 1)						
North Athabascan	202	17 3	48 5	31 2	3 0	0 42
Tlingit	82	19 5	46 3	31 7	2 5	0 44
South-west U S A						
Apache (ref 2)	98	34 6	48 0	17 4	0	0 59
Navajo (ref 3)	267	21 3	46 1	32 6	0	0 44
Central America (ref 4)						
Non-Mayan	189	32 8	49 2†	17 9	0	0 57
Mayan	418	33 4	50 2†	15 8	0 6	0 59
Lacandon	31	77 4	12 9	0	9 7	0 93
South America						
Venezuela (ref. 5)						
Hybrids	208	27 4	54 8	17 8	0	0 55
Irapa	129	0 8	39 5	59 7	0	0 21
Paraujano	114	28 6	49 6	21 8	0	0 53
Macoita	74	16 2	54 1	29 7	0	0 43
Maquiritare	59	45 8	30 5	23 7	0	0 61
Chile (ref 3)						
Araucanian	34	58 8	32 4	8 8	0	0 75
Peru						
Aymara	56	50 0	42 9	7 1	0	0 71
Quechua	117	54 7	38 5	6 8	0	0 74

* No visible haptoglobin, or unclassified
† Includes two examples of *Hp* 2–1 (mod)

117 specimens from the Quechua-speaking Indians of Paucarcolla From work on their blood groups, these subjects were calculated to have a White admixture of about 1 1 per cent and a Negro admixture of about 14 6 per cent[7,8], although no aberrant hæmoglobins or deficiencies in glucose-6-phosphate dehydrogenase were encountered[9] The details of collection and distribution of samples have already been described[8]

Starch-gel electrophoresis was performed by Smithies' method[10], using Poulik's discontinuous buffer system[11] and a staining procedure outlined elsewhere[12]

No difficulties in interpreting the haptoglobin patterns were encountered, since all specimens contained adequate quantities of the hæmoglobin-binding protein

There were no unusual phenotypes, such as the *Hp* 2–1 (mod) type which is a characteristic of 10 per cent of American Negroes[13]

Transferrin phenotype *C* was found in all the subjects except one, whose phenotype was B_2C Transferrin CD_1, which occurs in about 10 per cent of American Negroes[14], and the recently described $B_{0-1}C$ type[3], found in about 8 per cent of Navajo Indians, were not encountered

The haptoglobin phenotype distribution in the two Peruvian groups is compared in Table 1 with data on other Indians of North, Central and South America The marked elevation in Hp^1 gene frequency cannot be considered to be due to Negro admixture in the Aymara-speaking Indians, and Negro admixture could only account for a moderate degree of elevation in the Quechua

It has been suggested[4] that an increasing frequency of the Hp^1 gene in native populations occurs in the following order Asia, Alaska, North America, Central and South America That this apparent cline might reflect some regional selective advantage(s) is an attractive hypothesis. Further work on these and other populations may eventually lead to the elucidation of mechanisms responsible for the maintenance of haptoglobin polymorphism throughout the world

This work was supported, in part, by U S. Public Health Service Grant *H*-5780.

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Serological Evidence of a Chromatographic Sub-fractionation of Human 7S γ-Globulin

PREPARATIONS consisting of the electrophoretically slowest migrating part of the 7*S* γ-globulin were prepared by means of preparative zone electrophoresis Individual preparations were obtained from normal serum and from the serum of an *Rh*-immunized individual Preparations from pooled plasma were obtained from commercially available Cohn fractionated γ-globulin (supplied by AB KABI, Stockholm) All preparations showed *Gm*(*a*+) character when tested in a serum-grouping system according to Grubb[1,2]. On immunoelectrophoresis with different rabbit antisera against human whole serum (supplied by Behringwerke, W Germany) the γ-globulin preparations showed the character of 7*S* γ-globulin No β_{2A} or β_{2M} was present

These 7*S* γ-globulin preparations were subjected to hydroxyl apatite chromatography according to Hjertén[3]. The protein preparations dissolved in 0 02 mole phosphate buffer, *p*H 7 9, were applied to columns equilibrated with the buffer The protein was eluted with the same buffer by a stepwise increase of the molarity of 0 03–0 65 As a rule all the protein was eluted in the steps up to 0 09 *M* After dialysis against buffered saline (*p*H 7 2) and adjustment of the sample by ultrafiltration to equal protein concentration (as determined by a modified Folin method and by the Kjeldahl method), the sub-fractions were analysed and compared in the following way

Immunoelectrophoresis revealed a somewhat higher electrophoretic mobility of the fractions eluted by phosphate buffer of lower molarity than of fractions eluted at higher molarity Furthermore, the former type of γ-globulin showed a double precipitin line consisting of two discrete parallel lines, while the latter type showed a sharp single precipitin line (cf Heremans[4]) This difference was more or less pronounced depending on which antiserum was used

As already pointed out the γ-globulin starting materials used in the experiments were known to inhibit agglutination in a *Gm* grouping test system This activity was now tested in the sub-fractions obtained by the hydroxyl apatite chromatography The agglutinating system used consisted of constant amounts of *O Rh*(+) human red cells sensitized by incomplete *Rh*-antibodies and a rheumatoid serum of anti-*Gm*(*a*) character The γ-globulin sub-fractions, of equal protein concentration, were added in two-fold serial dilutions to the agglutinating system In all cases a higher agglutination-inhibiting activity was

Table 1 SEROLOGICAL ACTIVITY, AT EQUAL PROTEIN CONCENTRATION, OF 7 *S* γ-GLOBULIN SUB-FRACTIONS FROM HYDROXYL APATITE COLUMNS ELUTED AT DIFFERENT MOLARITY OF PHOSPHATE BUFFER (*p*H 7 9) EXPRESSED AS (TITRE)$^{-1}$

Fraction eluted at molarity	*Gm*-agglutination inhibition*	Sensitizing activity towards *O Rh*(+) red cells revealed by Anti-γ serum†	Sensitizing activity towards *O Rh*(+) red cells revealed by Rheumatoid arthritis serum†
0 04	4	—	—
0 05	16	—	—
0 06	32	1	—
0 07	128	2	1
0 08	64	4	2

* To an agglutinating system consisting of *O Rh*(+) red cells sensitized by incomplete anti-*Rh* antibodies and diluted rheumatoid arthritis serum of anti-*Gm*(*a*) character two-fold dilutions of the different *S* 7 γ-globulin sub-fractions were added

† A constant amount of *O Rh*(+) cells was added to two-fold dilutions of the different 7 *S* γ-globulin sub-fractions After incubation at 37° for 2 hr and subsequent washing, the agglutinability of the cells was tested with a rabbit anti-human γ-globulin serum and with the anti-*Gm*(*a*) rheumatoid arthritis serum

found in the sub-fractions eluted by buffers of higher molarity than in fractions eluted by buffer of lower molarity. In the different experiments, the largest difference in this respect between sub-fractions originating from the same γ-globulin preparation varied 2–5 titre steps.

The possibility that this difference could be explained by an enrichment in the more active sub-fractions of aggregated 7*S* γ-globulin, which may have a high agglutination-inhibiting activity in the *Gm* grouping test system[5], was excluded by the following experiment. γ-Globulin sub-fractions obtained by hydroxyl apatite chromatography were submitted to zone ultracentrifugation in a sucrose gradient. The top fractions in this way freed from any fast-sedimenting 7*S* γ-globulin aggregates were recovered and tested in the *Gm* grouping system. The same difference in agglutination inhibition was observed as before ultracentrifugation.

The serological investigation of the γ-globulin sub-fractions of the serum containing incomplete *Rh*-antibodies is shown in Table 1. Besides the differences mentioned above, an enrichment of the *Rh*-antibodies in the sub-fractions eluted by buffer of high molarity was observed.

These observations thus indicate that hydroxyl apatite chromatography of human 7*S* γ-globulin may give sub-fractions differing in serological character.

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PATHOLOGY

Intracellular Response to an Iso-immune Reaction at the Surface of Ascites Tumour Cells

THE cytotoxic effect on mouse ascites tumour cells treated *in vitro* with various antibodies has been shown to require the presence of complement[1,2]. In the absence of complement the cells appear to be normal when viewed by phase contrast microscopy[3], they retain their capacity to exclude trypan blue[3] and to metabolize glucose[4]. The only changes which have been reported are slight alterations in the fine structure of the cell surface, which were thought to favour agglutination by rabbit antibody directed against ascites cells[3].

There is strong evidence, from hæmagglutination work, that some iso-antigens of the *H*-2 system[6] are present on the surface of mouse erythrocytes[5]. The present investigation was undertaken to determine whether such iso-antigens could be demonstrated on the surface of *EL*4 ascites cells, and whether histochemical methods could be used to reveal the effect on the cell of an iso-immune reaction at its surface, in the absence of complement.

Iso-antisera were prepared in *BALB/c* strain mice against *EL*4 tumour cells resident in *C57BL* strain mice by immunization with tumour cells mixed with Freund's complete adjuvant. The mice and tumour were the generous gift of the late Dr. P. A. Gorer. The cytotoxic titres[2] of these antisera, in the presence of complement, varied from 1/64 to 1/256. In the absence of complement, iso-antibody caused negligible agglutination of cells even when present at as high a concentration as 1 vol. antiserum : 1 vol. cell suspension containing 2×10^7 cells/ml. This was in marked contrast to the strong agglutinating effect of rabbit antibody which was observable even with rabbit antiserum diluted 256 times.

It was first necessary to confirm that the mouse iso-antibody did react with surface antigens. This was effected by incubating suspensions of *EL*4 cells with mouse antiserum (preheated for 30 min. at 56° C.) in the absence of added complement. That the antibody was located exclusively on the cell surface was observed by subsequent staining of air-dried smears of washed cells with a rabbit anti-mouse globulin which had been conjugated with fluorescein isothiocyanate.

It was remarkable that in these circumstances, which were expected to produce purely surface effects, marked cytoplasmic changes were observed when histochemical techniques were applied to air-dried smears. Thus, exposure of the cells to iso-antiserum alone, at 37° C. for 15 min., in the absence of complement, resulted in marked activation of lysosomal acid phosphatase[7]; there was also simultaneous unmasking of cytoplasmic phospholipid, particularly in discrete granules, as shown by the acid hæmatin technique[8]. The stimulation of glucose dehydrogenase activity observed under similar conditions could have been a non-specific effect since it was also observed after incubation of these cells with normal serum.

The immunofluorescent results indicated that this iso-antibody acted solely at the surface of the cells under these experimental conditions. If this were true, then the effect of the immune reaction at the cell surface in causing changes in intra-cytoplasmic particles was surprising.

In homograft rejection, circulating antibody may act synergically with immunologically competent host cells, though the extent to which complement is involved is uncertain. It is possible that intra-cytoplasmic effects of iso-antibody acting at the donor cell surface may play some part in the mechanism of rejection.

We are indebted to Prof. E. G. L. Bywaters, Dr. L. E. Glynn and Dr. E. J. Holborow. One of us (L. B.) wishes to thank the Trustees of the Prophit Fund for a research studentship.

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A Carcinogenic Action of Testosterone, provoking Uterine Tumours in Mice

THE literature concerning the induction of tumours by hormones is very extensive[1-4]. Œstrogens especially appeared to play an important part in hormonal carcinogenesis[5]. Evidence of a carcinogenic action of androgens is very scant. Horning[6] observed 3 theca-cell ovarian tumours in rats and 2 adrenal cortical tumours in hamsters after long-term treatment with testosterone. Also, Kirkman[7] suggested that testosterone propionate may act as a non-specific carcinogen in the hamster.

In our experiments one pellet of 1–2 mgm. testosterone propionate was subcutaneously implanted into female mice of the $F1$ hybrid ($C57BL \times dba$) twice a week. The treatment was started at the age of 1½–3 months and continued until the mice had to be killed because of their bad condition.

At post-mortem examination a surprisingly high percentage of uterine tumours was found (Table 1).

Table 1. UTERINE TUMOURS INDUCED BY TESTOSTERONE

	No. of mice*	No. of mice with tumours	No. of mice with lung met.	Average survival time (months)†
♀♀ F_1 (*BD*)	14	6	3	18·8
♀♀ F_1 (*BDf*)	16	9	3	14·7
♀♀ F_1 (*BDf*)	12	11	4	15·3

* Surviving the testosterone treatment for 10 months or more.

† No difference was found in the survival time of the tumour-bearing and the non-tumour-bearing mice.

In 26 of the 42 mice treated with testosterone uterine tumours were observed, and in 10 of the tumour-bearing mice metastases were present in the lungs.

Most of these tumours were found either in the uterine cervix or in the unpaired uterine part. Only a few tumours were located in the horns of the uterus. The size of these tumours varied considerably. The largest diameter was about 25 mm. and the smallest 4 mm. The tumours were always strictly limited to the uterus and the vagina and no other tissue of the peritoneal cavity was involved. The surfaces of the cut tumours showed mostly a pink colour, but sometimes necrotic or hæmorrhagic areas were seen.

The histological pattern of the testosterone-induced uterine tumours was very characteristic and similar for the several tumours we have observed. In most of these tumours large trabeculæ of cells were found next to more solid parts. The trabeculæ formed locally branched papillæ inside large sinuses. Most of the tumour cells had a diameter varying from 20 to 30µ and contained an abundant fine granular cytoplasm which stained mostly basophilic. The nuclei were large and relatively clear with prominent nucleolus (Fig. 1).

Most of the tumours showed an infiltrative growth in the muscular layers of the uterine wall. This fact together with the appearance of lung metastases are distinguishing marks of the malignancy of these tumours.

In most of the mice bearing a uterine tumour as well as in those without a gross uterine tumour one or more small pink nodules were observed in the uterus. Macroscopically and microscopically these nodules showed close similarities to the gross uterine tumours. We think we are justified in considering these nodules as stages preceding the malignant uterine tumours.

These nodules developed in the stroma of the endometrium. The epithelium and the muscular layers were not involved. The origin and the histological aspects of the nodules are similar to the histological changes in the stroma of the endometrium during the first stages of pregnancy. On these similarities we base our hypothesis that the uterine tumours induced by testosterone are related to the decidua cells. The histological characteristics of these specific uterine tumours differ from the uterine carcinomas and uterine sarcomas so far described.

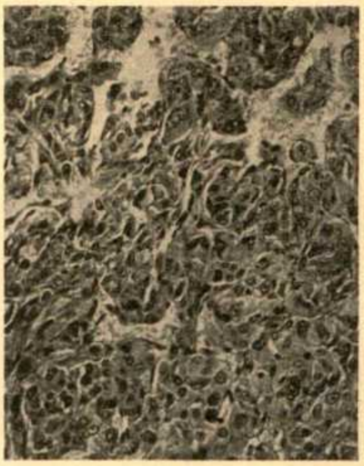

Fig. 1. Histological picture of a uterine tumour. (× 200)

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Deiodination of Thyroxine-^{131}I in Tumour-bearing Rats

IT has been shown that hyperthyroidism induced by the administration of large doses of triiodothyronine increased the radiosensitivity of tumours in rats and in human beings[1,2]. Later it was found that hyperthyroidism induced in this way significantly enhances the growth of a solid non-metastatic myeloid leukæmia tumour of the Sprague–Dawley rat, a rat fibrosarcoma of a local strain and the Ehrlich mouse ascites tumour[3].

In view of the profound effect of severe hyperthyroidism on tumour growth and its response to radiation, it became of interest to investigate the turnover of thyroxine in animals bearing these tumours.

The rate of deiodination of thyroxine was investigated by administration of a tracer quantity of labelled thyroxine and the amount of the inorganic iodide formed was determined by radioactive assay. To ensure complete retention of the iodide formed in the circulation, rats were surgically thyroidectomized 48 hr. before the turnover experiment. Tracer thyroxine, labelled with iodine-131 in the 3′ and 5′

positions, was injected into the vena cava following a bilateral nephrectomy. Blood samples were collected into heparinized test-tubes at 45-min. intervals for 5–8 hr. The serum was then separated and the iodine bound to protein was determined[4]. From the total values of serum iodine and protein-bound iodine the serum iodide concentration (per cent of injected dose per gm. serum) was calculated. The increase in iodide concentration with time was taken as an index of the rate of deiodination of thyroxine.

In the experiments performed on 150 rats it was found that the rate of deiodination (per cent thyroxine deiodinated per unit time) was higher in the animals bearing tumours than in the respective controls. Since age was one of the major factors affecting the rate of deiodination, all rats selected were of identical age. The differences between the average rates were 50 ± 10 per cent in the leukæmia tumour and 45 ± 10 per cent in the rats bearing fibrosarcoma, with a statistical significance of more than 99 per cent. In hypothyroid animals the difference in the rate of deiodination was even greater, amounting to 80 ± 15 per cent in the animals bearing fibrosarcoma as compared with their hypothyroid non-tumorous controls. In a similar manner old age was found to be another condition in which the difference between tumorous and control animals manifests itself emphatically; a difference of more than 150 per cent in the rates of deiodination was observed here. On the other hand, no significant difference in the rate of deiodination was found between animals with or without tumours in which hyperthyroidism had been induced artificially.

After the deiodination experiments the animals were killed and the specific activity of the tumours (per cent of injected dose per gm. tissue) was compared with that of the gastrocnemius muscle. The specific activity of the tumours was 3–5 times higher than that found in muscle and was comparable with that in the ovaries. A similar finding has been reported in the mouse mammary tumour using triiodothyronine[5].

The weights of the tumours ranged from 5 to 40 gm., constituting 2–25 per cent of the total weight of the rats bearing tumours. However, when the increase in rate of deiodination was related to the weight of tumour in each animal, or to the per cent body-weight constituted by the tumour, no correlation could be established.

Physiological variations between normal and tumour-bearing animals as described here are rare and are worth careful consideration. An increased rate of deiodination is generally associated with the hyperthyroid state, yet no indication of such a state is apparent in animals bearing tumours. As little is known about the mechanism of deiodination of thyroxine at the cellular level, it is premature to put forward an explanation for the enhanced deiodination.

The lack of correlation between the degree of enhanced deiodination and the mass of the tumour strongly suggest that the effect is due either to a change in the hepatic metabolization of thyroxine or to a certain factor of a systemic nature mediated by the presence of the tumour. The tumour thus does not appear to be the site at which the excess deiodination takes place. Should this effect prove to be associated with the presence of malignant tumours in general and should it occur also in human subjects afflicted with cancer, then the demonstration of an increase in the rate of deiodination of thyroxine may be of value for the early diagnosis of malignant growths which are not otherwise detectable.

This research was supported, in part, by the Ann Langer Cancer Research Foundation, Chicago.

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HISTOLOGY

Role of the Thymus in Polysaccharide Metabolism

IT has been shown by a series of experiments[1-8] that the thymus plays an active and significant part in polysaccharide metabolism. It was demonstrated[1,3,4,8] that, unlike other lymphatic organs, stress and processes of tissue proliferation induced the emigration of cells from, and a mast-cell reaction in, the thymus. This reaction was preceded by the appearance of periodic acid–Schiff-positive cysts and of thymocytes containing periodic acid–Schiff-positive matter. It was possible to confirm these findings by demonstrating in tissue cultures[2] that the cells of the thymus showed a marked affinity for heparin[5] and decomposed heparin[2], which was much stronger than that of other cells (liver, spleen, heart, kidney) and even more pronounced than the heparin affinity of the lymph node, which ranks, in this respect, next to the thymus. Although these experiments revealed the prominent role of this organ in the metabolism of mucopolysaccharides, it seemed necessary to measure this activity by means of a more exact method.

It was with an indirect method and the use of isotopes that we investigated the correlation between thymus and acid mucopolysaccharides in the present experiments. Instead of labelled heparin we used labelled thionine (a dye): it combines with heparin *in vivo* so that radioactivity indicates the extent to which thionine is taken up by the organs and thus can be used as an indicator of their heparin metabolism. Thionine was labelled with sulphur-35 in our experiments for which 10 rats of 150 gm. body-weight were used. Each animal received intraperitoneally 4 mgm. of the dye. The animals were killed by a blow on the head 24 hr. after the injection; thymus, lymph node, spleen, liver and heart were removed, weighed and homogenized; radioactivity was then measured with an end-window Geiger–Müller tube and a scaler of type *EMG* 1872. Radioactivity in the blood was ascertained also. Results are shown in Table 1; values are given as percentages of total radioactivity introduced and refer to 100 mgm. of wet tissue.

Table 1

Organ	Radioactivity (percentage of total)	Standard deviation	Result of Student's t test
Thymus	2 52	0 18	Highly significant, $P \leqslant 0\ 1$ per cent
Lymph node	0 152	0 012	Highly significant, $P \leqslant 0\ 1$ per cent
Spleen	0 11	0 011	
Liver	0 12	0 003	
Heart	0 12	0 003	
Blood	0 007		

It can be seen that all tissues investigated, with the exception of the thymus and lymph node, took up approximately the same small quantity of dye This seems to indicate active uptake or the combination of the dye with the existing acid mucopolysaccharides, since radioactivity in the blood was quite negligible. Though quantitatively low, activity was significantly higher in the lymph node, and outstandingly high in the thymus, about 20 times as high as in other tissues

These figures are in good agreement with the results of earlier experiments· they show that the thymus plays a prominent part in the metabolism of acid mucopolysaccharides (of heparin in particular) and furthermore they show that although morphologically similar, the thymus on one hand, and the spleen and lymph node on the other, have different functions and are involved in different biological processes It seems, moreover, that as regards polysaccharide metabolism, there is also a difference between lymph node and spleen; this, too, is in agreement with earlier observations according to which the lymph node is more or less capable, and the spleen incapable, of forming mast cells[2,5]

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Fixation of Muscle

I HAVE investigated recently the effect of various fixation methods on mammalian skeletal muscle For this purpose the biceps brachii muscle of the albino mouse was chosen because of its convenient size and the fact that it can be easily dissected out All the mice used were females weighing about 34 5 gm , the biceps muscles were weighed in each case, and all were found to weigh about 0 0155 gm

The fixatives used were of the normal concentrations, 2 per cent osmium tetroxide being used in Champy's fluid and in Flemming's fixative Following fixation, etc , the paraffin-embedded material was cut at 7 5µ and sections taken through the belly of this muscle were stained with Mallory triple stain The outlines of the fibres were drawn on squared graph paper with the aid of a camera lucida The average cross-sectional area and the standard error were determined in each case from a sample of 25 figures taken at random In the case of fibre measurements of the living muscle, the fibres were teased out in Locke's mammalian Ringer solution, and their diameters were measured immediately As the fibres were seen to be circular in transverse section, their cross-sectional area was obtained by treating them as cylinders Direct cross-sectional area measurements were found to be impracticable because the living material tended to contract on cutting The standard error of the living fibre sample was 106 sq µ The results are represented in the histogram (Fig 1)

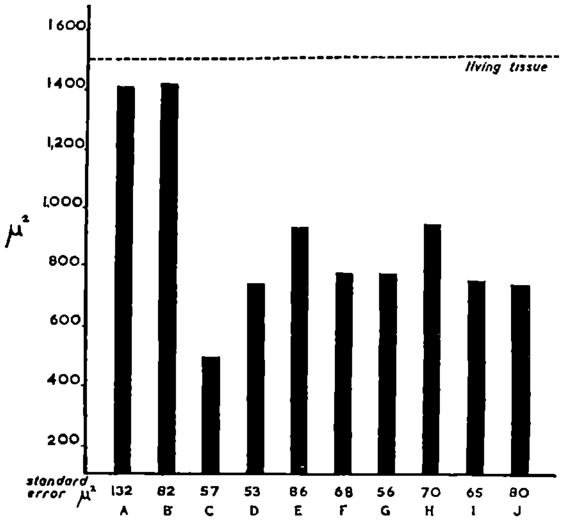

Fig 1 Effect of various fixatives on fibre cross-sectional area (fixation time in parentheses)
A, Champy's fluid (42 hr)
B, Flemming without acetic acid (42 hr)
C, Formol calcium (12 hr)
D, Bouin's (12 hr)
E Carnoy (3 hr)
F, Dalton's (1 hr and postchrome for 6 hr)
G, Neutral formalin, neutralized with sodium hydroxide (48 hr)
H, 2 per cent osmium tetroxide in acetone } freeze substitutions (1 week)
I, Carnoy
J, Methanol

The results of the freeze substitution technique[1] were surprisingly poor, these were repeated but the same extent of distortion occurred Sections of kidney prepared at the same time and in the same way showed no distortion In the case of muscle treated by the methanol freeze substitution technique, extensive breakage of the sarcolemma was noted Champy's fluid and Flemming's without acetic acid are seen to cause little shrinkage, and the results obtained from these two osmium tetroxide fixatives are very close to those for the living tissue, the histological appearance of such sections is extremely good and the muscle spindles are very well preserved It is often not practicable to use osmium tetroxide on a large scale, as it is relatively expensive and has only very low powers of penetration, thus limiting severely the size of the tissue which can be fixed It would seem that a slight shrinkage is inevitable, as the fibres will lose their turgidity on fixation

The other fixatives caused great distortion This could be readily seen because of the polygonal shape of fibres in transverse sections as compared with the spherical or ovoid shape of those fixed in Flemming without acetic acid, Champy's fluid, and the living fibres This distortion is probably due to a chemo-shortening of the fibrous connective tissue around the bundles

In the light of these results, it seems most unfortunate that much of the work on the shape and size of muscle fibre has been carried out on material fixed in formalin

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RADIOBIOLOGY

Detecting Reversion in T4rII Bacteriophage to r^+ induced by Ultra-Violet Irradiation

DURING investigations into the mechanism of ultra-violet mutagenesis in extracellular phage, the following test was developed for estimating the extent of reversion of bacteriophage *T4B* from *r*II to r^+ induced by ultra-violet irradiation.

All *T4r*II mutants were obtained in this laboratory by ultra-violet irradiation of $T4Br^+$ phage 12 hr cultures of *Escherichia coli B* and *K*–12 (λ), obtained in 1958 from E Freese, were used in assays of *r*II and r^+ phage respectively Agar media were those of Chase and Doerman[1], broth media contained, per litre ion-free water, 10 gm tryptone, 8 gm sodium chloride, and 1 gm glucose Phage were diluted and irradiated in this broth All assays were performed by the agar layer method of Adams[2] To detect as few as 20–30 r^+ phage among 5×10^8 *r*II phage the concentration of bacterium *K*–12 (λ) per soft agar tube approached 2×10^9

A 15-watt 'Sylvania' 17-in low-pressure germicidal lamp was used to irradiate all samples at a distance of 67 cm. and at 4° C Phage samples were distributed as two-drop aliquots on glazed white porcelain spot test plates which were agitated during exposure Irradiated phage were handled in dim light to avoid photoreactivation Under these conditions a standard inactivation curve, graphed as the logarithm of percentage survivors against ultra-violet dose, was linear to 0 001 per cent survivors, 55 sec of ultra-violet irradiation resulted in a survival-level of e^{-1}

Each of 12 *r*II mutant stocks was assayed on *B* and on *K*–12 (λ) to determine accurately the titre of total and of r^+ phage Triplicate samples of each phage stock were irradiated to various survival-levels as indicated in Table 1 Two to three aliquots of these samples were then assayed on bacterium *K*–12 (λ) to obtain the titre of r^+ phage (background plus ultra-violet-induced mutants) Remaining aliquots of these samples were assayed on bacterium *B* to provide the exact survival-level

Results are presented in Table 1 Clearly 5 of the 12 mutants revert to r^+ when irradiated with ultra-violet The extent of reversion ranges from 1 81 $r^+/10^6$ survivors to $4 2 \times 10^5$ $r^+/10^6$ survivors Two other mutants (*UF*54 and *UF*147) may be weakly revertible but cannot be definitely classed as such since the proportion of r^+ phage per 10^6 surviving phage is so low as to make an accurate assay of their number unfeasible by this technique

Control experiments by Krieg[3] ruled out the possibility that the observed increase in r^+ phage might be due to ultra-violet-induced selection for r^+ phage present in the stocks before irradiation

This technique for detecting reversion differs from Krieg's[3] in the following points (1) Ultra-violet-irradiated phage are directly assayed for their r^+ content with no intervening cycle of phage growth (2) Our average number of lethal hits per phage was considerably lower than Krieg's[3], yet reversion was still demonstrable (3) Our multiplicities of infection of bacterium *K*–12 (λ) with ultra-violet-irradiated phage during r^+ assay were of necessity less than 1 Krieg's[3] multiplicities of bacterium *B* during the first cycle of ultra-violet-irradiated phage growth were considerably greater than 1, and lead to multiplicity reactivation The results presented here lead one to surmise that multiplicity reactivation is not a prerequisite occurrence for the ultra-violet-induced reversion of *r*II to r^+ phage

Table 1 EXTENT OF REVERSION OF *T4r*II TO r^+ INDUCED BY ULTRA-VIOLET RADIATION

Mutant	*n* where $e^{-n} = S/S_0$	Stock titre $\times 10^9$	r^+_s	r^+_s (S/S_0)	Total r^+ after ultra-violet irradiation	r^+ ultra-violet per 10^6 *S*	Reversion group
*UF*2	0 60	5 6	970	533	360	0	*NR*
*UF*2	9 9	5 6		0 003	0	0	
*UF*54	0 97	4 8	470	199	290	0 06	*NR*
*UF*54	6 3	4 8		0 8	0	0	
*UF*147	0 88	2 0	805	338	630	0 35	*NR*
*UF*147	5 3	2 0		4 03	1 plaque	—	
*UF*125	0 57	8 3	1,670	950	1,180	0	*NR*
*UF*125	7 4	8 3		1 01	2 plaques	—	
*UF*211*A*	1 6	15 0	6,150	1,230	1,010	0	*NR*
*UF*211*A*	7 7	15 0		1 35	0	0	
*UF*344*A*	3 4	10 0	10,700	353	950	1 81	*Rw*
*UF*165	1 9	10 0	3,300	495	480	0	*NR*
*UF*114	3 0	2 5	1,010	50 5	300	2 01	*Rw*
*UF*144	13 0	6 0	$1 7 \times 10^7$	49 4	$6 84 \times 10^8$	$4 2 \times 10^5$	*Rs*
*UF*173	5 8	13 0	$1 1 \times 10^6$	$3 2 \times 10^2$	$3 5 \times 10^3$	83	*Rs*
*UF*518*B*	8 0	14 0	$1 1 \times 10^7$	$3 8 \times 10^3$	$3 7 \times 10^4$	$7 44 \times 10^2$	*Rs*
*UF*50	0 39	2 1	$0/10^9$	0	0	0	*NR*

n, Average number of ultra-violet 'hits' per phage where $e^{-n} = S/S_0$ S/S_0, percentage of survivors, r^+_s, titre of r^+ phage present per ml, phage stock before irradiation, r^+, ultra-violet, total r^+ after ultra-violet $- r^+ (S/S_0)$ Reversion group *NR*, non-reverter, *R*, reverter *s*, strong, *w*, weak

The possibility is not ruled out that, when present, multiplicity reactivation may of itself lead to the production of revertants

Different *r*II mutants appear by this simple test to differ appreciably in their ability to be induced to revert by ultra-violet-irradiation Among the 5 *r*II mutants which could be induced to revert, the extent of reversion ranged from 1 81 to $4 2 \times 10^5$ r^+ per 10^6 surviving phage It is interesting that all those mutants which are most easily reverted by ultra-violet irradiation are also high-frequency spontaneous reverters The compartmentalization of this small sample into ultra-violet-revertible and ultra-violet-non-revertible is suggestive of some specific mutagenic effect of ultra-violet light upon free phage Investigations concerning this specific ultra-violet mutagenic effect on free phage, from r^+ to *r*II and from *r*II to r^+, will be published elsewhere

This work was supported by a research grant (*G*–14,209) from the National Science Foundation

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Effect of X-Irradiation on Self-Stimulation of the Brain

RECENTLY it has been shown that the brain is sensitive to low doses of ionizing radiation[1,2]. Prolonged exposure to X-irradiation decreases lever-pressing by rats[3]. Conditional aversion to saccharin results from exposure of rats to γ-radiation[4,5]. Because radiation affects psychological tests based upon food or fluid intake, we studied the effects of X-irradiation on rats trained to press a lever for electrical stimulation of the brain[6]. A single electrode was permanently implanted in the posterior hypothalamus of 8 *CFN* rats. After a training period of 6 months, the rats received 600 r. acute whole-body X-irradiation. Radiation characteristics were 250 kVp., 15 m.amp., focus-object distance 70 cm., filters, 0·21 mm. copper inherent, 0·5 mm. copper parabolic and 1·0 mm. aluminium, half-value layer 1·92 mm. copper; 22·9 r./min. measured in air.

Calibration of the machine was done with a Victoreen thimble r.-meter and output during irradiation was measured with a Radocon unit. On completion of the experiment, histological sections of the brain were compared with the de Groot atlas[7] to locate the electrode tip.

The responses of the animals can be divided into two groups. Those with high pre-irradiation rates (Fig. 1) had highly significant increases in the rate of lever-pressing on the day of irradiation. This was followed by return to, and a fluctuation within, the control values until the 17–19 post-irradiation day when there was a significant decrease in the rate of lever-pressing.

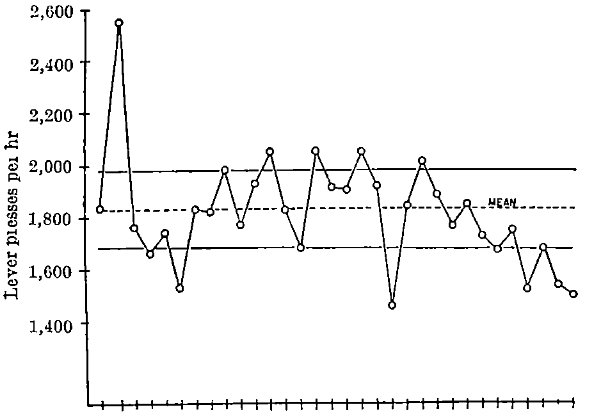

Fig. 1. Response of high-rate lever-pressing rat to 600 r. acute whole-body X-irradiation. The horizontal lines indicate the mean and standard deviation determined over a period of 6 months. The elevation of lever-pressing activity is highly significant ($P > 0{\cdot}001$). *C*, Control; *R*, irradiation day

The other group of 3 animals had low pre-irradiation rates (Fig. 2) and showed highly significant decreases in rate on the first post-irradiation day. These animals had difficulty in again attaining their pre-irradiation rates throughout the 30-day period of observation. Histological examination of the brains revealed that the rate of lever-pressing was not associated with any specific nucleus in the hypothalamus, because both high and low rates were obtained from nucleus arcuatus hypothalamic, nucleus dorsomedialis hypothalamic and area lateralis hypothalamic.

Although the usual transient loss in weight produced by the irradiation was seen, this effect apparently had nothing to do with the changes in performance of both groups of animals because they showed slight increases in weight during the 30-day observation period. These results indicate that self-stimulation of specific nuclei in the posterior hypothalamus may give high or low rates of response depending on the particular nucleus stimulated.

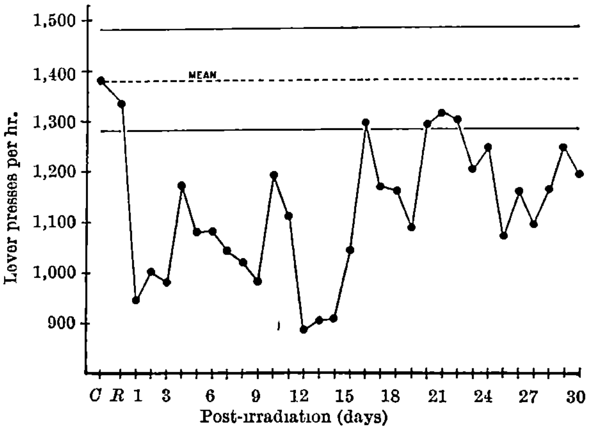

Fig. 2. Response of low-rate lever-pressing rat to 600 r. acute whole-body X-irradiation. The horizontal lines indicate the mean and standard deviation determined over a period of 6 months. The depression of lever-pressing activity is highly significant ($P = 0{\cdot}01$). *C*, Control; *R*, irradiation day

This confirms the observations of Hess[8] on the autonomic and somatomotor responses in the diencephalon, those of Gellhorn[9] on the autonomic areas and also those of Slusher and Hyde[10] on diencephalic control release of adrenocorticotrophic hormone. In all these cases, diametrically opposite actions were obtained by stimulation of similar areas. The depression of lever-pressing activity is much more prolonged than the stimulation of such activity. Thus, it would appear that the nuclei involved in these opposite actions may be directly affected by radiation, although the present experiments do not rule out the possibility that indirect and/or humoral agents from other areas in the body actually produced the effects observed. The question of direct versus indirect effects of the irradiation on the brain as well as the use of other electrode arrangements is now under investigation.

We wish to thank Dr. M. A. Slusher for her assistance. This work was supported by contract *AT* (04-1) *GEN*-12 between the Atomic Energy Commission and the University of California.

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Concentration of Inhaled Cerium-144 in Pulmonary Lymph Nodes of Human Beings

In the course of an investigation of fission products deposited in human lungs[1], we have also measured the radioactivity of pulmonary lymph nodes. In most cases investigated so far, the amounts of fission products found were too small to permit deductions about concentration of these substances in the lymph nodes However, in some cases larger activities were observed and the occurrence of a concentration process could be clearly recognized

Concentration of radioelements in lymph nodes may, in certain circumstances, produce high local radiation dosages In evaluating radiation hazards due to inhaled radioelements it is therefore necessary to find out whether lymph nodes have to be considered as a 'critical organ' Many factors must be taken into consideration in this connexion[2,3], but the data required for this are only partly available so far Data about the concentration of inhaled radioelements in lymph nodes of human beings have been reported only in one case, that of a man who had occupationally inhaled plutonium-containing aerosols for several years[5] Further data on concentration in lymph nodes have been obtained in experiments with animals, chiefly with plutonium and polonium[2,3,5,6]

We wish to report three cases, in which our measurements give information about concentration of cerium-144 in human lymph nodes (Table 1). The lungs investigated were obtained from deceased persons, who had—like all people—inhaled the radioactive aerosol produced in nuclear test explosions in recent years The major part of the cerium-144 detected in our measurements on lungs was probably produced in the high-yield test explosions carried out in 1957 and 1958 Cerium-144 is particularly suited for studying the behaviour of inhaled 'fall-out'-particles for the following reasons (1) This fission product is present in fall-out largely in insoluble form[7] (2) Because of its half-life (285 days) and fission yield, cerium-144 is present in larger activity than other fission products in the period of approximately 1–4 years after formation by nuclear fission (ref 8)

The organs investigated came from older persons (58–81 years), who had lived in Vienna for at least several years and died here After the routine autopsy pulmonary lymph nodes (Lymphonodi bronchopulmonales, bifurcationis, tracheobronchiales, paratracheales) were removed This was facilitated by their dark coloration due to deposited anthracotic pigment, which is commonly present in city inhabitants Lymph nodes and lungs were then ashed separately In the ashes cerium-144 ($E\gamma$ = 134 keV) was determined by gamma-spectrometry using a well-type NaI(Tl)-crystal The spectrum was obtained in detail from 84 to 156 keV by measuring 32 min in each channel of 8-keV width In the cases reported here, the photo-peak at 134 keV was clearly identified We determined the area of the photo-peak above a 'background continuum', due in large part to the Compton continua of other radioelements and environmental radiation, and then calculated the amount of cerium-144 present by comparing with samples of known activity

In the first group of samples listed in Table 1, the lymph nodes from three lungs were combined in order to increase the activity to be measured Thus the concentration ratio observed for this group of samples represents a mean value for three individuals In the two other groups listed, activities of lung and lymph nodes of one individual were compared As shown in the last column but one, the cerium-144 activities found in the lymph nodes were between 1/8 and 1/3 of the activities of the lungs Since the mass of the lymph nodes is quite small, the specific activity (concentration of radioelement) in these organs is many times greater than in the remainder of the lung The concentration ratios calculated are given in the last column Because of the statistical error of the measurements, the values are only approximate, but it is apparent that an increase in specific activity of one to two orders of magnitude has occurred Rather similar values were found in the investigations cited above[3–6].

Table 1. Cerium 144-Contents in Lungs and Lymph Nodes

Date of death	Sample*	Wet tissue mass, W (gm.)	Ash mass (gm)	Date of measurement	Cerium-144 activity of whole sample, A ($\mu\mu$c) and statistical error†	Ratio of specific activities (lymph nodes lung) V‡
	BI/N	45	2 8	14/12/60	16 4 × (1 ± 0 34)	
29/11/60	BH/L	800	7 46	13/12/60	17 0	
30/11/60	BG/L	500	6 68	13/12/60	16 5	
29/11/60	BF/L	1,000	11 39	19/12/60	10 2	
	Sum	2,300			43 7 × (1 ± 0 30)	19
17/ 8/60	BQ/N	11	0 15	13/ 2/61	6 6 × (1 ± 0 69)	
17/ 8/60	BP/L	1,020	10 1	21/ 2/61	52 0 × (1 ± 0 23)	12
12/ 5/60	CG/N	5	0 25	11/ 2/61	5 6 × (1 ± 0 76)	
12/ 5/60	CF/L	1,150	11 8	24/ 2/61	20 0 × (1 ± 0 68)	64

* /N, lymph nodes, /L, lung

† From calibration measurements for cerium-144 1 c p m = 5 1 $\mu\mu$c Formula for calculation of statistical error, σ

$$\sigma = \sqrt{4(A_{88} + A_{152})^2 + (A_{120} + A_{128} + A_{136} + A_{144})^2} \text{ (c p m)},$$

where A_E stands for the counts accumulated (in 32 min) in the channel between (E – 4) keV and (E + 4) keV.

‡ $V = \frac{A_N \quad W_N}{A_L \quad W_L}$

We thank Primaria B Uiberrak (Prosektur "Am Steinhof") for putting the lungs at our disposal, Dr H Houtermans (International Atomic Energy Agency) for calibrating a cerium-144 solution and Mrs Ch. Friedmann for ashing the samples Prof H Hayek, director of the Institute for Anatomy, has kindly advised and supported us

This investigation is being carried out under a research contract with the International Atomic Energy Agency

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Pituitary Effect on Synthesis of Deoxyribonucleic Acid in Regenerating Rat Liver following Whole-body X-irradiation

WHOLE-BODY X-irradiation delivered within 6 hr of hepatectomy will inhibit the synthesis of deoxyribonucleic acid (DNA) in regenerating liver. This inhibition has been elegantly demonstrated to be a result of the failure to synthesize certain enzymes concerned with the formation of DNA[1] The nature of this inhibition of enzyme formation is suggested by two recent reports Myers[2] has reported that synthesis of DNA in regenerating liver is not completely inhibited following X-irradiation of liver only, and Benjamin and Yost[3] have observed that the uncoupling of oxidative phosphorylation seen in liver mitochondria following whole-body irradiation is due to a pituitary stimulation with subsequent activation of the adrenal cortical hormones These observations suggest that this interesting and rather unusual effect of low doses of whole-body X-irradiation on synthesis of DNA in the intact animal may be due to a pituitary stimulation which ultimately results in a decreased amount of energy being available for enzyme synthesis The possibility of a pituitary effect on synthesis of DNA in regenerating liver following whole-body X-irradiation was confirmed in the experiments reported here

Male Sprague–Dawley rats (180–200 gm) underwent partial hepatectomy by a standard method[4], and then were irradiated within 2 hr of the operation The animals were given 15 μc radioactive phosphate intraperitoneally 3 hr before they were killed (see Table 1 for schedule). Following killing, the livers of all animals within a group (2–3) were pooled, the DNA isolated, and the specific activity determined as suggested by Hecht and Potter[5] All irradiated animals received 750 r delivered by a General Electric 'Maxitron 250' operated at 30 m amp , 250 kVp with added filtration of 1 mm aluminium, 0·5 mm copper, delivering 106 r /min at 50 cm Shielding of animals was accomplished in a restraining box covered with 1 5 cm lead located outside the radiation beam

The results of these experiments are summarized in Table 1

Table 1 SPECIFIC ACTIVITY OF DNA ISOLATED FROM REGENERATING RAT LIVER FOLLOWING VARIOUS RADIATION PROCEDURES

Procedures	Time after death (hr.)	Specific activity (c p m /mgm)
Non-irradiated	18	0
Non-irradiated	22	777
Whole-body	22	0
Head only	22	0
Body only	22	38
Body only + 10 units of adrenocorticotrophic hormone	22	0
Body only + 1 unit of thyroid stimulating hormone	22	0

These experiments show that under conditions in which synthesis of DNA is active (19 hr postoperative), 750 r delivered either to the whole body or to the head will suppress synthesis of DNA in regenerating liver Head shielding resulted in a markedly reduced synthesis and the administration of pituitary hormones immediately following irradiation further abolished DNA synthesis The reciprocal experiments were also tried using commercial hypophysectomized rats but none of these animals survived the course of the experiment The conclusion from this series of experiments is that irradiation of the head alone produced the same inhibition of DNA synthesis in regenerating liver as irradiation of the whole body, and that DNA synthesis, although reduced, was present when the head was shielded The reduction of DNA synthesis in animals with their heads shielded may be the result of adrenal stimulation following X-irradiation

In view of the importance of regenerating liver as a system for studying synchronous formation of DNA, these experiments serve as a reminder that contributions from other organs in modifying synthesis of DNA in liver should not be ignored This is especially true in radiation work on synthesis of DNA as well as on population changes in 'radiation-sensitive' tissues

This work was supported by a United States National Health Service grant 3925 (c–2)

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Radioprotective Effects of Reserpine and its *N*-oxide

CERTAIN amine oxides exert a protective effect against radiation injury[1,2] Reserpine has also been shown to have this action[3] This suggested a comparison between reserpine and its *N*-oxide Groups of 20 *CF*–1 mice were administered various doses of the two compounds 24 hr before X-irradiation with 600 r (Table 1) Radiation conditions were the same as described previously[1] Both compounds produced typical reserpine effects, sedation and ptosis, but the *N*-oxide was less active per dose All three doses of reserpine *N*-oxide significantly increased the ST_{50} day, whereas only one dose of reserpine had this effect (Table 1) On the basis of total survival it requires twice as much reserpine as its *N*-oxide to decrease mortality by 25 per cent, and neither compound is as effective as quinoxaline di-*N*-oxide[1] or anhydroerythromycin *N*-oxide[2] Mechanisms of action are probably different, since the reserpine compounds exert no antibiotic effects However, they have the proper chemical structures postulated for removing oxidizing radicals[2] Protection might be partially the result of the release of known protective compounds, adrenaline and 5-hydroxytryptamine[5] The release of catecholamines and 5-hydroxytryptamine is essentially complete 16 hr after reserpine administration, and the binding sites, as well as the plasma content, do not begin to contain appreciable amounts of these amines for at least 4 days[6–9] Metabolism and excretion are also going on at this time, and in all probability the amount of such amines available for exerting a protective effect is below that necessary for such an action On the other hand, reserpine has been found in body tissues for at least 48 hr following a single dose[10]. Most of the reserpine was found in the tissue cells where, in the present radiation experiment, it could act as a protecting compound The fact that reserpine and its *N*-oxide exert only a slight protective effect against

Table 1 ORALLY ADMINISTERED RESERPINE AND ITS *N*-OXIDE AND SURVIVAL AFTER X-IRRADIATION

Treatment	Dose (mgm / kgm)	ST_{50}* and range	Slope and range	Mortality	
				Final (per cent)	Days
Water control		10 0 (8 4–12 0)	1 52 (1 33–1 73)	100	28
Reserpine	10	13 8 (12 0–15 9)†	1 38 (1 24–1 54)	90	30
	15	9 1 (6 8–12 1)	1 95 (1 58–2 39)	95	30
	20	13 0 (10 0–16 9)	1 78 (1 44–2 21)	75	30
Reserpine *N*-oxide	10	15 5 (12 7–18 9)†	1 56 (1 32–1 84)	75	30
	15	13 2 (10 9–16 0)†	1 54 (1 34–1 78)	90	30
	20	13 8 (12 8–14 9)†	1 20 (1 13–1 27)	100	19

* ST_{50} day on which 50 per cent of animals are expected to be still alive

† Differs significantly from control values $P = 0\ 05$ (ref 4)

lethal X-irradiation points out the need for continuing the search for compounds having low toxicity and greater protective effects at the cellular level

We thank Ciba Pharmaceutical Products, Inc , Summit, N J , for the reserpine compounds This work was supported by contract *AT*(04–1)*GEN*–12 between the Atomic Energy Commission and the University of California

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BIOLOGY

Requirement of Essential Fatty Acids and Avian Atherosclerosis

ALTHOUGH Reiser in 1950[1] claimed that chickens needed a source of essential fatty acids during growth, the same author later concluded[2] that mature hens, maintained for a full year on a fat-free diet, did not require essential fatty acids for egg production or hatchability More recently Bieri *et al* [3,4] showed that chicks could be grown to maturity on an essentially fat-free ration without marked deficiency symptoms and suggested that Reiser's purified chick ration may have been deficient in thiamine and vitamin A In contrast with the chicken, exclusion of essential fatty acids from the diet of several mammals leads to a clear deficiency disease which, among more apparent external symptoms, is also characterized by an increase in liver and epidermal cholesterol and a decrease in serum cholesterol in the rat[5], and by aortic lesions in the pig[6]. Such observations lend support to the view of a possible connexion between deficiency of essential fatty acid and atherosclerosis[7] Since atherosclerosis develops in the chicken on low-fat as well as on normal rations[8], it seemed of interest to determine if rigid exclusion of essential fatty acid from the diet would aggravate aortic lesions At the same time more information regarding the requirement of essential fatty acids and deficiency symptoms would be obtained from a group of birds maintained continuously on a diet deficient in essential fatty acids from the day of hatching until 19 months of age

Day-old female chicks (10 per lot) were given either fat-free (albumen-glucose) or normal, fat-containing (about 3 per cent corn oil) rations for 19 months. On the fat-free diets the birds developed less rapidly (Table 1) than on the normal ration, but were otherwise asymptomatic. The early weight differences were largely overcome after 18 months. In our opinion the extreme dryness of the fat-free ration interfered with prehension and deglutition in the birds consuming this diet In order to improve the physical texture of the fat-free diet, starch was substituted for part of the glucose Although this improved the texture and increased feed consumption, it was found by analysis[9] that the starch supplied 0 06 per cent dienoic acid to the ration so that the starch was again removed Acid hydrolysis was used to determine any residual fat since conventional solvent extraction procedures were ineffective

Table 1 EFFECT OF THE EXCLUSION OF ESSENTIAL FATTY ACIDS FROM THE DIET OF CHICKENS

	Diet free of essential fatty acids	Normal diet containing essential fatty acids
Body-weight (gm)		
8 weeks	367 ± 16	695 ± 26
12 months	1,550 ± 74	1,773 ± 91
18 months	1,678 ± 53	1,735 ± 108
Age at first egg (weeks)	24	20
Fatty acid composition of egg lipid (percentage of fat*		
Saturated	30 8	40 9
Monoenoic	66 8	45 9
Dienoic	0 9	9 8
Trienoic	1 4	0 4
Tetraenoic	0 1	1 7
Pentaenoic	0	0 5
Hexaenoic	0	0 9
Plasma cholesterol (mgm /100 ml)	248 ± 23	160 ± 12
Atherosclerosis		
Aorta score †	1 7 ± 0 2	2 1 ± 0 2

* From eggs collected after one full year of production.

† Of the 10 chicks started on each diet there ultimately were 5 and 8 left at the end of 19 months on the fat-free and normal diets respectively Only non-specific causes were responsible for the deaths. For details regarding the scoring technique, see Fisher *et al* (ref 11)

The time of onset of egg production was delayed by one month in the birds on the fat-free diet, as shown in Table 1 Egg production was good, but sporadic, on the fat-free diet, and there was only one bird on this régime that never laid On autopsy, however, this bird was found to have been an internal layer with an active ovary at time of killing Egg production on the conventional ration was somewhat better It is also worth noting that fertilized eggs from the hens on the fat-free ration hatched apparently healthy chicks

Even after one year of egg production the eggs from the birds on the fat-free diet still contained small amounts of dienoic acid, as shown in Table 1 Even when the trace of dienoic acid (supplied by the starch which had been fed for a short time) is taken into account we cannot explain the origin of the dienoic acid in the eggs other than to suggest biosynthesis However, Murty *et al* recently showed that linoleic acid could not be synthesized from acetate by the laying hen[8] Compared with the eggs from birds on the normal ration, the trienoic acid content of eggs from birds maintained on a fat-free diet is almost 3 times as high Such elevated levels of trienoic acid in the tissue lipids have been considered an excellent indicator of the state of deficiency of essential fatty acids in the rat[10]

The plasma cholesterol values of the hens shown in Table 1 indicate a clear elevation in the fat-free versus the normal hens. By contrast, and in agreement with the observation of Horlick *et al.*[8], on a low-fat diet the visual aortic scores were actually lower in the fat-free, compared with the normal birds (Table 1).

In summary, it appears that in the chicken the exclusion of essential fatty acids from the diet does not aggravate atherogenesis even though the level of cholesterol in the blood is elevated. Moreover, no overt deficiency symptoms such as skin lesions or impaired reproductive functions were encountered.

This work was supported, in part, by grants-in-aid from the U.S. Public Health Service and New Jersey Heart Association.

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Anthropometric Correlations between Adult Brothers

ANTHROPOMETRIC measurements have been made on 32 pairs of brothers. All subjects are adult men. The average age of the younger brothers is 32·3 yr., that of older brothers, 39·4. Age differences between members of the same pair vary from 1 to 20 years. The brother–brother correlations are shown in Table 1. Some coefficients are low but, unless below 0·35, they are significant at the 5–1 per cent level. Low coefficients are to be expected because in a panmictic population a brother–brother correlation should not significantly exceed 0·50 (or 0·42 for autosomal dominant characters). Other coefficients are higher but, as for low significant correlations, with two exceptions there is no significant departure from 0·50. For head breadth and cephalic index, correlations are surprisingly high and significantly above 0·50. Such values can be expected if, instead of random mating, there is a fairly high degree of homogamy. In man, assortative mating exists primarily for body-height, secondarily for some characters correlated with stature. In our sample, fraternal correlation for body-height is low and does not differ significantly from 0·50, the limiting value compatible with panmixia. One can guess that assortative mating is directly related to some other anatomical traits, but it is hard to believe that cephalic index, which gives the strongest correlation, can be sexually attractive. A quite different explanation seems to be more credible.

Table 1. CORRELATIONS BETWEEN PAIRS OF ADULT BROTHERS

	This sample r	Howells's data* r
Stature	0·35	0·47
Sitting height	0·50	0·39
Trunk height	0·62	—
Vertex-suprasternale	0·64	—
Neck length	0·32	—
Upper limb length	0·15	—
Lower limb length	0·40	—
Head length	0·54	0·38
Head breadth	0·72	0·48
Head heigth	−0·11	0·49
Cephalic index	0·76	—
Minimum frontal breadth	0·34	0·47
Bizygomatic breadth	0·16	0·29
Face height	0·16	0·59
Nose height	0·27	0·51
Nose breadth	0·44	0·25
Mouth breadth	0·41	—

* Corrected figures published by J. M. Tanner (ref. 1).

Our sample does not represent a definite geographical area. As for many other samples, it is fortuitously mixed, because subjects come from different parts of the country. Two groups of fraternal pairs come from two distinct areas, the populations of which are anthropologically different. One is far more dolichocephalic than the other. In our small sample, these two groups are strong enough to increase the coefficient of correlation. The mingling of two more or less dissimilar groups—panmictic but 'allopatric'—can exaggerate fraternal correlations in a way which simulates homogamy. After all, in this case, excessively high correlations are due to a kind of assortative mating, not by choice, but by geographical isolation. Therefore, both exceptionally high fraternal correlations are not intrinsically wrong, but their numerical value should be reduced in some way.

On the whole, significant correlations are in accordance with other data. Unfortunately, so far as I know, only three series of fraternal correlations have been published, with not entirely comparable assortments of characters[1]. Seven correlations of Table 1, without corresponding values in Howells's data, have been calculated probably for the first time. In 22 pairs, the eye colour, classified as light (blue, grey) and dark (all other shades), is similar ($\chi^2_1 = 4{\cdot}57$; $P = < 0{\cdot}05$). But for hair colour there is no fraternal similarity.

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Goblet Cells in the Swim Bladder of *Ophiocephalus striatus* (Bloch)

DURING investigations on the mechanism of gas secretion by the gas gland epithelium in the swim bladder of teleosts, a preliminary investigation was made of the histology and histochemistry of the gas gland to identify the types of cells which are involved in the secretion of gases.

Adult *Ophiocephalus striatus* were caught as and when required and stunned by a blow on the head and the swim bladder rapidly dissected out and fixed in Zenker's, Helly's and Champy's fluids, a saturated solution of mercuric chloride in 70 per cent alcohol with 2·5 per cent potassium dichromate, a mixture of equal parts of saturated aqueous mercuric chloride and 2·5 per cent aqueous potassium dichromate, and a solution of equal volumes of 8 per cent aqueous basic

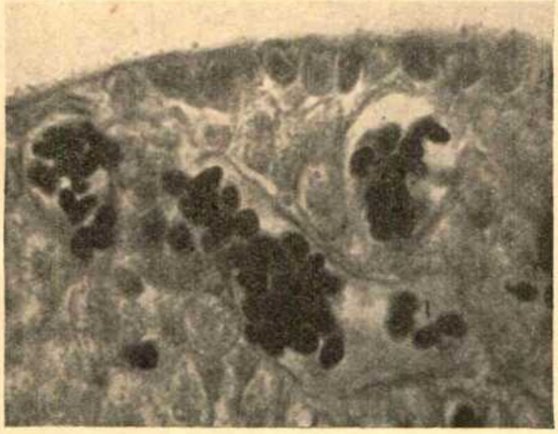

Fig. 1. Swim bladder epithelium of *Ophiocephalus striatus* (× *c.* 970). Fixed in saturated mercuric chloride with 2·5 per cent potassium dichromate and stained with Bensley's neutral gentian. Note along the border of the epithelium rounded, deeply stained cells with thick striations in the cytoplasm. The very dark bodies are blood cells

lead acetate and 16 per cent formalin. They were washed, dehydrated and embedded in paraffin as usual. Sections 2–6μ thick were stained with hæmatoxylin-eosin, Mann's stain, rapid phloxine-methylene blue, Bensley's neutral gentian and Delafield's hæmatoxylin.

These stains revealed a type of cell which is different from the main types of cells found in the swim bladder epithelium. This type of cell is found only towards the lumen of the bladder and is seen distinctly as a one-cell border in places where the cells are present in large numbers (Fig. 1). They are not seen in the depths of the glandular mass. In the region of the main glandular mass these cells are relatively less numerous but more abundant where the epithelium is thin.

The cells are usually rounded or ovoid and similar to goblet cells seen elsewhere. The nucleus is seen pushed to the base of the cell away from the lumen. In all the stains used the nucleus has taken a blue colour. Clear cytoplasm is seen just around the nucleus and above this is the condensed matter with very fine granules filling almost the entire cell. In some cells the condensed matter appeared to be crescentic. The space around these cells is clear of stain. In the rapid-phloxine methylene-blue stain the cytoplasm of the cell is stained a bright pink. In sections stained with Mann's stain these cells have taken less eosin than the main glandular mass adjacent to them. The condensed matter is stained violet with Bensley's neutral gentian, and often a clear border is present between the condensed matter and the cell wall. In some cells, probably spent ones, there can be seen violet round bodies, while in many others violet striations along the long axis are seen in addition. In the slides stained with hæmatoxylin-eosin, only a slight difference is seen in the staining between these cells and the rest of the gland cells.

These cells resemble the goblet cells seen in the epithelial lining of the intestines. Hence some sections were stained in 0·1 per cent toluidine blue in 1 per cent alcohol and others in 0·1 per cent toluidine blue in 30 per cent alcohol and some others in 0·2 per cent Ehrlich thionin for mucoproteins. There was no metachromasia in the cells of the swim bladder epithelium while there was well-marked metachromatic staining of the intestinal goblet cells which was more pronounced with the dye made in 30 per cent alcohol and still better in the thionin. Cells similar to those observed have not been described by previous workers on the fish swim bladder. George[1], who has studied the swim bladder epithelium of four species of *Ophiocephalus*, fails to mention their occurrence. Such cells have, however, been described in the alimentary tract of *Labeo* by Girgis[2] and in the epithelium of the accessory respiratory organs of *Clarias*, including the air chamber, by Moussa[3]. Their function is that of secreting mucin, and so it seems quite likely that the cells seen in the swim bladder also have a mucus-secreting function. It is evident, however, that there is some qualitative difference in the products of secretion produced by the cells described here and those occurring in the intestinal mucosa.

In the swim bladder of *Gadus morhua* "a copius secretion of a viscid, slimy opaque material" has been described by Vincents and Barnes[4]. Fange[5] worked on this material and he says "that very probably the secretory product discovered by Vincents and Barnes in some way originates from vacuoles of the gas gland cells". He has not, however, identified any particular type of cell causing this secretion. The presence of a slimy secretion in the lumen of the swim bladder has been reported by many other workers also. Scholander[6] notes that the bubbles of gas are secreted into the mucus covering the gland. The mucus may, therefore, have a very important function with reference to gas secretion. It is also quite likely that there has been some confusion in the material examined by these workers as they think that these secretions are in the nature of myelin and related to the secretory vacuoles. Vacuoles and granules have also been observed in the gas gland of this fish in the cytoplasm of the main type of cells; but no vacuoles have been seen in the cells described here. The vacuoles of the gland cells are probably related to the gas-secreting activity of the gland. Thus the cells described here seem to be different in function and to be responsible for a mucoid secretion. Further work is progressing under the supervision of Prof. C. M. Francis, of this College.

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Natural Occurrence of *Leptospira icterohæmorrhagiæ* in the Eastern Grey Fox and the Eastern Raccoon

PREVIOUS investigations of wild life in the United States have established the grey fox and the raccoon as natural hosts of leptospires. *Leptospira ballum*[1], *L. australis A*[2], and *L grippotyphosa*[2] have been isolated from the grey fox. *Leptospira ballum*[1], *L. pomona*[1], *L. australis A*[3], *L. grippotyphosa*[2], *L. autumnalis*[4], and *L. mini-Georgia*[5] have been isolated from the raccoon.

This communication concerns the first isolations in the United States of *L. icterohæmorrhagiæ* from the

Table 1. LEPTOSPIRAL ISOLATIONS FROM KIDNEY SUSPENSIONS

Animal	Day growth detected	Mediums supporting growth
Grey fox—38	Day 12 post-inoculated	Semi-solid Fletcher—horse; semi-solid Chang's—horse
Raccoon—7	Day 5 post-inoculated	Semi-solid Fletcher—horse
Raccoon—76	Day 12 post-inoculated	Semi-solid Chang's—horse and rabbit; 10^{-4} diluted semi-solid Fletcher—rabbit
Raccoon—80	Day 8 post-inoculated	Semi-solid Fletcher—horse; semi-solid Chang's—horse; 10^{-2} and 10^{-3} diluted semi-solid Fletcher—rabbit

eastern grey fox and the eastern raccoon. The isolations were made during the course of an epizootiological investigation of leptospirosis in Pennsylvania wild-life.

The species of wild-life investigated were caught in No. 2 coil spring steel traps. Specimens were removed alive from the traps and transported to the School of Veterinary Medicine, University of Pennsylvania. On arrival at the Laboratory, the animals were asphyxiated in a carbon monoxide chamber. Blood specimens for serological testing were collected and a portion of one kidney was removed aseptically from each animal.

Each kidney specimen was ground with sterile mortar and pestle and diluted to a 10 per cent suspension with Stuart's liquid medium base (Difco). One to three drops of the suspension were inoculated into one tube of each of the following media: Fletcher's semi-solid medium (Difco) with 10 per cent rabbit serum, Fletcher's semi-solid medium with 15 per cent horse serum, Chang's semi-solid medium with 15 per cent horse serum, and Stuart's semi-solid medium[7] with 10 per cent rabbit serum.

Serial ten-fold dilutions of the kidney suspension were made, with Stuart's liquid medium base, to approximately 10^{-1} to 10^{-5} final dilutions. One to two drops of each dilution were inoculated into a tube of Fletcher's semi-solid medium with 10 per cent rabbit serum. All inoculated mediums were incubated at 29° C. and examined by dark-field microscopy at 10–14-day intervals. Culture results are summarized in Table 1.

Sub-cultures of media containing growth of Leptospires were made into Fletcher's semi-solid medium with 10 per cent rabbit serum and Stuart's liquid medium with 10 per cent rabbit serum. Sub-cultures of each isolate were sent to the World Health Organization Reference Laboratory for Leptospirosis, Walter Reed Army Medical Center, Washington, D.C., and to the Leptospirosis Unit, Animal Disease Eradication Division, Ames, Iowa, where they were identified as *L. icterohæmorrhagiæ*.

Microscopic agglutination tests were conducted on serum specimens collected from each animal at the time of culturing using an antigen bank of eleven leptospiral serotypes: *L. bataviae*, *L. pomona*, *L. autumnalis*, *L. ballum*, *L. canicola*, *L. icterohæmorrhagiæ*, *L. alexi*, *L. grippotyphosa*, *L. australis A*, *L. sejroe* and *L. hyos*. Test results are summarized in Table 2.

Dr. Clark and Mr. Kresse are on assignment from Laboratory Services, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture.

This work was supported in part by United States Public Health Service grant *E*-2821.

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Synergid Haustoria of *Quinchamalium chilense* Lam.

DECAISNE[1] pointed out that in *Santalum album*, *Nanodea*, *Myoschilos*, *Osyris* and more obscurely in *Quinchamalium*, the tip of the embryo sac extends beyond the ovule into the ovarian cavity. The present account is based on an investigation of *Quinchamalium chilense*, a native of the South American Andes.

The ovary is inferior with a free-central placental column, bearing three hemianatropous, unitegmic ovules, the nucellus being reduced to one or two epidermal cells capping the megaspore mother cell. The thick integument does not grow beyond the nucellus and thus a micropyle is not formed. The embryo sac is of the Polygonum type and extends beyond the ovular epidermis at the 4-nucleate stage. At maturity it comprises the egg apparatus, two polar, and three antipodal nuclei. The latter are separated from the rest of the embryo sac by a wall, but do not become organized into individual cells.

In most of the Santalaceae, the synergids are inconspicuous, have small nuclei, and degenerate before or soon after fertilization. In *Q. chilense*, on the contrary, they are unusually large and pear-shaped with prominent nuclei. Even in unpollinated flowers they protrude beyond the embryo sac (Fig. 1*a*) and eventually extremely long (approximately 1,200μ) tubular structures reaching up to one-third the length of the style are formed (Fig. 1*b*). These are densely cytoplasmic and sometimes persist until the endosperm has become quite massive. Dahlgren[2] has recorded that in some members of the Compositae, namely, *Calendula arvensis*, *Mutisia candolleana* and *Ursinia anthemoides*, the tips of the synergids extend into the micropyle and even beyond it, sometimes reaching as far as the funiculus. Paliwal's[3] observation that in *Santalum album* dichotomously branched synergid haustoria develop after fertilization has been contradicted by Bhatnagar[4]. The

Table 2. MICROSCOPIC AGGLUTINATION TITRES OF SERUM SAMPLES OBTAINED AT THE SAME TIME AS KIDNEYS WERE CULTURED

Animal	Leptospiral serotypes										
	Bat.	*Pom.*	*Autum.*	***Bal.***	*Canicola*	*Ictero.*	*Alexi*	*Grippo.*	*Aust. A*	*Sejroe*	*Hyos*
Grey fox-38	—	—	—	—	—	100	—	—	—	trace	—
Raccoon—7	—	100	trace	—	—	—	trace	trace	trace	trace	trace
Raccoon—76	—	—	—	—	—	400	—	—	trace	—	—
Raccoon—80	—	—	—	—	trace	400	—	—	—	—	—

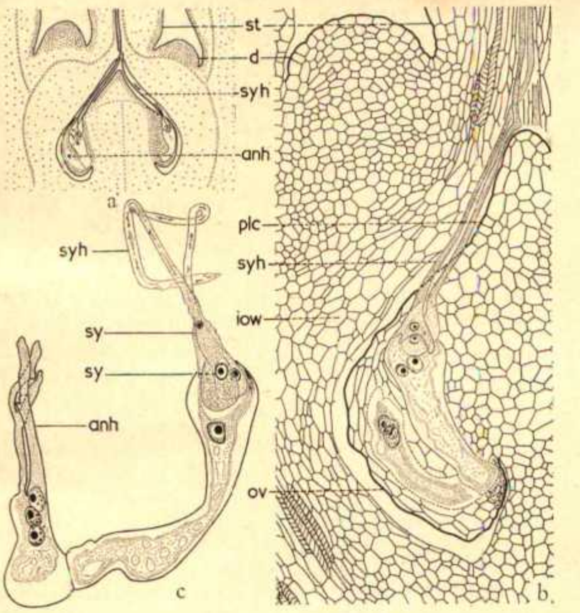

Fig. 1. *a*, Longisection of base of flower showing floral parts and extension of synergid and antipodal haustoria (× 40); *b*, semi-diagrammatic enlargement of 1*a* to show the details of embryo sac in relation to ovary and style (× 124); *c*, whole mount of embryo sac showing the synergid and antipodal haustoria (× 262). anh, Antipodal haustorium; d, disk; iow, inner ovary wall; ov, ovule; plc, placental column; st, style; sy, synergid; syh, synergid haustorium

latter author has pointed out that in this plant the synergids degenerate soon after fertilization and that Paliwal had mistaken the persistent pollen tubes which remain adhering to the embryo sac (see also Griffith[5,6]) for synergid haustoria. Thus, *Quinchamalium* has perhaps the longest synergid haustoria so far known in any angiosperm.

In addition to very long and conspicuous synergid haustoria *Quinchamalium chilense* also shows a much-branched antipodal haustorium. As mentioned earlier, the antipodal nuclei do not become organized into cells. Instead, they enlarge, become hypertrophied and multinucleolate and may even divide. Later the chalazal end of the embryo sac extends as far as the placental column and branches profusely (Fig. 1c). These branches ramify into the placental tissue in the vicinity of the vascular supply. In the Santalaceae an extension of the chalazal end of the embryo sac into a cæcum leaving the antipodal cells *in situ* is a common feature. In *Quinchamalium* the antipodal nuclei are carried at the tip of the haustorium until it branches (Fig. 1c). The activity of the synergid and antipodal haustoria declines with the progressive development of the endosperm.

The embryology of other species of *Quinchamalium* and allied genera, namely, *Arjona* and *Myoschilos*, is as yet unknown and it would be worth while to investigate them to ascertain if prominent synergid haustoria develop in these plants also.

I shall be grateful if botanists could help me in procuring the preserved materials of all stages of buds, flowers and fruits of different species of *Arjona* and *Myoschilos*.

I am indebted to Dr. B. M. Johri and Prof. P. Maheshwari for guidance. Thanks are also due to Dr. S. P. Bhatnagar for comments, to Mr. V. Garcia, University of Buenos Aires, Argentina, now working at the University of Delhi, for providing the material, and to the Council of Scientific and Industrial Research for financial assistance.

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Relation between Organophosphorus-Resistance and Non-Diapause in Spider Mites

As has been shown by Lees[1], the females of *Tetranychus urticae* enter into diapause under the influence of a short-day treatment during their development. High temperatures tend to prevent diapause even if the photoperiod is short.

An investigation into the diapause-pattern of several spider mite populations originating from places in different countries (the Netherlands, Belgium and Germany) proved that the critical area of the diapause-pattern of seven populations was significantly smaller than may be expected. Only four populations showed a diapause-pattern in accordance with the data of Lees.

In work on the susceptibility of the populations to organophosphorus compounds it appeared that the seven populations with the deviating diapause-pattern were organophosphorus-resistant; the four

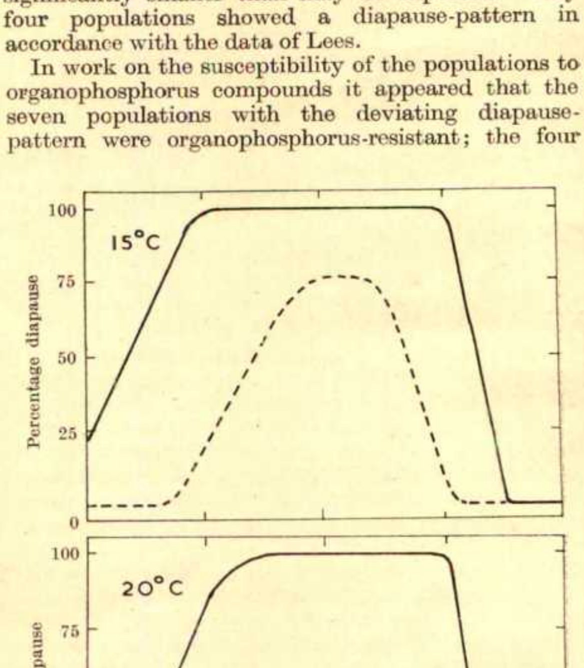

Fig. 1. The effect of photoperiod on the incidence of diapause in two populations of *Tetranychus urticae*. The dotted line represents an organophosphorus-resistant population, the continuous line a susceptible population

populations with a normal diapause-pattern showed a high susceptibility to organophosphorus compounds.

Fig. 1 shows the difference in diapause-pattern between a susceptible and an organophosphorus-resistant population. At 20° C. a régime of short photoperiods evoked only a small percentage of diapause forms in the resistant population. At 15° C. resistant mites entered into diapause, but the percentage of diapause forms was significantly lower with the resistant population than with the susceptible one.

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Effect of Copper Deficiency in White Clover on Nitrogen Fixation

IN view of a recent report by Hallsworth *et al.*[1] that copper stimulates nitrogen fixation by subterranean clover (*Trifolium subterraneum*), the following evidence from a field trial is presented as indicating a similar function of copper in nitrogen fixation by white clover (*T. repens*).

White clover was dissected from mixed herbage samples taken on November 27, 1956, from a rates of copper trial conducted by one of us (F. H. v. d. E.) on a pasture recently established on raw peat on the Moanatuatua swamp. The samples were analysed for copper and total nitrogen contents.

Many clover plants in the control plots showed characteristic copper deficiency symptoms—die-back with bleaching of the margins and rolling and collapse of the leaflets. The samples analysed were apparently healthy whole leaves (leaflets plus petioles).

Table 1. YIELDS OF MIXED HERBAGE AND COPPER- AND NITROGEN-LEVELS IN WHITE CLOVER

Treatment		Dry matter yield (lb. per acre) (Spring 1956)	Copper (p.p.m. in dry matter)	Nitrogen (percentage in dry matter)
Control		2,090	2·5	4·25
5 lb. Bluestone		3,330	3·6	4·88
10 lb. Bluestone		3,410	4·0	4·94
20 lb. Bluestone		3,540	6·6	5·05
40 lb. Bluestone		3,520	7·2	5·00
80 lb. Bluestone		3,520	8·2	5·03
Significant	5%	350	0·78	0·16
differences	1%	470	1·05	0·21

The copper treatments resulted not only in increased growth of white clover and elimination of deficiency symptoms, but also in significantly higher concentrations of total nitrogen. Incubation tests of the peat soil with copper additions failed to demonstrate any increase in mineral nitrogen-levels. The clover nitrogen-levels in the controls were relatively low for succulent spring growth under local conditions, but normal in the copper-treated plants. The presence of copper deficiency symptoms together with low nitrogen- and very low copper-levels suggests that the increases in nitrogen concentrations in the clovers were due to a specific effect of copper on nitrogen fixation by white clover.

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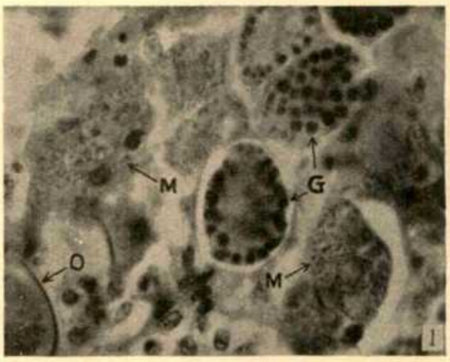
Limitations of the Periodic Acid–Schiff Technique for the Demonstration of Stages of *Eimeria* in Tissues

IN a recent communication, Jennings[1] suggested that the periodic acid–Schiff technique is useful for the demonstration of all stages in the life-cycles of four species of coccidia occurring in the intestine of the fowl. These species were *Eimeria acervulina*, *E. maxima*, *E. necatrix* and *E. tenella*.

The periodic acid–Schiff technique was first applied to *Eimeria* by Lillie[2], who obtained positive reactions in the cytoplasm of oocysts of *E. stiedae* from the rabbit. More recently, this technique was used by Cheissin[3] on *E. magna*, *E. media* and *E. intestinalis* from the rabbit, Pattillo and Becker[4] on *E. acervulina* and *E. brunetti* from fowls, Gill and Ray[5] and Tsumoda and Itikawa[6] on *E. tenella* from fowls. These authors all failed to obtain positive reactions by the periodic acid–Schiff technique with some stages of these life-cycles. Cheissin found that isolated microgametes were negative and that microgametocytes gave only an insignificant reaction. Pattillo and Becker obtained no positive reactions using the Hotchkiss[7] periodic acid–Schiff technique with the microgametocytes and schizonts of both *E. acervulina* and *E. brunetti*, although the merozoites of *E. acervulina* gave a weak reaction. Developing macrogametocytes were often negative.



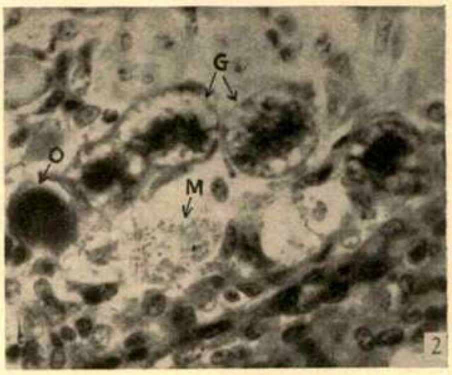


Fig. 1. Section of small intestine infected with *E. maxima* and fixed in formol saline; stained by Hotchkiss periodic acid–Schiff technique and counter-stained with hæmalum. Note the intense staining of the 'plastic granules' (*G*) in the macrogametes and oocyst wall (*O*). The microgametocytes and microgametes (*M*) have stained with the hæmalum but not with the periodic acid–Schiff reagent. (× 525)

Fig. 2. Section of small intestine infected with *E. maxima* and fixed with Bouin's fluid; stained by Hotchkiss periodic acid–Schiff technique and counter-stained with hæmalum. Note the lack of preservation of the plastic granules in the macrogametes (*G*); the microgametocytes and microgametes (*M*) have stained with the hæmalum, but not with the periodic acid–Schiff reagent. (× 525)

In our experience, the invading sporozoites and merozoites of *E. tenella* give only weak reactions and consequently the technique is of little value in demonstrating these stages in tissues. Long and Rootes[8], using the Hotchkiss periodic acid–Schiff technique, showed that the reactions of the prominent so-called 'plastic granules' of the macrogametes depend on the type of fixative used (see Figs. 1 and 2). According to Pattillo and Becker, these granules probably consist of a protein–carbohydrate complex, which is unaffected by pre-treatment with saliva or α-amylase. Fixation with Bouin's fluid fails to preserve these granules, and negative results are obtained with the periodic acid–Schiff technique although there is no interference with the staining of the glycogen in the cytoplasm. For these reasons the periodic acid–Schiff reaction is not an appropriate technique for the demonstration of all stages of the life-cycles of *Eimeria*, and when used on sections of very heavily parasitized intestinal tissue, large amounts of mucus giving a positive reaction with the periodic acid–Schiff technique may mask the parasites. The same difficulty may be encountered when the technique is applied to sections of rabbit liver containing trophozoites and small stages of the asexual and sexual cycles of *E. stiedae*, because of the possible masking effect of the stained liver glycogen.

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CYTOLOGY

Identification of the Chromosome Complements of New-born and Adult Living Mice

Chromosome abnormalities similar to those known in man are now being found in mice[1]. Identification of the chromosome complement of these mice in cells from bone marrow[2] or corneal epithelium[3] involved the death of the animals. The advantage in identifying the chromosome complement of living mice is obvious, and the present communication describes techniques to achieve this in new-born and in adult mice, chiefly by the use of primary outgrowths of cells from pieces of tissue cultured *in vitro*.

The chromosome complement of new-born mice aged 1–3 days has been identified in cells from small pieces of tail. Loss of their tail-end does not obviously affect the development of these mice. Attempts to obtain cells in mitosis for chromosome counts directly from the tail-pieces were not uniformly successful, hence the pieces have been cultured *in vitro*. Before removal from the mouse, the tail-ends are washed in a mixture of alcohol and ether; this serves as a partial anæsthetic and to clean the tails. The excised tail-ends are then washed in Gey's solution containing mycostatin, penicillin and streptomycin, cut into four or five small pieces, and mounted separately in tiny plasma clots on a 3/4 in. cover-slip. The cover-slip is inverted in a cavity cell, and growth medium is added. Antibiotics and mycostatin are necessary to keep down bacteria and fungi respectively. Initially, Gey's solution containing 15 per cent calf serum, 2 per cent chick embryo extract, and 0·5 per cent lactalbumin hydrolysate was used as the growth medium, but this was later superseded by medium 199 (Glaxo Laboratories), plus 15 per cent calf serum. The medium is changed every other day, and all cavity cells are gassed with 5 per cent carbon dioxide.

Cells emerge from the grafts within one or two days (Fig. 1). After 4–5 days, the cell monolayer in many of the cultures is ready for cytological preparation. The small pieces of tail, still easily recognizable, are gently pulled off the cover-slip with least disturbance to the cell monolayer, and are re-embedded in tiny plasma clots on another cover-slip to serve as a reserve if needed. The cover-slip containing the cell monolayer is then re-inverted into the growth solution and treated for chromosome processing in a manner similar to that described in ref. 4: 'Colcemid' (Ciba Pharmaceuticals) at a concentration of 0·25–0·50 μgm./ml. for 2 hr., hypotonic saline for 15 min., and fixation in 45 per cent acetic acid. In practice, after removal of the growth medium 'Colcemid' mixture, 1 ml. saline is placed in the cavity cell, 2·5 ml. distilled water is added dropwise, and the acetic acid added slowly 15 min. later. This concentration of hypotonic saline, and the gradual addition of water and fixative, were found to give good spreading of the chromosomes with minimal cell breakage. After fixation, the cells were dried in air and stained by the Feulgen technique.

Mitoses are found in the great majority of the preparations. The clarity of many mitoses is sufficient to count the chromosomes easily (Fig. 2), and more than thirty of them can be selected in some cultures. If this number is not available, or if more are required, the reserve culture is available a few days later. The mitotic index is especially high in cultures fixed 10–11 hr. after change of growth medium. Details of the counts on mitoses in six tails are given in Table 1. Almost all were diploid; a few were found with less than the diploid number, and were presumably a result of cell breakage. The technique obviously

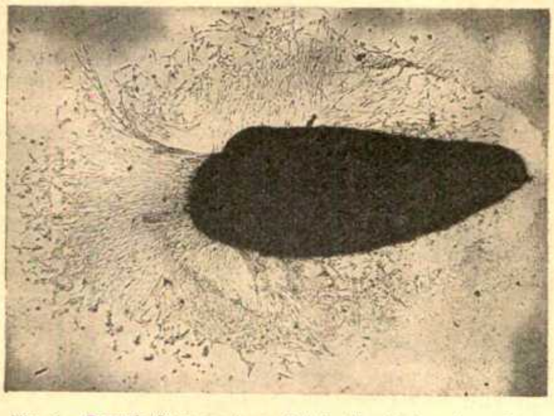

Fig. 1. Growth from a piece of tail after 3 days in culture. (× 22)

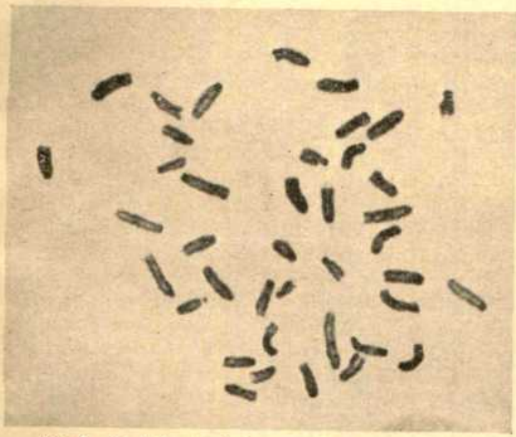

Fig. 2. Diploid mitosis from a tail culture. (× 756)

permits a fairly rapid identification of the chromosome complement of new-born mice, and twenty-two such mice have recently been examined here using an arbitrary limit of twelve clear mitoses for classification. Thirteen were classified within 5 days, six others within 7 days, and two more after 10 days; the last culture was lost through bacterial contamination.

Table 1. CHROMOSOME COUNTS ON CELLS IN OUTGROWTHS FROM TAIL-TIP AND EAR-CLIP CULTURES

Tissue	No. of mitoses examined	Chromosome counts 31	33	39	40
Tail	68	1		2	65
Ear	45		1	1	43

Table 2. GROWTH FROM EAR CULTURES

Ear tissue	Proportion of successful cultures after 12 days	Mean interval (days) until: First appearance of fibroblasts	Ready for processing
Normal	7/10	7·4 ± 0·3	14·5 ± 0·8
Regenerating for 2 days	13/13	3·6 ± 0·2	9·6 ± 0·6
Regenerating for 4 days	11/12	3·4 ± 0·3	10·2 ± 0·4

The chromosomes of adult mice were also counted in cells grown *in vitro*. Mitotic cells can be obtained directly from mice by partial splenectomy, but such an operation is often undesirable. Pieces of skin are not very suitable for obtaining cell outgrowths; growth is very slow or absent, and bacterial and fungal infections are frequent. Small pieces clipped from the ears produce a better growth. The most successful method is to make a small cut in the ear of the mouse and, 2 or 4 days later, remove small pieces containing the regenerating tissue. Cells now emigrate from the ear tissue much sooner (Table 2), and the cell monolayer can be processed for chromosome counts after approximately 10–15 days. Details of the counts made on four ear cultures are given in Table 2. The technique clearly serves to obtain an indication of the chromosome complement of the adult mouse with the minimum of discomfort to the animal.

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GENETICS

A Heterocaryon possessing Haploid and Diploid Nuclei

IN general, a heterocaryon between a white and a yellow mutant of *Aspergillus* fungi gives rise to white, yellow, and green progeny. It was found recently, however, that five heterocaryons produced between two of the seven colour mutants of *A. sojae* strain *K* could give brown progeny besides the white, yellow, and green. Evidence that the brown strain appears to be a heterocaryon possessing haploid and diploid nuclei in a common cytoplasm is to be described.

Among the five heterocaryons, a heterocaryon between a white histidineless mutant and a yellow leucineless mutant was investigated extensively. The heterocaryon was produced by the method reported in ref. 1. When conidia of this heterocaryon were plated on minimal medium (Czapek agar), there appeared four kinds of colonies, namely, white 12, brown 6, green 10, and heterocaryotic colonies 4,410. Eight of the heterocaryotic colonies had brown sectors.

A conidial suspension prepared from a whole brown colony, in which more than 90 per cent of conidia were single, was spread on complete medium (Koji extract agar), and the conidial colour and nutritional requirement of each colony were examined after one week. In approximately one thousand colonies, 84 per cent were yellow, 13 per cent brown, and 3 per cent green. Using one of the brown colonies thus obtained, plating was done again on the complete medium. Results are shown in Table 1 together with the results of the third experiment. It is noteworthy that no white colony was found among the conidia of the brown strains. In addition, the yellow colonies were dependent on leucine and the brown and the green colonies were prototrophic. In contrast with this, for normal heterocaryons there was no green colony, so far as was tested, although they gave white, yellow, and heterocaryotic colonies (Table 1).

Table 1. PATTERN OF SEGREGATION OF THE BROWN STRAINS AND NORMAL HETEROCARYONS

	Brown strain					Normal heterocaryon				
	Segregation ratios (per cent)				No. of colonies examined	Segregation ratios (per cent)				No. of colonies examined
No. of transfer	*W*	*Y*	*B*	*G*		*W*	*Y*	*X*	*G*	
1	0	84	13	3	1,100	44	43	13	0	1,680
2	0	88	9	3	1,010	41	48	11	0	986
3	0	87	12	1	1,166	40	50	10	0	802

W, White histidineless; *Y*, yellow leucineless; *X*, heterocaryotic prototroph; *G*, green prototroph; and *B*, brown prototroph.

In order to estimate ploidy of the yellow and the green segregants, numbers of nuclei/conidium were examined by the method described in ref. 2. Results were that the yellow conidium contains on the average 3·5 nuclei and the green conidium 1·7. These values approximate to those in a haploid (3·5) and a heterozygous diploid conidium (1·6) which was produced by the authentic method[3,4]. Moreover, the yellow clone was stable, and the green clone gave white and yellow progeny after a long cultivation. The appearance of white progeny from the green strain might be due to either somatic recombination or reduction[5]. It is concluded, therefore, that the nuclei in yellow conidia are haploid and the nuclei in green conidia diploid.

It is known that a white haploid nucleus and a yellow haploid nucleus in a heterocaryotic cell can conjugate under natural conditions to form a heterozygous diploid nucleus which develops green colour. In the heterocaryon described before (the original heterocaryon), it seems that the diploid nuclei are seldom segregated and transmitted to progeny together with the yellow nuclei. The resulting progeny are brown. Because a single conidium of the brown strain gives rise to yellow and green progeny, the conidium is considered to be a heterocaryon possessing both haploid and diploid nuclei. The average number of nuclei in a brown conidium is about 3·0.

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ANTHROPOLOGY

Possible Origin of the Use of Fire by Early Man

THOUGH many animals can become accustomed to the presence of fire, man alone has learned to produce, to maintain and to control it. However, this achievement is not peculiar to *Homo sapiens*. Fire was known also to *Homo neanderthalensis* and *Pithecanthropus pekinensis*[1]. From the study of fire-myths and the cultures of primitive races, it is usually presumed that fire was first obtained from such natural sources as volcanoes, bush fires, lightning, sparks struck from stones, or dry branches rubbing together in the wind[2–5]. Myths, however, are not necessarily reliable as guides to the distant past, while the reaction of animals, even of modern man, to volcanoes and forest fires is one of terror. Even when fear has been mastered, the practical difficulties of obtaining fire from these sources are still immense. Natural production of fire by friction or percussion is too rare to be readily observed by members of a small and scattered population.

An alternative to present theories is that use of fire itself is secondary to use of heat derived from bacterial decomposition of plant material. The heat produced by a haystack or compost heap is a matter of common knowledge. This heat may well become great enough to result in fire. Since many animals use as beds piled and trampled grasses, early man may well have slept in a similar manner. Individuals leading a purely nomadic existence would not use their bedding heaps sufficiently long for the warmth of decay to become apparent, but so soon as a more settled mode of life was adopted bacterial decay of the bedding could become important. Accumulation of sun-dried vegetation in a bed, perhaps a communal one, could lead to the 'compost heap' situation, presenting man with a small-scale source of heat. At first this would merely increase the temperature of his bed; later it might lead to the idea of the peat fire or even of earth-oven cooking. Occasionally, no doubt, such beds would burst into flame, providing at once the source and the idea of a blazing fire. On this theory, other means of fire-production can be regarded as being developed at a much later period, either from observation of natural events or as a by-product of chipping and grinding processes in toolmaking industries.

There can be no direct evidence for any theory of the origin of the use of fire, but it seems not unreasonable to regard the awe-inspiring character of natural fires and the rarity of small-scale fire production in Nature as militating against acceptance of present theories. In contrast, heat or flame produced by the method here suggested would be, at most, only mildly startling, while the care accorded by some primitive races to their existing fires could be interpreted as deriving from a period when an active compost heap was a most precious family possession. The 'compost heap' theory would also allow for widespread discovery of the use of fire by early man, even in regions where natural sources of fire were not available.

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PSYCHOLOGY

Validity of Prediction based on Cross-Sectional Analysis

CROSS-SECTIONAL analysis, by means of which is investigated the behaviour of a cross-section of the population at a given point in time, is a convenient method in many fields of research where age or maturational differences are of interest. Implicit in the findings of such investigations are predictions about the future behaviour of the groups concerned. Such predictions are, however, based on the assumption that the behavioural differences between groups are due to the age differences involved, and that future age changes will bring about similar behavioural differences. The fallibility of such predictions in the field of demography has been pointed out[1]. This investigation is aimed at checking similar findings in the field of absence behaviour in an industrial situation.

The absence records of a group of 140 continuous service men engaged on manual work in an engineering firm during the period 1949–58 formed the material of the investigation. The sample was divided into four age-groups, each covering an approximate 10-yr. span, so that the median age of one group at the end of the period was approximately equal to that of the next oldest group at the beginning of the period. The records for each group were first analysed cross-sectionally for a 2-yr. period 1949–50, and the same groups were then followed longitudinally for a further 8 years. Two measures of absence were used in the analysis: (*a*) the number of occasions of absence, and (*b*) the average length of absences. The data were divided into five 2-yr. periods. The results for the first and last of these periods are summarized in Tables 1 and 2.

For the length of certified sickness absence (Table 2), the cross-sectional analysis based on the 1949–50 data shows a sharp increase in the length of absence for the oldest group. This agrees with the findings of previous investigators, for example, ref. 2. The prediction from this finding would be that, as age increased, group 3 would show a similar increase in length of sickness absence, and an examination of the

Table 1. FREQUENCY OF ABSENCE FOR REASONS OTHER THAN SICKNESS

Age group	No in group	Median age at 1/1/49	1949–50 Average No of absences per man	1949–50 No and percentage of men with 0 absences	1957–58 Average No of absences per man	1957–58 No and percentage of men with 0 absences
1	20	23	2·35	3 (15)	2·75	3 (15)
2	51	31	1·61	16 (31)	1·71	18 (35)
3	34	40	1·18	18 (53)	1·71	14 (41)
4	35	51	1·63	15 (43)	1·49	15 (43)
All groups	140	35	1·61	52 (37)	1·8	50 (36)

Table 2. LENGTH OF CERTIFIED SICKNESS ABSENCES

Age group	No in group	Median age at 1/1/49	1949–50 Average length of absence (days)	1949–50 Percentage of absences lasting more than 2 weeks	1957–58 Average length of absence (days)	1957–58 Percentage of absences lasting more than 2 weeks
1	20	23	7·43	14	3·83	0
2	51	31	7·65	18	7·47	13
3	34	40	7·82	18	13·12	53
4	35	51	14·22	67	12·64	55
All groups	140	35	9·9	34	10·9	41

data for the end of the period (1957–58) shows that such an increase has, in fact, occurred. In this case the longitudinal method supports the findings of the cross-sectional method, making it probable that this is a true ageing effect.

In the case of frequency of absence (Table 1) this is not so. The cross-sectional analysis (1949–50) shows the youngest group to have a frequency of absence for reasons other than sickness higher than the other groups. Similar findings by other investigators are reported[3]. On this basis one would expect their absences to become less frequent as they grew older, but the data for the end of the period show that this has not happened and that their absence frequency has, in fact, increased relative to the average. The higher absence frequency of this group seems to be a continuing characteristic, independent of age.

The conflict of evidence arising from the use of these two methods indicates the need for greater caution in the interpretation of the results of cross-sectional investigations. The differences shown by this method may be due to the age differences involved, but the possibility that other factors are operating must also be borne in mind.

This work will be published in greater detail in *Occupational Psychology*.

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Role of Experience in Misreaching produced by Visual Cortex Lesions

WHEN the macular projection area—that part of the visual striate cortex to which the macular region of the retina projects—is removed in monkeys a characteristic symptom can be demonstrated. The animal frequently misreaches for small pieces of food when they are presented to him on a flat surface[1,2]. The error involved may be as much as three inches, and two inches is common. It is the direction of the object, rather than the distance, which is usually gauged incorrectly by the animal, that is, his fingers fall to the sides of the object rather than beyond or in front of it. The direction of the error appears to depend primarily on which hand is used, misreaching to the left tending to occur when the left hand is used and misreaching to the right when the right hand is used. The position of the food relative to the animal's body is also important. This type of behaviour is often called 'past-pointing', but since the error tends to be one of direction rather than distance, and also because past-pointing is also used to refer to vestibular or cerebellar derangement, we prefer to use the term 'misreaching'.

Misreaching is easily demonstrated during the first few days after operation. It declines, and almost invariably disappears within ten days. Afterwards, operated animals are as adept as normal animals at detecting and picking up small objects.

It has never been shown whether practice is necessary for this recovery to occur or whether it is the result of a spontaneous re-organization of the visual system after part of it has been damaged. The latter hypothesis might seem rather improbable were it not for the fact that we now know that the rhesus monkey is not blind in the centre of its visual field after this operation, although this region is less efficient than in the normal animal[3]. Whichever hypothesis is correct, it is interesting to contrast the high degree of plasticity in such monkeys with the lack of correction in orientation after eye rotation or similar alterations in Amphibia[4].

In order to distinguish between these two views about the recovery process—spontaneous recovery or practice—an animal was kept in total darkness for ten days after the macular projection area had been removed; he was then tested for misreaching. The result was clear. The animal misreached in exactly the same manner and for the same length of time as previous animals which had been tested without a delay after surgery. A control animal which was kept in the dark for 10 days showed no misreaching when it emerged. These statements are based on daily observations made in an experimental situation which permits quantitative estimates of misreaching from ciné film records.

It is concluded that, although the cause of misreaching is still not perfectly clear, recovery depends on experience in the light. It cannot be said without further experimental work whether mere exposure to the light is sufficient, or whether specific practice in reaching for objects is necessary. The latter appears more likely in view of recent work on the recovery of misreaching by human subjects wearing prisms[5].

This investigation was supported by the U.S. Air Force under Contract *AF* 61 (052)–185. One of us (A. C.) held a Medical Research Council fellowship while doing this work.

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MATHEMATICS

Equation of Motion in Five Space

THERE exist many models for the description of the gravitational and electromagnetic field Perhaps the most successful formalism developed is still that of Kaluza[1], with notable improvements by Bergmann[2] since it reproduces exactly the classic equations of the electromagnetic field with gravity However, there still remained the well-known difficulties associated with the classic treatment of Nature so that generalizations which lead to substantially different equations were made in the hope that there could be included within the new frameworks an adequate theory encompassing quantum-phenomena as well These hopes have not been realized; but nevertheless it is most difficult to relinquish a theory which by and large has been subject to confirmation—quite strikingly in recent times using techniques based on the Mossbauer effect[3] In addition to numerous verifications of the original version of the theory, Einstein, Infeld and Hoffmann[4] have shown that there exists a most æsthetic feature, namely, that the field equations themselves determine the classic equations of motion of 'singularities' or 'particles' in the field Thus it is not necessary to supplement the field equations with ponderomotive equations.

It seems to be of some interest to investigate the equations of motion in a five-dimensional setting to examine departures from the usual results. I will assume that the additional dimension is space-like and take for the field equations

$$R_{\mu\nu} - g_{\mu\nu}R/2 = 0 \qquad (1)$$
$$\mu,\nu = 0, 1, 2, 3, 4$$

where $g_{\mu\nu}$ is the metric tensor, $R_{\mu\nu}$ the Riemann-Christoffel curvature tensor and R its contraction '0' indices will be considered temporal, the others spatial with the '4' index reserved for the additional dimension Now if we seek approximate solutions of the field equations corresponding as closely as possible to the four-dimensional case it is found on reckoning differentiation with respect to X_4 as leading to quantities of the same order of smallness as differentiation with respect to $X_0(= ct)$, and then proceeding in much the same fashion as in reference 2 and 4 'co-ordinates' of the 'particles' will involve X_4 as well as X_0, which would imply that the equations of motion for the position of the 'particles' will be partial differential equations rather than total differential equations In addition to a field, which, in view of the Kaluza theory, may be interpreted as that arising from a charged 'particle,' there arises another which I make no attempt to interpret here

For purposes of comparison let us record the results of the calculations for the metric tensors to that order essential to obtain the Newtonian approximation These are

$$g_{00} = 1 - c^{-2} \sum_j((12\varkappa M_j + 6\varkappa P_j)/13) V_j$$

$$g_{0s} = (6\varkappa/13)c^{-3} \sum_j(3M_j \partial\xi_j^s/\partial t - (13/2\varkappa)^{1/2}(cQ_j)\partial\xi_j^s/\partial X^4) V_j$$

$$g_{4s} = (6\varkappa/13)c^{-3} \sum_j((13/2\varkappa)^{1/2}Q_j\partial\xi_j^s/\partial t - 3P_jc\partial\xi_j^s/\partial X^4) V_j \qquad (2)$$

$$g_{40} = - 6c^{-2}(\varkappa/26)^{1/2} \sum_j Q_j V_j$$

$$s = 1, 2, 3$$

where

$$V_j = ((X^s - \xi_j^s)(X^s - \xi_j^s))^{-1/2} \qquad (3)$$

ξ_j^s, the sth co-ordinate of the jth 'particle,' and M_j and Q_j its mass (gm) and charge (E S U) respectively. P_j may be considered another parameter describing another property of the jth particle It is to be noted that $\varkappa$ is Newton's constant of gravity and that the dimensions of the P_j is that of $(\varkappa)^{-1/2}$ times charge (E S.U) M_j, Q_j and P_j could be functions of $X_0(= ct)$ and X_4 satisfying

$$\begin{aligned}\partial M_j/\partial t - (13/18\varkappa)^{1/2}c\partial Q_j/\partial X^4 &= 0\\ \partial Q_j/\partial t - (18\varkappa/13)^{1/2}c\partial P_j/\partial X^4 &= 0\end{aligned} \qquad (4)$$

while the partial differential equations of motion for the jth particle in the Newtonian approximation turn out to be·

$$\partial^2\xi_j^s/\partial t^2 - (1/3)(cQ_j/M_j)(26/\varkappa)^{1/2}\partial^2\xi_j^s/\partial X^4\partial t + (P_jc^2/M_j)\partial^2\xi_j^s/\partial X^{42} \qquad (5)$$
$$- \sum_{i\neq j} (\varkappa M_i + Q_iQ_j/M_j - 4\varkappa P_jM_i/(13M_j) + \varkappa P_iP_j/M_j - 4\varkappa P_i/13)\partial V(i,j)/\partial\xi_j^s = 0$$

where

$$V(i,j) = ((\xi_i^s - \xi_j^s)(\xi_i^s - \xi_j^s))^{-1/2} \qquad (6)$$

with the time t in seconds units and X^4 the additional co-ordinates expressed in cm units

It is to be noted that even for an isolated 'particle' or systems of particles' far removed from one another the co-ordinates ξ_j^s satisfy the second-order equation obtained by considering only the first three terms of equation 5 which may be expressed as.

$$(\partial/\partial t - c_{j+}\partial/\partial X^4)(\partial/\partial t - c_{j-}\partial/\partial X^4)\ \xi_j^s = 0 \qquad (7)$$

if M_j, Q_j. and P_j are presumed constant. In equation 7 c_{j+} is given by

$$c_{j\pm} = (Q_j/M_j)(18\varkappa/13)^{-1/2} \pm ((Q_j/M_j)^2(18\varkappa/13)^{-1} - P_j/M_j)^{1/2})c \qquad (8)$$

which implies with the f_j^s and g_j^s arbitrary functions of the indicated arguments that

$$\xi_j^s = f_j^s(c_{j+}t + X^4) + g_j^s(c_{j-}t + X^4) \qquad (9)$$

a wave motion for the ξ_j^s which is propagated with velocities $- c_{j+}$ in our $t - X^4$ space

The insistence that equation 7 be a hyperbolic partial differential equation has as consequence $P_j/M_j < 0$ to assure this property even when $Q_j = 0$ For an electron the velocity of propagation attains a value of the order 10^{21} times the velocity of light One would be forced to conclude that if it were possible to communicate in such a space it would be a relatively simple task to assess the state of the far reaches of the universe within a considerably shorter interval of time than heretofore and so indeed to consider occurrences almost anywhere to be of a contemporary nature In any event the structure of the equations of motion which have emerged even for 'free particles' may perhaps provide us with other conceptual possibilities the starting point of which may lead to a theory with less startling consequences

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FORTHCOMING EVENTS

Tuesday, January 2

Royal Geographical Society (joint meeting with the Geographical Association and the Institute of British Geographers, at 1 Kensington Gore, London, S W 7), at 3 p m —Prof David Linton "Some Contrasts in Landscapes in the F I D S Sector of the Antarctic"

Wednesday, January 3

British Institution of Radio Engineers (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 10 a m —All-day Symposium on "Data Transmission"

Institution of Mechanical Engineers, Lubrication and Wear Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "Wear of Rubbing Electrical Contacts"

Wednesday, January 3—Friday, January 5

Science Masters' Association (at Imperial College, London, S W 7), at 10 a m daily—Annual Meeting

Thursday, January 4

Institute of Refrigeration (at the Institute of Marine Engineers, The Memorial Building, 76 Mark Lane, London, E C 3), at 5 30 p m —Mr S I Neuman "Refrigeration in Dam Cooling"

Institution of Electrical Engineers (at Savoy Place, London, W C 2), at 5 30 p m —Mr F J Lane "The Transmission of Power at High Voltages" (Fourth Hunter Memorial Lecture)

Royal Society of Medicine, Neurology Section (at 1 Wimpole Street, London, W 1), at 8 p m —Meeting on "Inborn Errors of Metabolism" Speakers Dr Richard H Dobbs, Dr L I Woolf and Dr G A Rose

Friday, January 5

Plastics Institute (at the Institution of Electrical Engineers, Savoy Place, London, W C 2), at 2 30 p m —Prof M Stacey, F R S "Nature's Plastics" (Annual Young People's Lecture)

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S W 1), at 6 30 p m —Mr F P Doyle "Recent Advances in the Chemistry of Penicillin"

Institution of Electrical Engineers, Medical Electronics Discussion Group (at Savoy Place, London, W C 2), at 6 p m —Discussion on "Membranes" opened by Dr H Davson

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Biochemist for the Wellcome Laboratory, situated at the Veterinary Hospital—The Secretary of University Court, The University, Glasgow, W 2 (January 12)

Ornithologist (with a good honours degree and/or high professional attainment in ornithology) at London Headquarters, for work on ornithological matters at National level—The Nature Conservancy (E), 19 Belgrave Square, London, S W 1 (January 12)

Lecturer (with an honours degree or equivalent qualifications, and preferably special interests and experience in general psychology, experimental psychology or statistics) in the School of Applied Psychology—The Agent-General for New South Wales, 56–57 Strand, London, W C 2, and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (January 15)

University Lecturer (with an honours degree in agriculture, or in agricultural science, together with experience in teaching and research, and preferably with interests in the field of animal husbandry) in Agriculture—The Secretary of the Appointments Committee of the Faculty of Agriculture, The University, Cambridge (January 16)

Research Fellow or Research Assistant in Physics at the University of New England, to assist in a programme of research into the physics of ice, water and clouds—The Registrar, University of New England, Armidale, New South Wales, Australia, and The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 19)

Lecturer in Geography—The Registrar and Secretary, The University, 38 North Bailey, Durham (January 20)

Lecturer in Physics—The Registrar and Secretary, The University, 38 North Bailey, Durham (January 20)

Lecturer (preferably with a major interest in applied mechanics, especially theory of machines, stress analysis and properties of materials, or applied thermodynamics) in Engineering (Mechanical) —The Registrar, The University, Manchester 13 (January 20).

Lecturers (2) (suitably qualified honours graduates) in Geography in the Liberal and Social Studies Department, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana—The Assistant Registrar, Kwame Nkrumah University of Science and Technology, 29 Tavistock Square, London, W C 1 (January 20)

Scientific Officer (with a first- or second-class honours degree in mathematics with statistics, or a Diploma in Statistics) in the Statistics Section, for duties mainly concerned with the design and analysis of field and laboratory experiments—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (January 20)

Associate Professor in Concrete Technology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 24)

Lecturer or Assistant Lecturer in the Sub-department of Computation—The Secretary and Registrar, The University, Southampton (January 26)

Demonstrators (graduates with some research experience) in the Department of Organic Chemistry—The Registrar, The University, Liverpool, quoting Ref CV/575/N (January 31)

Demonstrators (2) (with a science degree with a major in zoology) in Zoology at the University of Melbourne, Australia—The Registrar, University of Melbourne, Parkville, N 2, Victoria, Australia (January 31)

Imperial Chemical Industries Research Fellows (one or more) —The Secretary, The University, Aberdeen (January 31)

Lecturer in Civil Engineering—The Registrar, The University, Sheffield (January 31)

Lecturer in Mechanical Engineering at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Hong Kong and London, January 31)

Senior Lecturer in Inorganic Chemistry at the University of Natal, Durban—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (South Africa and London, January 31)

Professor of Botany, and a Professor of Zoology—The Secretary, University College, Galway, Republic of Ireland (February 5)

Imperial Chemical Industries Research Fellows in the various branches of Chemistry, Chemical Engineering, Engineering, Technology, Physics and allied subjects—The Registrar, The University, Manchester 13 (February 14)

Turner and Newall Research Fellows in Engineering, Inorganic Chemistry, Physics and allied subjects—The Registrar, The University, Manchester 13 (February 14)

Reader in Mathematics, and a Lecturer/Senior Lecturer in Mathematics at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, February 17)

Lecturers (2) in Fuel Technology and Chemical Engineering —The Registrar, The University, Sheffield (February 28)

Senior Lecturer in Electrical Engineering, and Lecturers (2) in Electrical Engineering at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, March 1)

Lecturers, Assistant Professors or Associate Professors in the Department of Mathematics (either at the Edmonton campus or the Calgary campus)—Prof M Wyman, Mathematics Department, University of Alberta, Edmonton, Alberta, Canada

Postdoctoral Assistant for the operation of a research mass-spectrometer for the detection of unstable reaction intermediates—J W Linnett, Inorganic Chemistry Laboratory, The University, Oxford

Research Assistant (science graduate), to work with tissue culture in relation to carcinogenesis—Prof H N Green, Department of Experimental Pathology and Cancer Research, School of Medicine, The University, Leeds 2

Scientific Officer or Senior Scientific Officer (with an honours degree and experience in microbiology) in the Bacteriology Department, for work on problems involving hygiene and the isolation and classification of micro-organisms—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref 61/17

Senior Histological Technician to take charge of a Research Laboratory—Chief Technician, Department of Morbid Anatomy, Institute of Orthopædics, 234 Great Portland Street, London, W 1

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Modern Science Memoirs, No 41 On Teaching Voltaic Cells and Electrolysis By H G Andrews Pp 11 (London John Murray, 1961 Published for the Science Masters' Association) 2*s* net [1710

Department of Scientific and Industrial Research Forest Products Research Record No 31 Non-pressure Methods of Applying Wood Preservatives By J Bryan and D F Purslow Pp iv+14+8 plates 2*s* 6*d* net Bulletin No 47 Working Stresses for Structural Timbers By J G Sunley Pp iv+18 1*s* 3*d* net (London H M Stationery Office, 1961) [1710

Bulletin of the British Museum (Natural History) Geology Vol 5, No 7 The Old Red Sandstone of Brown Clee Hill and the Adjacent Area 1 Stratigraphy By Harold William Ball and David Lawrence Dineley 2 Palaeontology By Errol Ivor White Pp 175–310+plates 33–48 (London British Museum (Natural History), 1961) 70*s* [1710

Building Research Station Digest, No 15, (Second Series) Pipes and Fittings for Domestic Water Supply Pp 5 (London. H M Stationery Office, 1961) 4*d* [1710

Royal Observatory Bulletins No 37 Observations of Southern RR Lyrae Stars By T D Kinman Pp i+E151–204 (London H M Stationery Office, 1961) 7*s* 6*d* net [1710

British Cast Iron Research Association Fortieth Annual Report for the year ending 30th June, 1961 Pp 68 (Alvechurch, Birmingham British Cast Iron Research Association, 1961) [1710

London County Council Public Health Department—Scientific Branch Annual Report of the Scientific Adviser for the year 1960 Pp 60 (London London County Council, 1961) 1*s* 3*d* [1710

University of Oxford Department of Forestry—Library List of Periodicals and Serial Publications Part 1 A–R Pp xvii+1–150 Part 2 S–Z Pp 151–284 (Oxford Department of Forestry, The University, 1961) [1710

Agricultural Research Council Radiobiological Laboratory ARCRL No 5 Surveys of Radioactivity in Human Diet and Experimental Studies, (Report for 1960) Pp xii+76 (London H M Stationery Office, 1961) 6*s* 6*d*. [1710

The British National Health Service a Short Survey with particular reference to the Pharmaceutical Services Pp ii+22 (London The Pharmaceutical Press, 1961) 5*s* [1810

Schools Television a Discussion Document Pp 18 (London National Union of Teachers, 1961) 1*s* [1810

Imperial College of Science and Technology Research Report of the City and Guilds College, 1958–1961 Pp v+96 (London Imperial College of Science and Technology, City and Guilds College, 1961) [1810

Advisory Council on Scientific Policy, *Committee on Scientific* Manpower, Statistics Committee The Long-Term Demand for Scientific Manpower Pp ix+26 (Cmnd 1490) (London H M Stationery Office, 1961) 1*s* 9*d* net [1810

Royal Institute of Chemistry Directory of Independent Consultants in Chemistry and Related Subjects Fourth edition Pp iii+34 (London Royal Institute of Chemistry, 1961) [1810

Hill Farming Research Organisation Second Report, 1958–61 Pp 96 (Edinburgh Hill Farming Research Organisation, 1961) [1810

British Drug Houses, Ltd "Union Carbide" Molecular Sieves for Selective Adsorption Second edition Pp 36 Phosphate Esters in Metabolism an *Account* of some Biologically Important Organic Phosphate Compounds Pp 39 (Poole British Drug Houses, Ltd , 1961) [1810

Ion Exchange Progress, Vol 1, No 1, (October, 1961) Pp 4 (Lane End, Bucks Elga Products, Ltd , 1961) [1810

Ministry of Housing and Local Government Pollution of the Tidal Thames Report of the Departmental Committee on the *Effects of* Heated and other Effluents and Discharges on the Condition on the Tidal Reaches of the River Thames Pp iv+68+map (London H M Stationery Office, 1961) 5*s* 6*d* net [1810

Negretti and Zambia, Ltd Publication No APP/26 Centralised Temperature Indication for Hotels, Offices, Schools, etc Pp 3 Publication No E/16 Vibration Damping Bracket for use with "Aetec" Instruments Pp 2 Publication No E/19/1 "Aetec" Recording Pyrometers and Resistance Thermometer Recorders Pp 8 (London Negretti and Zambra, Ltd , 1961) [1810

Annual Report for 1960 (No 73) of the Marine Biological Station of the University of Liverpool at Port Erin, Isle of Man Drawn up by J S Colman Pp 29+1 plate (Liverpool The University Press, 1961) 5*s* [2310

Department of Scientific and Industrial Research and Fire Offices' Committee Joint Fire Research Organisation Fire Note No 1 Fire-resistance of Floors and Ceilings By G I Bird Pp ii+14 (London H M Stationery Office, 1961) 2*s* net [2310

The Inheritance of Hairy Ear Rims By R Ruggles Gates and P N Bhaduri (Mankind Monographs, No 1) Pp 38 (Edinburgh *The Mankind Quarterly*, 1 Darnaway Street, 1961) 6*s* , 1 25 dollars [2310

Institute of Personnel Management Annual Report, 1960–61 Pp 31 (London Institute of Personnel Management, 1961) [2310

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Fossils a Colour Filmstrip By Douglas G A Whitten, George R Raine, and Philip R Gurr With notes, 30*s* Drawings of 180 of the Commoner British Fossils Set of 20 sheets in case, 30*s* (Surbiton Marian Ray, 36 Villiers Avenue, 1961) [2310

Other Countries

Canada Department of Mines and Technical Surveys Geological Survey of Canada Map 1085A Firedrake Lake, District of Mackenzie, Northwest Territories, (Sheet 75 H) Paper 61–16 Preliminary Report on Borings through Pleistocene Deposits, Cochrane District, Ontario By O L Hughes Pp i+5 25 cents (Ottawa Geological Survey of Canada, 1961) [910

Chicago Natural History Museum Fieldiana Zoology Vol 42, No 10, (August 15, 1961) Philippine Zoological Expedition, 1946–1947 New and Interesting Odonata from the Philippines By M A Lieftinck Pp 119–149 (Chicago Chicago Natural History Museum, 1961) 1 dollar [910

Canada Review of the National Research Council, 1961 Pp 377 (NRC No 6421) (Ottawa National Research Council, 1961) 75 cents [910

State of Illinois Department of Registration and Education Natural History Survey Division Bulletin, Vol 27, Article 6, (August, 1961) Sex Ratios and Age Ratios in North American Ducks By Frank C Bellrose, Thomas G Scott, Arthur S Hawkins and Jessop B Low Pp vi+391–474 (Urbana, Ill Department of Registration and Education, Natural History Survey Division, 1961) 1 dollar [1710

Organization for European Economic Co-operation Rural Manpower and Industrial Development Adaptation and Training General Report by Prof H Krier Pp 130 (Paris Organization for European Economic Co-operation, 1961) 7 N F , 10*s* , 1 75 dollars , 5 80 D M [1710

Provincia de Buenos Aires Comision de Investigacion Cientifica Sistema Simetrico (MKSQ) de Unidades y Medidas Electromagneticas Por Enrique Loedel Palumbo Pp 90 (La Plata Comision de Investigacion Cientifica, 1960) [1710

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Chicago Natural History Museum Fieldiana Zoology Vol 39, No 51, (August 31, 1961) A New Peruvian Race of *Crypturellus obsoletus* By Emmet R Blake Pp 573–575 10 cents Vol 41, No 4 (August 25, 1961) The Land Snail Genus *Amphidromus*—a Synoptic Catalogue By Frank Fortescue Laidlaw and Alan Solem Pp ii+505–677 4 50 dollars (Chicago Chicago Natural History Museum, 1961) [1810

Republic of the Sudan Ministry of Agriculture Report of the Forests Department for the period July 1959 to June 1960 Pp v+115. (Khartoum Forests Department, 1961) [1810

Annual Report of the Board of Regents of the Smithsonian Institute showing the Operations, Expenditures, and Condition of the Institution for the year ended June 30, 1960 (Publication 4435) Pp x+605+106 plates (Washington, D C Government Printing Office, 1961) [1810

National Academy of Sciences—National Research Council, Nuclear Science Series NAS–NS 3039 The Radiochemistry of Niobium and Tantalum Pp v+57 (Washington, D C Office of Technical Services, Department of Commerce, 1961) 75 cents [1810

United States Department of the Interior Geological Survey Professional Paper 305-K Core Tests and Test Wells, Barrow Area, Alaska By Florence Rucker Collins With a section on Temperature Measurement Studies by Max C Brewer (Exploration of Naval Petroleum Reserve No 4 and adjacent Areas, Northern Alaska, 1944–53 Part 5 Subsurface Geology and Engineering Data) Pp iv+569–644+plates 39–43 (Washington, D C Government Printing Office, 1961) [1810

United States Department of Commerce Weather Bureau Technical Paper No 40 Rainfall Frequency Atlas of the United States for Durations from 30 Minutes to 24 Hours and Return Periods from 1 to 100 Years Prepared by David M Hershfield Pp ii+115 (54 charts) (Washington, D C Government Printing Office, 1961) 1 75 dollars [1810

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United States Department of the Interior Geological Survey Bulletin 1032-E Petrography of Radioactive Tertiary Igneous Rocks, Front Range Mineral Belt, Colorado By J D Wells Pp iv+223–272+plate 10 Water-Supply Paper 1475-E Ground Water in the Western Part of the Cow Creek and Soldier Creek Grazing Units, Malheur County, Oregon By R C Newcomb Pp iii+159–172 (Washington, D C Government Printing Office, 1960 and 1961) [2010

Australia Commonwealth Scientific and Industrial Research Organization, Melbourne Soil Publication No 17 The Development and Distribution of the Soils of the York-Quairading Area, Western Australia, in Relation to Landscape Evolution By M J Mulcahy and F J Hingston Pp 43+3 plates (Melbourne Commonwealth Scientific and Industrial Research Organization, 1961) [2310

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Forschungsberichte des Landes Nordrhein-Westfalen Nr 972 Uber den Einfluss des Interferenzfeldes auf die Schallabbildung in Festkorpern, Von Gunter Radig Pp 29 (Koln und Opladen Westdeutscher Verlag, 1961) 11 70 D M [2310

Canada Department of Mines and Technical Surveys *Geological* Survey of Canada Paper 61–17 Age Determinations by the Geological Survey of Canada Report 2 Isotopic Ages Compiled by J A Lowdon Pp vi+127 50 cents Paper 61–19 Surficial Geology of the Ottawa Area—Report of Progress By N R Gadd Pp ii+14 25 cents (Ottawa Queen's Printer, 1961) [2310

State University College of Forestry at Syracuse University Graduate Theses, 1955–1960 Compiled by Vincent Thirgood Pp 111 (Syracuse, N Y State University College of Forestry at Syracuse University, 1961) [2310

United States Department of Commerce Coast and Geodetic Survey Special Publication No 226 Control Leveling Pp iii+20 (Washington, D C Government Printing Office, 1961) 15 cents [2310

Population Reference Bureau, Inc *Population Bulletin*, Vol 17, No 6, (October, 1961) Population Trends in the U S S R Pp 109–128 (Washington, D C Population Reference Bureau, Inc , 1961) 50 cents [2310

United States Department of Commerce Coast and Geodetic Survey Publication 62-1 Tellurometer Manual By Austin C Poling Revised (1961) edition Pp v+76 (Washington, D.C Government Printing Office, 1961) 45 cents [2310

Tea Research Institute of East Africa Pamphlet No 19 Proceedings of the Seventh Conference, 17th and 18th November, 1960 Pp. 77 (Kericho Tea Research Institute of East Africa, 1961) [2310

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